Hematopoietic Stem Cell Transplantation for the Pediatric Hematologist/Oncologist

Valerie I. Brown
Editor

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Contents

Part I Introduction, History and the Basic Principles of Pediatric Hematopoietic Stem Cell Transplantation (HSCT)

1 **Introduction** 3
Valerie I. Brown

2 **Historical Overview of Pediatric HSCT** 7
Valerie I. Brown

3 **Brief Introduction to the Basic Scientific Principles of Hematopoietic Stem Cell Transplantation (HSCT)** 19
Valerie I. Brown

Part II Topics Related to the Pre- HSCT Period

4 **Pretransplantation: Indications and Timing** 57
Alicia McFarren and Michael A. Pulsipher

5 **Spotlight on Minimal Residual Disease (MRD): Impact of MRD on HSCT Outcomes for Pediatric Leukemia** 77
Hisham Abdel-Azim and Michael A. Pulsipher

6 **HSCT Recipient Pretransplantation Evaluation** 91
Carrie Eichelberger and Valerie I. Brown

7 **How to Select a Donor and Hematopoietic Stem Cell Source: Related Versus Unrelated Donors for Allogeneic HSCT** 97
Malika Kapadia and Robert Greiner

8 **Donor Evaluation, Selection and Hematopoietic Stem Cell Mobilization, Procurement, and Manipulation** 111
William Ferguson and Aleksandar Babic

9 **Preparing the Patient for HSCT: Conditioning Regimens and Their Scientific Rationale** 139
Carrie-Lynn Kitko, Katie Gatwood, and James Connelly

Part III The Peri- HSCT Period: Pre-Engraftment (Days 0–30) and Early Post- HSCT (Days 30–100) Periods

10 Engraftment and Chimerism 177
Valerie I. Brown

11 Graft Failure 187
Valerie I. Brown

12 Engraftment Syndrome and Associated Cytokine Storm and Capillary Leak Syndrome 195
Mala K. Talekar and Jason L. Freedman

13 Nutrition in the Peri-HSCT Period 201
Arun Gurunathan, Judy Bailer, and Jason L. Freedman

14 Mucositis and Pain in the Peri-HSCT Period 209
Arun Gurunathan, Neil S. Patel, and Jason L. Freedman

15 Hepatotoxicity in the Peri-HSCT Period 215
Valerie I. Brown

16 Renal Toxicities in the Peri-HSCT Period 235
Malika Kapadia, Terry Wikle Shapiro, and Robert Greiner

17 Infectious Complications and HSCT 241
Karen L. Bride, Ellen Levy, Anne Wohlschlaeger, and Jason L. Freedman

18 Acute Graft-Versus-Host Disease: Diagnosis, Prophylaxis, and Treatment 257
Karen L. Bride, Neil S. Patel, and Jason L. Freedman

Part IV The Late Post- HSCT Period (> 100 Days)

19 Chronic GvHD 269
Terry Wikle Shapiro and Malika Kapadia

20 Hematologic Complications Associated with HSCT 283
Mala K. Talekar and Timothy Olson

21 Pulmonary Complications Associated with HSCT 301
Malika Kapadia and Terry Wikle Shapiro

22 Renal Complications Associated with HSCT 327
Lena E. Winestone, Alix E. Seif, and Benjamin L. Laskin

23 Cardiac Complications Associated with HSCT 333
Valerie I. Brown

24 Neurologic and Sensory Complications Associated with HSCT 343
Valerie I. Brown

25 HSCT-Associated Complications of the Skin, Hair, and Nails 363
Valerie I. Brown

Part V Life After HSCT

26 Immune Reconstitution After Hematopoietic Stem Cell Transplantation 371
Mala K. Talekar and Timothy Olson

27 Life After HSCT: Survivorship and Long-Term Issues 385
Smita Dandekar

Part VI Pharmacologic-Related Issues of Pediatric HSCT

28 Medications Commonly Used in Pediatric HSCT 405
Kevin M. Mulieri, Ashley Teusink-Cross, JoEllen Weilnau, Krisoula Spatz, and Katie S. Gatwood

Index 449

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Part I

Introduction, History and the Basic Principles of Pediatric Hematopoietic Stem Cell Transplantation (HSCT)

1 Introduction

Valerie I. Brown

Abstract

This book stemmed from the concept of a symposium I organized and moderated a few years ago for the annual meeting of the American Society of Pediatric Hematology/Oncology. As the only member of the program committee who had expertise in pediatric hematopoietic stem cell transplantation (HSCT) at the time, I was charged to organize the joint Pediatric Blood and Marrow Transplantation Consortium (PBMTC)/ASPHO symposium for the following year's annual meeting. Generally, we try to make this session less esoteric in order to address the educational needs of a broader audience beyond pediatric HSCT specialists. At that time, the other committee members and I really appreciated that there can be a significant disconnect between the pediatric HSCT subspecialist and the rest of the pediatric hematology/oncology community with the biggest issues being which patients should be referred to the pediatric HSCT subspecialist for consideration of HSCT and when this referral should be made. Bridging this gap has become more and more important as the indications and accessibility to HSCT continue to expand. The symposium in its final format had one pediatric HSCT subspecialist present the data regarding the indications and the timing of evaluation for HSCT of pediatric patients with malignant conditions. The other symposium speaker addressed the same topics but for patients with nonmalignant disorders for which HSCT may be a treatment option. Overall, this symposium was well attended and well received. Based upon the positive responses received from attendees, this book was conceived and subsequently written with similar objectives

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and target audience in mind: This book is to provide an in-depth reference guide for not only pediatric HSCT subspecialists but also pediatric hematology/oncology specialists, fellows, residents, nurses, and advanced practitioners.

In general, this book is organized chronologically in terms of the "HSCT course." However, it is important to understand the past in order to understand the present practices and the challenges to moving the field of HSCT forward, and so Chap. 2 summarizes the "history" of HSCT from both the nonclinical and clinical aspects, starting with the first fundamental discoveries in immunology that led to the scientific understanding of immunology and transplantation biology and the development of HSCT in humans. While research in the areas of transplantation biology and immunology were being conducted in the first half of the twentieth century, it was not until the 1940s with the detonation of the two atomic bombs and the dawn of the Atomic Age that concerted efforts to apply transplantation biology to feasible patient care accelerated. The focus of research and funding transitioned to the investigation of the effects of exposure to radiation at varying levels on humans and how to treat these exposures. With the harnessing of radiation, physicians and scientists were exploring strategies to utilize radiation in a controlled fashion to treat a variety of diseases. This pioneering work led the way to the landmark clinical trials of the late 1960s and 1970s that are summarized in Chap. 2. It was also around this time that scientists and physicians who were pioneers of this burgeoning field began to form national and international organizations. In 1972, the International Bone Marrow Transplant Registry (IBMTR) was established and evolved into the Center for International Blood and Marrow Transplant Research (CIBMTR) in 2004. In 1974, the European Society for Blood and Marrow Transplantation was started. Organizations such as these were established in order to provide a formal mechanism by which these investigators could exchange their findings and pool their HSCT-related data in order to accelerate advances in HSCT. The 1980s and early 1990s were the times when alternative donors, such as umbilical cord blood and mobilized peripheral blood stem cells as well as graft manipulation and alternatives to myeloablative conditioning regimens, were being explored in order to expand the donor pool while reducing life-threatening side effects that can accompany HSCT. As a result of all of these efforts, more than 50,000 HSCTs are performed annually worldwide with much success currently.

Because HSCT is an immunotherapy, a basic understanding of the fundamental principles of hematopoiesis and transplantation biology is presented in Chap. 3. This chapter includes a discussion of hematopoiesis, the hematopoietic stem cell niches contained within the bone marrow, and how this bone marrow microenvironment that is hospitable to HSCs is created and maintained. This chapter also covers the fundamentals of transplantation biology with a focus on the immune response to allografts and the mechanisms of allograft rejection and tolerance. The pathophysiology of graft-versus-host disease can be found in Chaps. 18 and 19.

Part II of this book focuses on topics related to the pre-HSCT period and includes a discussion of the indications and timing of HSCT (Chap. 4). Chapter 5 is dedicated to the impact that minimal residual disease (MRD) status has on HSCT. In addition, this section of the book addresses other important pre-HSCT topics including how a potential patient is determined to be a suitable HSCT recipient (Chap. 6) and how the most suitable donor and donor HSC source are selected (Chaps. 7 and 8). Finally, this section concludes with an in-depth discussion of the need for conditioning prior to HSCT, the different types and intensities of conditioning regimens, and how the appropriate conditioning regimen is selected for an individual patient (see Chap. 9).

Part III centers on the key events that occur during the peri-HSCT period which spans the pre-engraftment period (days 0–30 post-HSCT)

through the period of early post-engraftment (days 31–100). The principles of engraftment and donor chimerism are discussed in Chap. 10, whereas potential complications encountered during this peri-HSCT period are covered in Chaps. 11, 12, 15, and 16 (including complications associated with engraftment as well as hepatotoxicity and renal toxicity), whereas Chaps. 13 and 14 focus more on the supportive care that is needed during these periods of the HSCT process (i.e., nutrition and the management of pain and mucositis). The prevention and treatment of infections are extremely important in HSCT, particularly during the peri-HSCT period. Chapter 17 is solely dedicated to the prevention and treatment of the most common and/or life-threatening infections encountered by pediatric HSCT patients. Finally, the last chapter of this section (Chap. 18) covers acute graft-versus-host disease (GvHD) which is a very common anticipated consequence of HSCT that can range from being self-limited to life-threatening. In addition to discussing the clinical features, diagnostic studies, and management of acute GvHD, this chapter addresses the approaches used for the prevention of acute GvHD.

Complications that occur during the late post-engraftment period (>100 days post-HSCT) are covered in Part IV of this book. The first chapter of this section (Chap. 19) focuses on chronic GvHD. Chronic GvHD can affect every organ system in the body but most commonly involves the skin, the eyes, the upper and lower GI tract, and the liver. The incidence, risk factors, clinical features, diagnostic studies, grading, treatment, and outcomes of chronic GvHD are detailed in this chapter. The remainder of the chapters (Chaps. 20–25) cover other common late post-engraftment complications and are organized by organ system. These include hematologic, pulmonary, renal, cardiac, and neurologic complications. In addition, non-GvHD-related issues of the skin, hair, and nails are addressed in Chap. 25.

Many medical issues may persist long after the actual infusion of the hematopoietic stem cells (i.e., the transplant). Part V of this book focuses on these topics. Chapter 26 details the immune reconstitution in terms of the different components of the immune system as well as the factors that impact this reconstitution. In addition, this chapter offers recommendations regarding revaccination post-HSCT. Chapter 27 provides a comprehensive review of long-term compilations of HSCT and how to approach the care of long-term survivors of HSCT.

Finally, Part VI contains a very comprehensive table of medications and agents that are commonly used in pediatric HSCT patients. It presents the medications by indications and provides pediatric dosing and schedule (where available) as well as common indications, side effects, and other relevant information for each agent. The information contained within this chapter is evidence-based. However, it provides general guidelines, and so the authors of this chapter and I strongly recommend that the reader follow their own institutional guidelines.

While other HSCT books are very comprehensive and "content" dense, this book was specifically designed to be a detailed guide to be used by all medical providers whose practice will intersect with a pediatric HSCT candidate, recipient, or donor. The authors and I hope that this book begins to bridge the disconnect that can exist between the pediatric HSCT specialists and other medical providers and trainees and promote a dialogue between these two groups.

2 Historical Overview of Pediatric HSCT

Valerie I. Brown

Abstract

With a better understanding of transplantation biology and immunology derived from animal models, hematopoietic stem cell transplantation (HSCT) in humans has become possible. Attempts at HSCT in humans were first reported as early as the 1930s. However, with the detonation of two atomic bombs at the end of World War II and advent of the "Atomic Age," interest in HSCT as a treatment modality for the effects of exposure to sub-lethal and lethal doses of irradiation on bone marrow function was reignited. Before the mid-1970s, the majority of HSCTs in humans were performed for nonmalignant conditions with 40% for severe aplastic anemia and 15% for primary immunodeficiencies. While attempts were made to treat advanced, refractory acute leukemia patients with HSCT, they were generally unsuccessful and used identical twin sibling as the donor initially. The first reports of successful sustained engraftment occurred in the early 1960s, but these patients died from complications associated with what is now known as graft versus host disease. It was not until 1968 that there were reports of three infants with primary immunodeficiency conditions that were long-term survivors after matched sibling donor bone marrow transplantation. Of note, all three patients are still alive today. In the late 1970s, Thomas and his colleagues reported their findings that of 100 patients with refractory acute leukemia, 13 were alive and leukemia-free 1–4.5 years after undergoing HLA-identical sibling donor bone marrow transplantation. These results showed that some patients with advanced acute leukemia could be cured of their disease with HSCT and that HSCT should be undertaken in the first or

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second remission (i.e., not with active disease) if the patient has an HLA-matched sibling donor because outcomes would be predictably better. Thus, by the mid-1980s, approximately 75% of all allogeneic HSCTs were performed to treat leukemia, and the vast majority were with HLA-identical sibling donors. As supportive care improved, drugs (such as calcineurin inhibitors) became available for graft versus host disease (GvHD) prophylaxis, and the use of alternative donor HSCTs (including matched unrelated donors, mismatched related and unrelated donors, familial haploidentical donors, and umbilical cord blood) was investigated, HSCT became a viable option for many more patients. Furthermore, the development of less intensive conditioning regimens and use of alternative hematopoietic stem cell sources made HSCT a feasible treatment modality for those who would otherwise be ineligible for HSCT. Nowadays, HSCT is a very important treatment modality for both pediatric and adult patients for a wide range of malignant and nonmalignant disorders. This chapter is divided into two major sections with the first part focusing on the seminal discoveries in transplantation biology and immunology using animal models (scientific and preclinical perspective) and with the second part highlighting key human clinical reports related to HSCT (clinical perspective).

Introduction

In the late 1860s, the pathologists, Neumann in Prussia (now Russia) and Bizzozero in Italy, independently reported the observation that mammalian blood cells are derived in the red, spongy areas of the bone, i.e., the bone marrow, and the blood cells exit the bone marrow via small blood vessels that traverse the bone cortex to the peripheral blood. Various attempts at replacing the bone marrow in patients who were perceived as having a deficiency in their bone marrow occurred early on; for example, in 1939, an attempt at treating a patient with aplastic anemia by injection of a few milliliters of the ABO-compatible bone marrow into the patient's sternum was reported. Of note, the patient had no response. It was not until a better understanding of transplantation biology and immunology was achieved first by using animal models and then in human clinical trials did HSCT become successful and eventually become a feasible treatment modality as we know it today.

The first half of this chapter focuses on the landmark observations and discoveries using animal models that led to the successful development of HSCT as we know it today, while the second half of this chapter highlights the studies in humans from the initial attempts at HSCT through the development of alternative donor and hematopoietic stem cell (HSC) transplants and the recognition and improvements in supportive care as well as other barriers to expanding HSCT to the majority of potential patients and how they were overcome.

Another aspect that has significantly contributed to the advancement of HSCT in humans has been the formation of national and international organizations to track and monitor HSCT. These include the establishment of the Center for International Blood and Marrow Transplant Research (CIBMTR) in 1972. It was formed with the goal of setting up a systematic method of collecting HSCT outcome data through collaboration. At the time, there were less than 50 patients who had been transplanted at 12 centers worldwide. Shortly thereafter, the European Society for Blood and Marrow Transplantation (EBMT) was established in 1974 to provide an organization in which scientists and physicians could cooperate to develop HSCT-related clinical studies. In 1993, the American Society for Blood and Marrow Transplantation (ASBMT) was formed. This association was established as a scientific and professional society for those dedicated to the advancement of HSCT. In conjunction with the International Society for Cellular Therapy (ISCT), the ASBMT cofounded the Foundation for the Accreditation of Cellular Therapy (FACT) in 1996.

FACT is a worldwide recognized accreditation program for HSCT centers.

In 1986, the U.S. Navy established the National Bone Marrow Donor Registry (now called the National Marrow Donor Program, NMDP) to establish an organization to facilitate the identification of unrelated HSC donors. Initially, 10,000 potential donors were registered, and the first donor search was performed in 1987. In 2004, CIBMTR and NMDP joined together. To date, the NMDP has facilitated over 74,000 marrow and umbilical cord blood transplants, with almost 6400 transplants a year. NMDP consists of over 150 HSCT centers and over 90 donor centers. Today, CIBMTR represents a large network of centers in over 50 countries and has collected data on more than 425,000 patients.

Scientific and Preclinical Perspective

Pre-World War II to the Mid-1940s

In the early twentieth century, Alexis Camel and colleagues noted that skin and organ transplants function for a time but were eventually rejected after 1–2 weeks. In the 1930s, Gorer, Snell, and colleagues were beginning to investigate the immunologic basis of tumor transplantation in mice; their work led to the discovery of the H2 antigen transplantation system [1, 2]. In the 1940s, Medawar and colleagues established the immunological basis of allograft rejection [3]. Owen et al. developed the concept of "immune tolerance," noting that freemartin bovine dizygotic twins had a mixture of red blood cells from each partner [4]. In the same decade, Billingham, Brent, and Medawar showed that donor-specific tolerance could be induced by injection of donor cells into newborn mice [5]. These seminal discoveries set the stage for further preclinical work performed after World War II that led to the feasibility of HSCT in humans.

Post-World War II to the Mid-1950s

Hematopoietic stem cell transplantation really started to take form post-World War II (late 1940s to early 1950s) when Jacobson et al. found that mice could survive lethal irradiation if the spleen were shielded (i.e., protected) with lead [6, 7]. Based upon this work, Jacobson and his colleagues proposed that humoral factors accounted for these observations that they termed the "humoral hypothesis." In contrast, Lorenz et al. [8] showed that lethally irradiated mice and guinea pigs could survive if they received a retroperitoneal injection of spleen or bone marrow cells that were harvested prior to the irradiation, thus supporting the "cellular hypothesis." These two reports spurred a great debate regarding the drivers of bone marrow recovery, i.e., humoral versus cellular mechanisms.

Further support for the cellular hypothesis was provided by the work of Barnes and Loutit in the mid-1950s. Their experiments showed that bone marrow recovery after spleen or bone marrow infusion was due to living cells and not "humors" [9]. In 1955, Main and Prehn [10] showed that cellular reconstitution (versus humoral factors) was protective against irradiation. They showed that mice that were lethally irradiated and rescued by an autologous bone marrow infusion did not reject subsequent skin grafts indefinitely even across major histocompatibility complex barriers. These experiments provided the proof of acquired tolerance and that this acquired tolerance was conferred by the transfer of living cells. In the following year, Trentin et al. [11] showed that tolerance of the skin graft was specific for the donor strain. Ford et al. [12] went on to show that the bone marrow of lethally irradiated mice rescued by the donor bone marrow or spleen cells displays the cytogenetic characteristics of the bone marrow donor; this was also the first report that used the term "radiation chimera" while referring to the resultant transplanted mouse. Also, in support of the cellular hypothesis, Nowell et al. [13] found that rat bone marrow protects mice against lethal irradiation. They found donor rat bone marrow cells in the bone marrow of the transplanted mice, indicating that the infused donor bone marrow cells can home to and take up residence in the host's bone marrow. In 1956, van Bekkum determined that intravenous administration of hematopoietic stem cells is the optimal route of administration to repopulate the bone marrow and

answered the key question of how to get bone marrow cells to grow in the recipient bone marrow.

Around the same time, Barnes et al. [9] successfully treated murine leukemia with supralethal doses of irradiation and normal bone marrow grafting, suggesting that bone marrow grafting could be used to treat human patients with leukemia. Barnes and colleagues went on to speculate that donor immune cells may have the capacity to destroy residual leukemia cells. This is the first written speculation of the concept of the graft versus leukemia effect.

The Late 1950s to the Late 1960s

The late 1950s ushered in a decade-long period of productive research in transplantation biology and immunology utilizing animal models, ongoing in mice and then in canine models. In 1957, Uphoff et al. [14] discovered that genetic factors control the severity of the immune reaction of donor cells against the host. They also showed that methotrexate can ameliorate the graft versus host reaction that had been noted in mice after they had received lethal irradiation followed by bone marrow or spleen infusion [15]. Billingham and Brent [16] also noted this phenomenon of engrafted donor cells mounting an immune reaction against the host, and they termed it "secondary disease." It was also referred to as "wasting syndrome" because of its associated symptoms of significant weight loss; the presence of poor, unhealthy fur; and generalized scruffiness [17]. This syndrome was subsequently recognized as graft versus host disease (GvHD). Shortly thereafter, Lochte et al. [18] demonstrated that methotrexate could be used not only to treat GvHD but also to prevent it. However, it was not until the development of better immunosuppressants, such as calcineurin inhibitors, that the control of GvHD became feasible.

In the early 1960s, Till and McCulloch [19] showed that the bone marrow contains clonogenic precursors capable of self-renewal and multi-lineage differentiation, i.e., hematopoietic stem cells (HSCs). Also, in the early 1960s, the important roles that the thymus, T-cells, B-cells, and other lymphoid subsets play in transplantation biology were beginning to be recognized by multiple investigators [20–22]. Eventually, Berenson et al. [23] found that the $CD34^+$ cell surface protein was a marker of a subpopulation of bone marrow cells enriched for the ability to engraft and give rise to all cell types of hematopoietic origin, i.e., $CD34^+$ is a marker for HSCs. In 1994, Korbling et al. reported that HSCs can be separated and purified based upon their $CD34^+$ expression of the cell surface [24].

While work continued in transplantation biology with inbred murine models, the use of outbred canine models expanded greatly during the 1960s, and this research yielded critical observations of not only basic transplantation biology but also of improvements in supportive care and better recognition and understanding of complications associated with HSCT. In the early 1960s, investigators showed that dogs could survive two to four times above lethal doses of total body irradiation (TBI) if they were given back previously harvested (fresh or frozen) autologous bone marrow cells after the exposure to TBI. As part of the work done with dogs, Cavins et al. [25] found that HSCs could be obtained from not only the bone marrow but also the peripheral blood.

By the late 1960s, research using dog models that determined the dog leukocyte antigen (DLA) system (which is equivalent to MHC in mice and HLA in humans) was critical in determining the outcomes of allogeneic bone marrow engraftment [26]. Irradiated dogs receiving DLA-mismatched bone marrow from littermates after lethal irradiation died from graft rejection or GvHD, whereas those who received DLA-matched bone marrow from littermates followed by post-HSCT methotrexate were long-term survivors [27–29]. Storb et al. [30] also found that dogs could successfully engraft after receiving chemotherapy alone (i.e., no TBI), with either cyclophosphamide or busulfan. Most of the dogs showed only donor cell engraftment, but some were healthy mixed chimeras. This work suggested that HLA-matched "sibling donor HSCTs" could lead to long-term, healthy chimeras.

The 1970s

As the 1970s began, studies continued to focus on understanding the causes of graft failure. For example, Storb et al. observed in dog models

that blood transfusions from the donor (related or unrelated) prior to transplant can result in sensitization of the recipient to donor transplantation antigens, resulting in graft failure [31].

Clinical Perspective

Post-World War II to the Mid-1960s

Early attempts in human HSCT were only successful in syngeneic HSCTs. In 1949, a report from Poland described the use of bone marrow infusion as a treatment in children with leukemia and other blood disorders [32]. The discovery of human leukocyte antigen (HLA) groups and the development of techniques to perform tissue typing were critical advances to the development of HSCT. In 1954, Miescher and Fauconnet [33] first described antibodies that were induced by transfusion or pregnancy that react with antigens on human white blood cells. In 1958, two other groups (Dausset et al. and van Rood et al.) observed that HLAs were inherited in codominant fashion [34, 35]. Serotyping was initially developed in dogs by Epstein et al. and was eventually developed for HLA [26, 36]. Shortly after these reports describing serotypes were published, international HLA workshops were held during which investigators exchanged reagents, standardized antigen definitions, established a common nomenclature, and developed standardized testing techniques. The advancements in typing techniques are discussed further in Chap. 7. These international workshops have continued on, and today, over 12,000 Class I and over 4500 Class II alleles have been identified [37].

The first attempts of treating humans with supralethal TBI followed by bone marrow grafting were reported in 1957 by Thomas et al., using an identical twin sibling as the donor [38]. In 1959, the same transplant group in Seattle reported the treatment of two patients with advanced leukemia with high-dose irradiation followed by a bone marrow infusion from their respective identical twin sibling [39]. The two patients engrafted and were "leukemia-free" for 4 months. However, they relapsed and succumbed to their disease. These landmark studies showed that TBI followed by an infusion of compatible bone marrow could reconstitute hematopoietic function as well as produce a snit-leukemic effect, albeit not durable, in these cases. Between the mid-1950s and the mid-1960s, approximately 200 HSCTs had taken place worldwide with generally dismal long-term outcomes, as reviewed in the landmark paper by Bortin which was published in 1970 [40].

These early failures were due to a lack of knowledge regarding human histocompatibility and typing, the use of inadequate radiation dosing to provide adequate immunosuppression, the lack of drugs to effectively prevent and treat GvHD, and the selection of patients with such advanced disease to undergo HSCT [41]. In addition, the lack of adequate supportive care such as effective antibiotics and antiviral agents as well as inadequate transfusional support with platelets contributed to these poor outcomes early on in HSCT. In a report from Leiden, the Netherlands, a child with severe combined immunodeficiency disease (SCID) was transplanted with a "matched" unrelated donor bone marrow infusion and four fetal thymuses [42]. While he appeared to show hematopoietic recovery, the child died 2 weeks later of fulminant bacterial pneumonia and possibly GvHD or a generalized autoimmune reaction. In another report from this same era, the Seattle HSCT group transplanted a patient with chronic myelogenous leukemia (CML) in blast crisis [43]. He was conditioned with TBI and received a bone marrow infusion from his "matched" sibling (who was later noted to be a one-antigen mismatch donor). The patient engrafted, but he subsequently died of cytomegalovirus (CMV) pneumonia. As a result, opportunistic infections in the post-HSCT patient population were recognized as a serious, life-threatening barrier for HSCT to succeed. The development of antiviral agents such as ganciclovir and sensitive CMV detection methods positively impacted HSCT outcomes significantly [44–46].

The Late 1960s to the Late 1970s

While the reports of the use of HSCT for the treatment of advanced leukemia in humans were very discouraging early on, the use of HSCT for nonmalignant disorders, particularly primary immu-

nodeficiencies, showed promise by the late 1960s. In 1968, Gatti et al. [47] reported the first successful allogeneic bone marrow transplant (BMT) in an infant with severe combined immunodeficiency disease (SCID) using an HLA-identical sibling as the donor. Two other reports of successful HLA-identical sibling BMTs for the treatment of a primary immunodeficiency were published shortly thereafter [48, 49]. All three of these patients remain long-term survivors today. Thomas et al. reported the first successful matched sibling donor allogeneic HSCT for severe aplastic anemia in 1972 [50]. HSCT was being attempted in very few pediatric patients with leukemia prior to 1975 because it was felt that there would be very little chance of a cure in this patient population that had been so heavily pretreated resulting in leukemia that would be very resistant to further treatment.

As the 1970s progressed, consistent donor bone marrow engraftment was achieved in patients with a variety of indications for HSCT. In a two-part series published in the *New England Journal of Medicine* in 1975, Thomas et al. reported the outcomes of the first 100 patients transplanted in Seattle [51, 52]. Of the 100 patients, 73 had advanced leukemia, whereas 37 had severe aplastic anemia. Overall, 50% of the patients with severe aplastic anemia had successful outcomes, and the number of patients with advanced leukemia who achieved remission post-HSCT was increasing. However, the consistent, successful allogeneic donor engraftment resulted in an increased incidence of GvHD, with acute GvHD occurring in approximately 50% of patients despite the long-term use of methotrexate. While the entity of GvHD had been recognized as a serious, potentially life-threatening consequence of allogeneic transplantation as a “wasting syndrome” in mice some 20 years prior, GvHD was not recognized as a serious barrier to moving HSCT forward in humans until the early 1970s with the advent of consistent successful engraftment of allogeneic matched sibling donor bone marrow in humans. Then, patients were dying from GvHD despite the use of methotrexate which was found to prevent GvHD in only about 50% of HSCT patients. It was not until 1978 when Powle et al. [53] described the first use of the calcineurin inhibitor cyclosporine A to treat GvHD in humans that the option of allogeneic HSCT in humans became more accessible to a larger group of patients.

In 1982, Deeg et al. [54] reported the successful use of a short course of methotrexate with cyclosporine A as GvHD prophylaxis in dogs. In 1986, Storb et al. [55] reported that a short course of methotrexate (days 1, 3, 6, and 11) in combination with daily cyclosporine A decreased the incidence of acute GvHD in matched sibling donor transplants to 20–30%. This combination is still considered the “gold standard” for GvHD prophylaxis today. Other approaches to reduce the incidence of GvHD were investigated at this time. In 1981, Reisner et al. [56] reported that T-cell depletion of the donor graft could decrease the risk of GvHD.

The Mid-1970s to the Late 1970s: Allogeneic HSCT Is Curative for Leukemia

In the first half of the 1970s, the advancement of HSCT as a viable treatment modality was somewhat stalled because the patients undergoing HSCT were typically patients with otherwise incurable, end-stage leukemia, and they either died from their disease or succumbed to GvHD or opportunistic infections, as described above. However, the HSCT group in Seattle published the results of 100 patients with advanced leukemia who had undergone matched sibling donor BMT [57]. Of these 100 patients, 13 were long-term survivors, demonstrating that some patients with end-stage, advanced leukemia could be cured with allogeneic HSCT. Thus, it was hypothesized that patients who undergo HSCT in the first remission (and not waiting until they relapse) may have a better chance for a cure. In Germany, in the late 1970s, the Berlin-Frankfurt-Munster (BFM) and the CoALL groups took this approach of transplanting patients with relapsed leukemia shortly after achieving a remission immediately after completion of induction chemotherapy [58]. Thomas et al. reported the successful treatment of patients with acute myeloid leukemia (AML) or with acute lymphoblastic leukemia (ALL) in the first remission with matched sibling donor HSCT [59, 60]. Dopfer et al. [61] reported that this treatment strategy was more beneficial for pediatric

patients with relapsed leukemia. Subsequent trials supported this observation. Now, it is well established that the lower tumor burden (i.e., low or no minimal residual disease (MRD) detected) is associated with superior outcomes [62].

Acute leukemias treated with HSCT were not the only type of leukemia being investigated. In 1979, Fefer et al. reported the disappearance of Ph+ chromosome in four patients with chronic myelogenous leukemia who were treated with chemotherapy and irradiation followed by an identical twin sibling donor BMT [63]. Two subsequent studies demonstrated that the treatment of CML in the chronic phase with chemotherapy and TBI followed by allogeneic BMT from a matched sibling donor was successful [64, 65]. Two large studies supported the successful outcomes of patients with CML treated with allogeneic HSCT [66, 67]. Treatment with HSCT of CML in the chronic phase was the standard of care until the development of BCR-ABL-targeted therapies such as imatinib and dasatinib. Nowadays, HSCT for CML patients in blast crisis is still considered the standard of care.

By the late 1970s, multiple reports noted that there was a decreased incidence in relapse of leukemia in patients with GvHD, and in a few patients, decreasing immunosuppression could lead to a remission of leukemia post-HSCT. These were the first inklings in humans of the concept of the “graft versus leukemia effect” in which the immune cells from the donor are capable of recognizing the leukemia cells as “bad” and eliminate (or at least disarm) them. This is the same concept proposed by Barnes after analyzing his leukemic mice studies over 40 years earlier [9]. The concept that HSCT serves as an immunotherapy (and not just a method to eliminate tumors cells) was supported by the observation that the infusion of donor lymphocytes along with the discontinuation of all immunosuppression can induce a remission. This approach of donor lymphocyte infusion (DLI) was first utilized successfully in CML and then in Epstein-Barr virus post-transplant lymphoproliferative disease (EBV-PTLD) [68, 69]. Kolb et al. reported that relapse of CML post-HSCT could be successfully placed back into remission with donor lymphocyte infusions (DLI) [70]. In 1994, Papadopoulos et al. reported the successful use of DLI for the treatment of EBV-PTLD [69]. These reports were among the first to suggest that the donor HSC graft not only “replaces” the recipient’s immune system that is eliminated by myeloablative conditioning but also acts as immunotherapy for the treatment of the underlying malignancy, i.e., creating a graft versus malignancy effect. It is now a well-established practice to use DLI if a post-HSCT patient shows signs of impending relapse (such as decreasing donor chimerism) or has a frank relapse of his/her leukemia (see Chap. 11).

The 1980s to the Present: Expansion of the Application of HSCT, Refinement of Conditioning Regimens, and Development of Alternative Donor HSCT

Expansion of the Application of HSCT: While HSCT was being actively investigated for the treatment of leukemia, HSCT was also being explored for the treatment of nonmalignant conditions beyond primary immunodeficiencies. It was not until the 1980s that HSCT was tested in humans as a curative treatment for hemoglobinopathies. In the early 1980s, the first successful matched sibling donor BMTs for thalassemia were performed. In 1982, Thomas et al. [71] reported the successful transplantation of a patient with thalassemia major using an allogeneic matched sibling donor. In 1984, Lucarelli et al. [72] reported the first successful outcomes of treating children with thalassemia with BMT. In that same year, Johnson et al. reported the case of an 8-year-old girl who underwent allogeneic matched sibling donor BMT for AML [73]. She also had sickle cell disease. The HSCT not only cured her of AML but also cured her of sickle cell disease. Currently, matched sibling donor HSCT is performed routinely for patients with thalassemia major and for patients with sickle cell disease with certain high-risk factors. Alternative HSCTs, such as familial haploidentical HSCTs, are currently being investigated (see Chap. 4).

Refinement of Conditioning Regimens: Initially, total body irradiation (TBI) was delivered as a single fraction alone as the conditioning

regimen. However, it was shown that TBI delivered in multiple fractions at a lower dose was superior to delivering it as a high-dose, single fraction [54, 74]. Because irradiation can cause such devastating long-term sequelae, particularly in young patients, conditioning regimens that avoid TBI such as busulfan/cyclophosphamide (Bu/Cy) were being explored in the early 1980s [75, 76]. In the following decade, multiple reports of the use of non-TBI conditioning regimens were reported but with mixed results. Clift et al. in 1994 reported no difference in a Bu/Cy versus a cyclophosphamide/TBI (Cy/TBI) regimen in event-free survival (EFS) [77]. In contrast, the first HSCT studies for patients with AML showed that outcomes were better with Cy/TBI versus cyclophosphamide/busulfan conditioning regimens; it is notable that this study was performed before the availability of intravenous busulfan [78]. In a subsequent study from 1997, Long et al. showed that cyclophosphamide/etoposide/TBI as a conditioning regimen demonstrated efficacy in patients with high-risk leukemia [79].

In the late 1990s, the concept of non-myeloablative (NMA) conditioning was being actively explored. Giralt et al. [80] demonstrated that patients conditioned with a purine analogue-based (i.e., fludarabine), NMA conditioning regimen resulted in successful engraftment. Shortly thereafter, multiple groups reported the use of NMA conditioning followed by HSCT in elderly patients with hematologic malignancies who would otherwise not tolerate a myeloablative conditioning regimen [81]. The use of NMA conditioning was also being actively investigated for patients who would have no benefit from the graft versus malignancy effect (and thus no need for GvHD), such as patients with primary immunodeficiencies or hemoglobinopathies [82]. However, many of the initial studies in patients with hemoglobinopathies were not very successful because a significant proportion of patients lost their grafts and reverted back to their chronic disease state despite initially engrafting.

In 2001, Giralt et al. introduced a conditioning regimen of fludarabine/melphalan as a reduced-intensity conditioning (RIC) regimen [83]. In 2005, Rao et al. [84] showed a significant survival advantage after RIC (versus myeloablative conditioning) followed by matched unrelated donor HSCT in children with primary immunodeficiency. Time to engraftment, chimerism, immune reconstitution, and incidence of GvHD were comparable. Of note, RIC was associated with increased viral reactivation.

Development of Alternative Donor HSCT: Because not every patient who may benefit from a HSCT has a suitable donor, alternative sources of donor HSCs have been actively pursued. These alternative donor sources needed to be safe and not result in increased morbidity and mortality. These types of HSCTs are referred to as alternative donor HSCTs (i.e., an alternative to matched sibling donors). In order for alternative donor HSCTs to be effective in humans, the mechanisms by which HSCs are regulated and donor grafts are rejected needed to be elucidated, and the pathophysiology of GvHD needed to be better understood. Work in these areas became actively investigated when Knudtzon et al. [85] first reported the in vitro growth of HSCs isolated from human umbilical cord blood in 1974. The first use of umbilical cord blood (UCB) as the HSC source for allogeneic matched sibling donor HSCT occurred in 1988 [86]. The patient had Fanconi anemia. In 1995, Broxmeyer proposed the use of unrelated UCB as an alternative HSC donor source. Shortly thereafter, UCB unit banks were established across the world. The first study of the use of unrelated UCB as the HSC source in 25 children with a variety of indications was reported in 1996 [87]. This study demonstrated that unrelated mismatched UCB HSCT was a feasible alternative donor HSCT. Because a UCB unit has a fixed HSC dose, its use was initially limited to children and small adults to minimize the risk of graft failure. However, Barker et al. reported the use of two unrelated UCB units in the same patient with no untoward effects [88]. These findings resulted in the expansion of UCB HSCT to adults as well as pediatric patients.

While it was known that HSCs can be found in the peripheral circulation, the absolute number of HSCs is low. However, it was observed that this number would be higher in a cancer patient's peripheral blood when recovering from chemotherapy. Investigators took advantage of this rebound effect and used low-dose cyclophospha-

mide to promote the release of HSCs into the peripheral blood, while others used endotoxin to evoke a similar response [89, 90]. With the availability of the cloned hematopoietic growth factors, granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF), mobilization of HSCs, and collection by apheresis became feasible [91–93]. Juttner et al. [94] reported the use of peripheral blood HSCs for autologous HSCT for AML. Shortly thereafter, it was shown that GM-CSF [92] and G-CSF [95] could be used in humans to stimulate and mobilize CD34$^+$ HSCs into the periphery for pheresis and then used for autologous HSCT. Nowadays, growth factor-mobilized peripheral blood HSCs are used as the stem cell source for both autologous and allogeneic HSCTs from both related and unrelated donors.

In addition, the late 1990s marked the advent of the use of partially mismatched donors [96, 97]. Furthermore, Reisner et al. [56] reported the use of a familial haploidentical donor as the HSC source for a patient with SCID that is now being actively investigated for multiple indications, including sickle cell disease, severe aplastic anemia, and leukemia, for patients who do not otherwise have a suitable donor.

Key Points

- With a better understanding of transplantation biology and immunology using animal models, HSCT in humans became feasible.
- With the detonation of the two atomic bombs at the end of World War II in the 1940s, interest in HSCT as a treatment for exposure to lethal doses of irradiation reached prominence.
- While HSCT was attempted as early as the 1940s, it was not until the late 1960s that HSCT resulted in long-term, disease-free survivors which consisted of three infants with primary immunodeficiency.
- In the late 1970s, allogeneic matched sibling donor HSCT was demonstrated to induce long-term remissions in a fraction of patients with advanced, end-stage leukemia, suggesting that HSCT may be more effective if performed in patients in the first or second remission. This proved to be true.
- In the 1980s and 1990s, advances were made that resulted in HSCT becoming widely available for patients who would otherwise be ineligible for HSCT. These advances include improvements in supportive care, the development of less intensive conditioning regimens, and the availability of alternative donors and HSC sources.

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Brief Introduction to the Basic Scientific Principles of Hematopoietic Stem Cell Transplantation (HSCT)

3

Valerie I. Brown

Abstract

The primary function of the immune system is to provide essential defense mechanisms against all foreign pathogens. The immune system has evolved in such a way that different immune responses are optimized to recognize and then eliminate or contain different types of foreign antigens which are expressed or secreted by foreign pathogens. It provides not only efficient and effective killing of microbes/pathogens via innate immunity but also specific long-lasting immunity against a particular microbe/pathogen to be triggered if the foreign microbe's antigen is encountered in the future via adaptive immune responses. Immunologic mechanisms are intimately involved in engraftment, engraftment rejection, graft versus host disease, and graft versus malignancy effect. In addition, immunologic tolerance is key for allogeneic immune reconstitution post-hematopoietic stem cell transplantation (HSCT). Because of a better understanding of the immune system and its different immune properties and responses, physicians and researchers have been able to perform successfully and safely HSCT in humans. While many of the concepts of basic immunology and transplant biology are intertwined into other chapters of this book, this chapter focuses on providing the fundamental principles of basic immunology and transplant biology, including the development of the components of the immune system (i.e., hematopoiesis), the molecules, cells, tissues, and organs that make up the immune system as well as their structural and functional organization and the types of immune responses along with their cardinal features. Key concepts related to HSCT including antigen presentation, alloreactivity, and tolerance and how these processes relate to HSCT

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will be described in brief. Firstly, though, this chapter begins with the definitions of some key terms and concepts related to basic immunology and transplant biology in order to establish the "vocabulary" of the immune system.

Introduction

The overarching function of the immune system is to serve as vital defense against foreign substances. Different mechanisms of defense have evolved against different pathogens. Knowledge of the immune system and its different responses has permitted physicians and researchers to successfully perform hematopoietic stem cell transplantation (HSCT) with long-term engraftment and success in humans. This chapter presents the major tenets of hematopoiesis, the organization of the immune system, different immune responses, and how this information relates to HSCT, starting with definitions of some of the key molecules, cells, functions, and concepts of the immune system.

Definitions

A vocabulary has been developed to describe the immune system and all of its components and processes. This section provides the definition of some of these keywords and concepts.

Immunity: Immunity refers to the protection against disease, particularly infections, that is mediated by a collection of cells, tissues, and molecules called the immune system. Immunity also refers to the ability to respond to any foreign substance, infectious and noninfectious.

Immune system: The immune system consists of the highly integrated collection of all the cells, tissues, organs, and molecules that provide protection against foreign organisms and substances. The immune system is responsible for immunity.

Immune response: An immune response refers to the collective and highly orchestrated response by immune molecules and cells to a foreign substance (e.g., microbes and their components), although noninfectious agents, such as proteins, polysaccharides, and chemicals, can elicit an immune response. An autoimmune response is the pathologic immune response to self-molecules that very often has detrimental effects.

Innate immunity: Innate immunity provides protection against infection via rapid, pre-existing responses to microbes with the same reaction (with the same intensity, time to initiation, and duration) to repeated infections. Components of innate immunity include cells (phagocytes, e.g., neutrophils, macrophages, and NK cells) and cytokines (predominantly produced by dendritic cells and mononuclear phagocytes), the complement system, and epithelial barriers.

Adaptive immunity: Adaptive (or acquired) immunity is stimulated by exposure to foreign substances and is characterized by exquisite sensitivity, specificity, and memory. Its specificity for distinct macromolecules and its memory allows for a more rapid and vigorous response with repeated encounters to the same foreign pathogen; it is mediated by lymphocytes.

Humoral immunity: Humoral immunity is a type of an adaptive immune response that is the principal defense against extracellular microbes and their toxins. Humoral immune responses are mediated by antibodies that are produced by activated B-cells.

Cell-mediated immunity: Cell-mediated (or cellular) immunity provides defense against intracellular microbes that either have infected a host cell or have been ingested by a phagocyte. Cell-mediated immune responses are mediated by T-cells predominantly of two different phenotypes: $CD4^+$ helper T-cells that mediate phagocyte activation and $CD8^+$ cytotoxic T-cells that are responsible for directly killing infected cells.

Homeostasis: Homeostasis is the state of the adaptive immune system that maintains a constant number and diverse repertoire of lymphocytes. It is a balance that is achieved by the regulation of death, inactivation, and expansion/proliferation of lymphocytes.

Tolerance: Tolerance is characterized by the unresponsiveness to antigens by the adaptive immune system that leads to inactivation or death of antigen-specific lymphocytes. Tolerance is the mechanism by which the immune system tolerates (or ignores) self-antigens and does not attack

self-tissues whereas tolerance of foreign antigens may be induced under certain circumstances and may be detrimental in the long term.

Antigen: A molecule that induces a specific immune response or is recognized by T-cells or B-cells as well as antibodies is an antigen. An antigen binds to an antibody or the T-cell receptor (TCR). An antibody can bind to an antigen alone whereas most TCRs bind to an antigen peptide fragment only when it is complexed with "self" MHC molecules.

Cytokine: Any secreted protein that regulates, stimulates, suppresses, and/or coordinates the activities of cells of the immune system is all classified as cytokines. Cytokines also mediate inflammatory reactions. Cells of the immune system secrete at least one cytokine and express specific signaling receptors for several cytokines. This expression is dynamic and often stochastic. Interleukins, chemokines, tumor necrosis factor (TNF), and interferons are all considered cytokines.

Chemokine: Chemokines are subsets of cytokines that regulate cell movement, migration, and chemotaxis. Chemokines maintain the localization of T-cell subsets and APCs within lymphoid organs.

Major histocompatibility complex (*MHC*)/*human leukocyte antigen* (*HLA*): Major histocompatibility complex (MHC) is the large genetic locus that contains the highly polymorphic genes which encode the peptide-binding molecules most commonly recognized by the T-cell receptor on the cell surface of T-cells. The human leukocyte antigen (HLA) locus is the equivalent to MHC in humans, and this locus is located on the short arm of chromosome 6 in humans. MHC molecules are expressed on the cell surface. The two major classes of MHC are Class I and Class II. MHC Class I molecules are polymorphic proteins that help to display peptide fragments of protein antigen derived from the cytosol on the cell surface of APCs for recognition by T-cells. This antigen peptide-MHC Class I complex is typically recognized by $CD8^+$ T-cells. MHC Class I molecules are expressed mostly on all nucleated cells. In contrast, the antigen peptide-MHC Class II complex, which is made up of polymorphic heterodimeric proteins, is also located on the cell surface but restricted to dendritic cells, macrophages, and B-cells, i.e., antigen-presenting cells. It displays antigen peptides derived from extracellular proteins that have been digested, processed into small peptide fragments, and then displayed on the cell surface of APCs for recognition by $CD4^+$ helper T-cells.

Alloantigen: An alloantigen is an antigen that is expressed on cells or tissues from one individual that is recognized as foreign by another individual.

Alloreactive: T-cells or antibodies that recognize and react to antigens (alloantigens) on cells or tissues from another individual are said to be alloreactive.

Effector cell: An effector cell is an immune cell with effector functions during an immune response, killing microbe-infected cells ($CD8^+$ cytotoxic T-cells), killing microbes (macrophages), secreting cytokines to enhance an immune response ($CD4^+$ helper T-cells), and secreting antibodies (differentiated B-cells).

Cluster of differentiation (*CD*) *nomenclature*: The cluster of differentiation (CD) nomenclature was established initially to name uniformly cell surface molecules in order to characterize cells of a particular lineage or stage of differentiation. They leave a defined structure and are recognized by a cluster of monoclonal antibodies. Each cell surface molecule is designated by CD. A specific constellation of CD molecules can identify a specific immune cell subtype, termed immunophenotype. For example, CD3 represents the T-cell receptor and is considered a marker for T-cells. While both helper and cytotoxic T-cells express CD3 on the cell surface, the expression of CD8 distinguishes cytotoxic T-cells from other T-cell subsets, whereas helper T-cells are $CD3^+$, $CD4^+$, and $CD8^-$. This CD marker system is used beyond cells of the immune system and is used to uniformly name molecules found on all cell types in the body.

Hematopoiesis, Its Regulation, and Cells of the Immune System

Hematopoiesis

Hematopoiesis refers to the highly regulated process by which all mature blood cells (i.e., leukocytes, erythrocytes, and platelets) are produced from pluripotent stem cells. Figure 3.1 represents a depiction of the lineage differentiation tree [1].

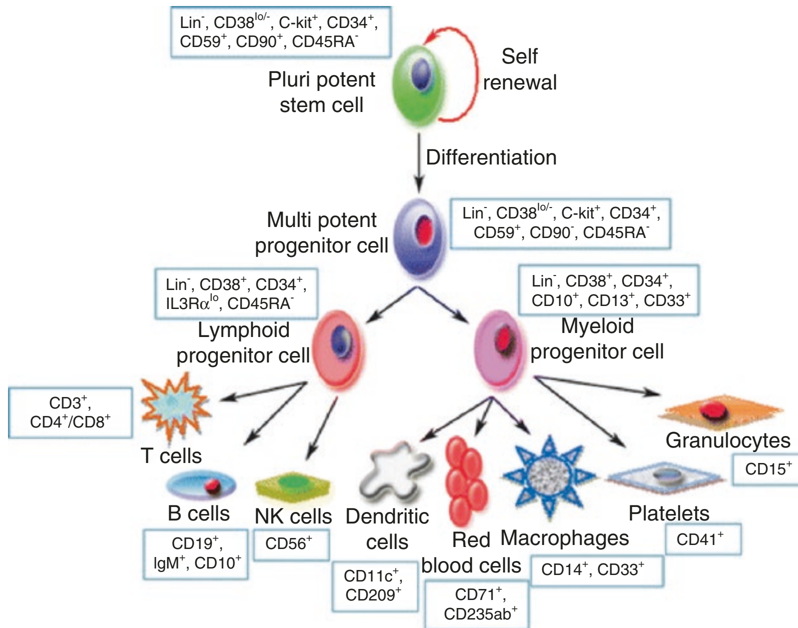

Fig. 3.1 Hematopoietic stem cell differentiation. Differentiation of hematopoietic pluripotent stem cells into multipotent progenitor cells which then differentiate into distinct hematopoietic lineages. This is the best example of stem cell differentiation, and the niche defines specific differentiation events in the bone marrow, spleen, or liver (Reprinted from: Vira, Darchni, Basak, Saroj K., Veena, Mysore S., Wang, Marilene B., Batra, Raj K., Srivatsan, Eri S. Cancer stem cells, microRNAs, and therapeutic strategies including natural products. Cancer and Metastasis Reviews. 31(3): 733–751, 2012, with permission from Springer) [1]

In humans, primitive hematopoiesis starts at d18 of gestation in the blood islands in the yolk sac. Only nucleated erythroblasts and, to a lesser extent, macrophages and megakaryocytes are produced there. Then, hematopoiesis moves to the aorta-gonad-mesonephros (AGM) region in the embryo where primitive hematopoietic stem cells (HSCs) are exposed to a microenvironment that promotes the transition to definitive HSCs. From there, these definitive HSCs migrate to the fetal liver where they undergo extensive expansion. During the second trimester, HSCs migrate to their specific niches within the bone marrow where they reside for the remainder of a person's life. Thus, humans are born with "adult" HSCs. At birth, hematopoiesis takes place in virtually all of the bones, but, as we age, hematopoiesis becomes more restrictive. By puberty, hematopoiesis occurs exclusively in the bone marrow of the flat bones, i.e., the sternum, vertebrae, iliac bones, and ribs. While the majority of hematopoiesis occurs in the bone marrow with the majority of HSCs residing in the bone marrow, HSCs can function and provide hematopoiesis in extramedullary sites, primarily in the liver and spleen (see Fig. 3.2, [61]).

HSCs that have the two properties of reconstituting and self-renewal capacity are referred to long-term hematopoietic stem cells (LT-HSCs) and are identified by the immunophenotype of Lin⁻, CD34⁺, CD38⁻, CD90⁺, and CD45RA⁻. The self-renewal property is defined as follows: when a stem cell divides, one of the daughter cells goes on to differentiate, while the other daughter cell does not go on to differentiate, but instead maintains the properties of a stem cell. In the homeostatic state, the majority of the cells that make up the LT-HSC pool are quiescent with only a small proportion undergoing cell division. Cellular senescence is the state in which cells no longer divide although they remain metabolically active. Senescence is governed by telomere length. Telomerase maintains the ends of chromosomes to protect telomere shortening that would otherwise occur with each cell division. Most mature cells do not express telomerase, and thus telomere shortening is associated with aging

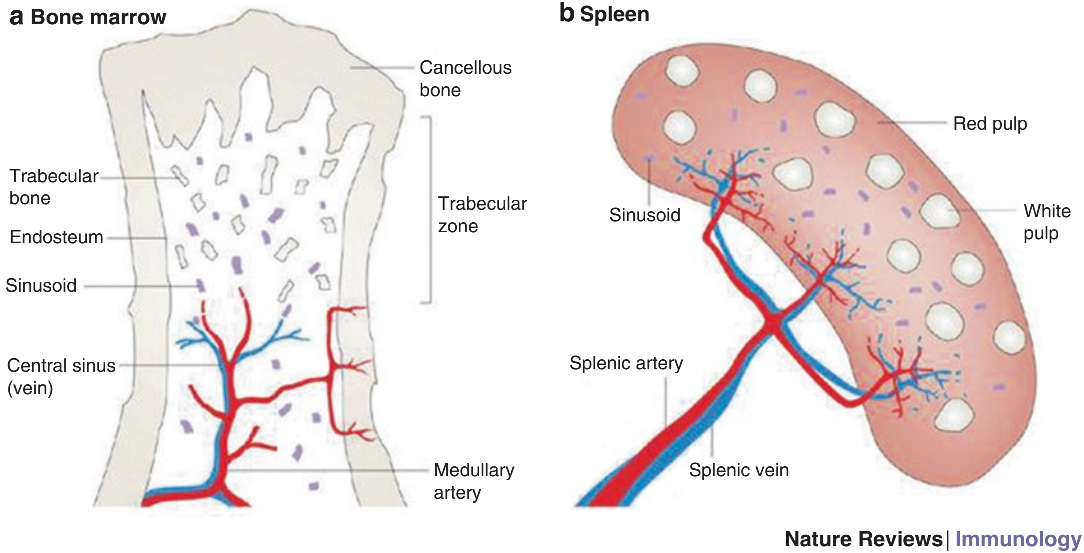

Fig. 3.2 Anatomy of the adult hematopoietic organs, bone marrow, and spleen. (**a**) Hematopoietic stem cells (HSCs) reside primarily within the bone marrow during adulthood. The bone marrow is a complex organ containing many different hematopoietic and non-hematopoietic cell types. Hematopoiesis occurs within the medullary cavity, surrounded by a shell of vascularized and innervated cancellous bone. Minute projections of the bone (trabeculae) are found throughout the trabecular zone of the bone, such that many cells in this region are close to the bone surface. The interface of the bone and bone marrow is known as the endosteum, and this is covered by bone-lining cells that can differentiate into bone-forming osteoblasts. Bone-resorbing osteoclasts are also present at the endosteum. Arteries carry oxygen, nutrients, and growth factors into the bone marrow, before feeding into capillaries and then sinusoids, which coalesce to form the venous circulation. Sinusoids are specialized venules that form a reticular network of fenestrated vessels that allow cells to pass in and out of circulation. (**b**) HSCs can also be found at low levels in extramedullary tissues such as the spleen and liver throughout adult life. When bone-marrow hematopoiesis is impaired by age, cancer, or myeloablation, expanded numbers of HSCs can engage in extramedullary hematopoiesis in the spleen. HSCs reside around sinusoids in the red pulp of the spleen, but not in the white pulp, which contains lymphocytes and antigen-presenting cells (Reprinted by permission from Macmillan Publishers Ltd: Kiel MJ and Morrison SJ. Uncertainty in the niches that maintain hematopoietic stem cells. Nature Reviews Immunology. 8:290–301, 2008 [61])

and cell senescence. Normal HSCs exhibit telomere shortening with serial transplantations [2]. These LT-HSCs give rise to multipotent cells referred to as short-term (ST-) HSCs. ST-HSCs have a limited to no capacity of self-renewal but can provide multilineage reconstitution, albeit transient. A higher percentage of ST-HSCs enter the cell cycle daily as compared to LT-HSCs.

ST-HSCs can go on to become the oligopotent progenitors, common myeloid progenitors (CMP) and common lymphoid progenitors (CLP). After multiple steps of differentiation, CMPs and CLPs will ultimately give rise to all terminally differentiated components of blood. Differentiation of CMPs will eventually lead to the development of platelets, erythrocytes, granulocytes, and macrophages, whereas all mature B-, T-, and NK cells are derived from CLPs. Dendritic cells can be derived from either CMPs or CLPs.

Hematopoiesis is a process that is strictly regulated by highly orchestrated interactions of molecular (noncellular) and cellular constituents. The regulation of proliferation and differentiation of these progenitor and precursor cells (i.e., hematopoiesis) is driven, for the most part, by cytokines and growth factors that are secreted by stromal cells and macrophages contained within the bone marrow. The major cytokines with their source, principal targets, and principal cell type induced are enumerated below and summarized in Table 3.1.

Key Cytokines of Hematopoiesis

Below is a list of cytokines that play important roles in hematopoiesis:

SCF: Stem cell factor (SCF) (otherwise known as c-kit ligand) is secreted by stromal

Table 3.1 Summary of key cytokines and growth factors that regulate hematopoiesis

	SCF	IL-3	IL-7	GM-CSF	G-CSF	M-CSF	Flt-3 ligand
Principal cellular source(s)	• Bone marrow stromal cells	• T-cells	• Fibroblasts • Bone marrow stromal cells	• Activated T-cells • Macrophages • Endothelial cells • Fibroblasts	• Activated T-cells • Macrophages • Fibroblasts • Endothelial cells	• Macrophages • Endothelial cells • Bone marrow cells • Fibroblasts	• Bone marrow stromal cells
Principal progenitor/ precursor target(s)	• HSCs	• Immature progenitors	• Immature lymphoid progenitors	• Immature and CMPs • Mature macrophages	• Committed granulocyte progenitors	• Committed progenitors	• HSCs • Dendritic cell progenitors • B-cell progenitors
Principal cell type(s) induced	• All cell types	• All cell types	• T-cells • B-cell precursors	• Granulocytes • Monocytes • Macrophage activation	• Granulocytes	• Monocytes	• Classical and plasmacytoid dendritic cells • B-cells

SCF stem cell factor, *HSCs* hematopoietic stem cells, *IL-3* interleukin-3, *IL-7* Interleukin-7, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *CMPs* common myeloid progenitors, *G-CSF* granulocyte colony-stimulating factor, *M-CSF* macrophage colony-stimulating factor

cells of the bone marrow. It acts on pluripotent hematopoietic stem cells, inducing maturation of all hematopoietic lineages. Its receptor is KIT.

IL-3: Interleukin-3 (IL-3) is principally secreted by T-cells and targets immature hematopoietic progenitor cells to induce the maturation of all hematopoietic lineages.

IL-7: Interleukin-7 (IL-7) which is preferentially secreted by fibroblasts and bone marrow stromal cells plays an important role in the proliferation of B- and T-cell precursors as well as differentiation of B- and T-cells. It also regulates the survival of naïve and memory T-cells.

GM-CSF: Granulocyte-macrophage colony-stimulating factor (GM-CSF) is produced by activated T-cells, macrophages, endothelial cells, and fibroblasts within the bone marrow stroma. The primary functions of GM-CSF are to stimulate the proliferation of macrophages, monocytes, and neutrophils.

G-CSF: Granulocyte colony-stimulating factor (G-CSF) is produced by activated T-cells, macrophages, and endothelial cells at the site of inflammation and/or tissue damage that acts on the bone marrow to stimulate proliferation and mobilization of neutrophils to replace those that have been consumed in inflammatory reactions.

M-CSF: Monocyte colony-stimulating factor (M-CSF) is secreted by macrophages, endothelial cells, bone marrow cells, and fibroblasts. It acts on committed hematopoietic progenitors to induce the maturation of monocytes. Its receptor is CSF1R.

Flt-3 ligand: Flt-3 ligand is secreted by bone marrow stromal cells. It targets HSCs as well as progenitors of dendritic cells and B-cells to induce the maturation to classical plasmacytoid dendritic cells and B-cells. Flt-3 ligand binds to the Flt-3 tyrosine kinase receptor on precursors of dendritic and B-cells.

Cells of the Immune System

Phagocytes (*neutrophils and macrophages*): The primary role of phagocytes is to ingest and destroy microbes as well as eliminate damaged tissue. Phagocytes are part of innate immunity. They respond in the same way to repeated exposures of the same microbe(s) in a stepwise fashion. After recruitment to the site of infection or tissue damage and the recognition of microbes, phagocytes are activated, resulting in the ingestion of microbes by phagocytosis and then the destruction of the ingested microbes. Phagocytes also play a role in the effector phase of some adaptive immune responses. Phagocytes consist of neutrophils (also called polymorphonuclear leukocytes) and mononuclear phagocytes.

Neutrophils: Neutrophils are the most abundant white blood cell type in the blood circulation. The cytoplasm of neutrophils is loaded with two types of granules filled with molecules that are poised to destroy ingested microbes and damaged cells. The majority of these granules are called specific granules. These granules are filled with lysozymes, collagenase, and elastase. The other predominant type of granule is the azurophilic granule which is a lysosome that contains enzymes along with other molecules (including defensins and cathelicidins which are microbicidal). Neutrophils typically are short-lived, just 1–2 days.

Macrophages: Mononuclear phagocytes include monocytes which are circulating mononuclear phagocytes and differentiate into macrophages when they reside in tissues. The most abundant type of monocyte is the classical monocyte. Classical monocytes are rapidly recruited to sites of infection or tissue damage and secrete abundant amounts of inflammatory mediators. In contrast, nonclassical monocytes promote tissue repair after injury and patrol along endothelial surfaces looking for areas in need of repair. Macrophages are derived from circulating monocytes and mature into specified macrophages once they migrate from the circulation. Once they enter a tissue, they become long-lived and specialized according to their tissue of residence. The most common tissues include those of the liver, brain, spleen, lungs, peritoneal cavity, and skin (see Fig. 3.3) (reviewed in [3]). For example, Kupffer cells are macrophages that live in the sinusoids of the liver, whereas microglial cells are macrophages

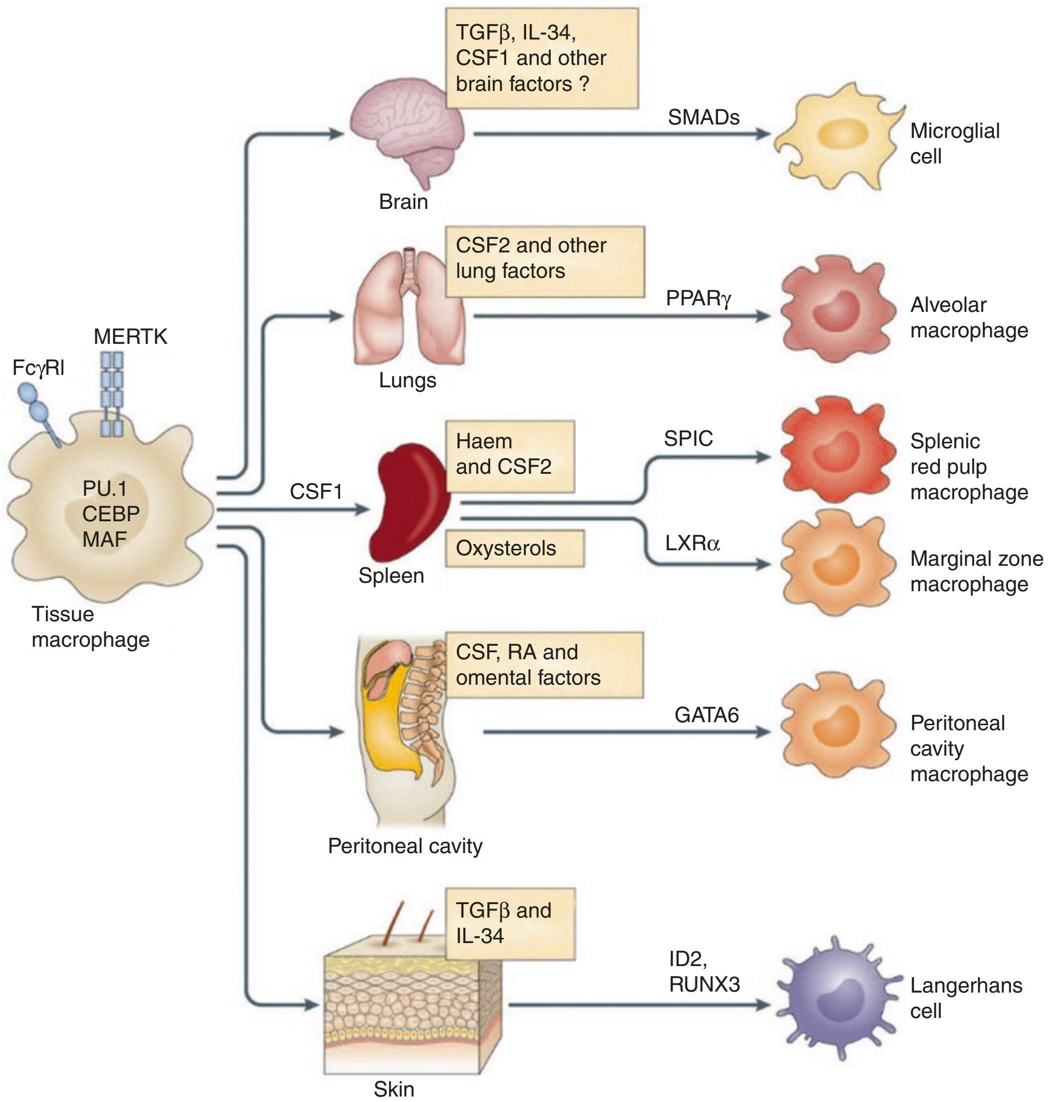

Fig. 3.3 The tissue microenvironment determines macrophage differentiation cues. During embryonic development, macrophages enter the tissues where they self-renew and proliferate. Macrophages in all tissues are characterized by expression of the cell surface marker FcγRI (also known as CD64), tyrosine-protein kinase MER (MERTK), and the transcription factors PU.1, CCAAT/enhancer-binding protein (CEBP) family members, MAF, and MAFB. In the tissues, macrophage identity and functions are shaped by cytokines and metabolites that are produced in the local environment and drive specific transcription factor expression. In the brain, incoming yolk sac-derived cells are exposed to locally express transforming growth factor-β (TGFβ), which drives Smad phosphorylation and the expression of genes that are unique to microglia. In the lungs, fetal monocytes that are exposed to colony-stimulating factor 2 (CSF2) express peroxisome proliferator-activated receptor-γ (PPARγ), which drives their differentiation into alveolar macrophages. In the spleen, haem drives SPIC expression, which controls the differentiation and maintenance of red pulp macrophages and the expression of key splenic red pulp macrophage-specific molecules, including vascular cell adhesion molecule 1 (VCAM1). In the marginal zone of the spleen, macrophage maintenance depends on liver X receptor-α (LXRα)-mediated signals. Retinoic acid (RA) and omental factors induce the expression of GATA-binding protein 6 (GATA6), which promotes the differentiation of peritoneal cavity macrophages. ID2, inhibitor of DNA binding 2; IL-34, interleukin-34; RUNX3, runt-related transcription factor 3 (Reprinted by permission from Macmillan Publishers Ltd: Lavin Y., Mortha A., Rahman A., Merad M. Regulation of macrophage development and function in peripheral tissues. Nature Reviews Immunology. 15:731–744, 2015 [3])

that reside in the brain. Splenic red pulp and marginal zone macrophages reside in the spleen and alveolar macrophages in the lungs. It was thought that Langerhans cells in the skin were macrophages, but data have shown that they are actually derived from dendritic cells (reviewed in [4]). The major function of macrophages is to ingest and destroy molecules by producing reactive oxygen and nitrogen species that are toxic to microbes and proteolytic degradation. Macrophages also ingest dead cells as well as apoptotic cells before they can release their toxic contents and trigger an inflammatory response. Activated macrophages secrete cytokines that promote recruitment of more monocytes and neutrophils into the infected and/or injured areas to amplify the immune response. Macrophages can also act as antigen-presenting cells (APCs) (see "Antigen-Presenting Cells" section below). In addition, they also promote the repair of damaged tissues, stimulating angiogenesis and fibrosis. Macrophages can undergo classical or alternative activation. Classical activation results in macrophages that are efficient in the ingestion and killing of microbes, whereas alternative activation results in macrophages that promote tissue remodeling and repair. Unlike neutrophils, macrophages are not terminally differentiated, and they can divide at the site of inflammation.

Mast cells, *basophils*, *and eosinophils*: Mast cells, basophils, and eosinophils make up a small percentage of white blood cells (or leukocytes) and are called granulocytes because their cytoplasm contains abundant granules filled with various inflammatory and microbicidal substances. Mast cells mediate allergic reactions. Their cytoplasmic granules are filled predominantly with histamine and are fused with the cell membrane. When activated, they release histamine extracellularly, inducing inflammation. They are located in the skin and mucosal epithelia with very few in the circulation. Basophils act similarly to mast cells but are not normally present in tissues. They make up less than 1% of leukocytes in the blood. They play a role in anaphylaxis, asthma, atopic dermatitis, and hay fever. They secrete histamine, proteoglycans, and serotonin to produce inflammation. They can perform phagocytosis. In contrast, eosinophils are known to play a key role in immune responses against parasites. The cytokines, GM-CSF, IL-3, and IL-5, promote myeloid precursors to differentiate into eosinophils. Eosinophils are found normally in the mucosal linings of the lungs, GI tract, and GU tract. Their numbers are increased in the setting of inflammation.

Antigen-presenting cells (*APCs*): Antigen-presenting cells (APC) are a critical component of adaptive immune responses. Professional APCs (e.g., dendritic cells, macrophages, and B-cells) ingest pathogens and foreign substances and then process these antigens into peptide fragments. These peptide fragments are then bound to MHC Class II molecules and displayed on the cell surface of APCs to naïve T-cells. If a T-cell's antigen receptor (the TCR) recognizes an antigen peptide-MHC Class II complex presented on the cell surface of an APC, then the T-cell is activated. An additional co-stimulatory signal is needed before full T-cell activation can occur. APCs also secrete cytokines that stimulate and induce the maturation of naïve lymphocytes (T- and B-cells). Dendritic cells are the predominant cell subtype of APCs that initiate T-cell-mediated immune responses. Macrophages and B-cells are also part of cell-mediated and humoral immune responses, respectively.

Dendritic cells as APCs: Dendritic cells play a key role in the activation of naïve T-cells and in innate immune responses to infections (reviewed in [5]). They also link innate and adaptive immune responses together. They arise from the myeloid lineage, directly from a precursor cell that can also differentiate into monocytes (but not granulocytes). The cytokine Flt-3 ligand induces differentiation into dendritic cells. Dendritic cells have long projections in order to ingest microbes and present antigens complexed to MHC molecules to naïve T-cells efficiently. Dendritic cells tend to reside within the skin, mucosal epithelia, lymphoid tissues, and organ parenchyma. Classical (or conventional) dendritic cells migrate to lymph nodes after ingesting microbes in order to display the processed antigen peptide fragments to naïve T-cells that are residing in the lymph node and stimulate the T-cells that recognize specifically the antigen peptide fragment-MHC complex. In contrast, plasmacytoid dendritic cells, which are another subtype of dendritic cells, are involved in immune responses to

viral infections. After recognition of viral nucleic acids, plasmacytoid dendritic cells secrete type I interferons which have potent antiviral activities. When they are located within inflamed tissues, monocytes can differentiate into inflammatory dendritic cells as well. Dendritic cells also play a role in peripheral tolerance, to help prevent the development of autoimmune disease. In addition, the Langerhans cell is a type of dendritic cell derived from embryonic tissue precursors. Langerhans cells specifically reside in the skin.

Macrophages as APCs: Another role of macrophages is to present antigens to CD4$^+$ helper T-cells at the sites of infection. Once CD4$^+$ helper T-cell activation occurs, they produce cytokines (including interferon-γ) that act on macrophages to greatly enhance their microbicidal properties [6]. This process is necessary in order to eliminate microbes ingested by phagocytes but resistant to killing.

B-cells as APCs: Among its functions, B-cells act as professional APCs, as they can internalize antigen that binds to the B-cell receptor on the cell surface of B-cells. After processing these antigens, the B-cell presents the antigen peptide fragments complexed to MHC Class II molecules along with co-stimulatory (such as B7 complex) to CD4$^+$ helper T-cells. This interaction promotes the cooperation between CD4$^+$ helper T-cells and B-cells to enhance B-cells' antibody responses to protein antigens (reviewed in [7]).

Nonprofessional APCs: Any nucleic acid can act as a "nonprofessional" APC in that they can display endogenous peptides in the context of Class I MHC molecules coupled to a β-2 microglobulin on the cell surface. This cell can be recognized by a CD8$^+$ cytotoxic T-cell that expresses the antigen receptor that recognizes its specific antigenic-peptide Class I MHC complex displayed by the nonprofessional APC, resulting in CTL activation.

Lymphocytes: Lymphocytes are derived from common lymphoid progenitor cells in the bone marrow shortly after birth. T- and B-cell precursors go through a highly regulated series of differentiation and then maturation steps that are distinguished by a series of different phenotypic and functional CD markers. Maturation of T- and B-cells takes place in different organs: B-cells mature in the bone marrow and migrate to the spleen, lymph nodes, and peripheral lymph tissues to undergo further maturation, whereas immature T-cells leave the bone marrow and go to the thymus where they undergo maturation. T-cells leave the thymus fully matured but naïve and go to the secondary lymph organs, i.e., the spleen, lymph nodes, and lymphoid tissues, to await interaction with foreign antigens presented to them by APCs. Naïve T-cells are called this because they have not yet encountered their foreign antigen which will bind their specific antigen receptor.

Lymphocytes are responsible for driving adaptive immunity (see section below in this chapter, "Adaptive Immunity"). They have the unique properties of diversity and specificity. In other words, each individual lymphocyte expresses antigen receptors that will only recognize a single antigen peptide fragment (or determinant) coupled with MHC molecules with high affinity (hence the property of exquisite specificity). Simultaneously, the tremendous diversity of lymphocytes is derived from the large number of lymphocytes (approximately 5×10^{11}) contained within the body, each expressing one specific antigen receptor but with the capability of recognizing a different antigenic peptide determinant. This diversity is often referred to as the "lymphocyte repertoire." This diverse lymphocyte repertoire enables an individual to recognize and respond to any foreign antigen it will potentially encounter. The mechanisms by which the lymphocyte repertoires develop and operate are discussed later in this chapter (below in this section and under the section "Adaptive Immunity"). The distribution of lymphocytes throughout the human body is as follows: approximately 65% in the lymphoid organs (the spleen and lymph nodes), 15% in lymphoid mucosal tissues (in the GI and respiratory tracts), 10% in the bone marrow, 4% within the skin, and 2% in the blood.

Lymphocyte subsets: The two major classes of lymphocytes are B-cells and T-cells. B-cells and T-cells are subdivided into subsets that are characterized by their function and/or cytokine production because morphologically they are quite similar. Table 3.2 summarizes the lymphocyte subsets and their functions.

B-cells: B-cells were lymphocytes named as such because they were first found to mature in the bursa of Fabricius in birds. Mammals,

Table 3.2 Summary of lymphocyte subsets and their functions

Class	Subsets	Antigen receptor	Receptor specificity	Selected immunophenotype markers	Functions
T-cell	• $CD4^+$ helper T-cell	• αβ heterodimer	• Diverse specificities for peptide-Class II MHC complexes	• $CD3^+$ • $CD4^+$ • $CD8^-$	• Humoral immunity – B-cell differentiation • Cell-mediated immunity – Macrophage activation • Stimulates inflammation
	• $CD8^+$ cytotoxic T-cell	• αβ heterodimer	• Diverse specificities for peptide-Class I MHC complexes	• $CD3^+$ • $CD4^-$ • $CD8^+$	• Kills cells that are infected with virus or intracellular bacteria
	• Regulatory T-cell	• αβ heterodimer	• Specific for self and some foreign antigens	• $CD3^+$ • $CD4^+$ • $CD25^+$ • $FoxP3^+$	• Suppresses functions of other T-cells • Maintains self-tolerance • Regulates immune responses – "Brakes" of the immune responses
	• NKT cell	• αβ heterodimer	• Limited specificity for glycolipid-CD1 complexes	• $CD56^+$ • $CD16^+$ • $CD3^+$	• Suppresses innate and adaptive immune responses • Activates innate and adaptive immune responses
	• γδ T-cell	• γδ heterodimer	• Limited specificities for peptide and nonpeptide antigens	• $CD3^+$ – $CD4^+$ or $CD8^+$ (variable)	• Helper and cytotoxic functions (innate) • Majority found in the gut mucosa • Prominent role in lipid-antigen recognition
B-cell	• Follicular B-cell	• Surface immunoglobulin	• Diverse specificities for many types of molecules	• Fc receptor • Class II MHC • $CD19^+$ • $CD23^+$	• Humoral immunity – Antibody production
	• Marginal-zone B-cell	• Surface immunoglobulin	• Limited specificities for a restricted set of molecules	• IgM • $CD27^+$	• Humoral immunity – Antibody production – Responds rapidly to blood-borne microbial antigens
	• B-1 cell	• Surface immunoglobulin	• Limited to T-cell-independent antigens	• CD5+ (Ly-1)	• Humoral immunity – Secretes IgM – Provides protective natural immunity
Innate lymphoid cell	• ILC-1 – Natural killer cell	–	–	• $CD56^+$ • KIR+	• Defense against viruses • Tumor surveillance
	• ILC-2	–	–	–	• Allergic inflammation
	• ILC-3 – LTi	–	–	–	• Intestinal barrier • Lymphoid organogenesis

ILC innate lymphoid cell, *LTi* lymphoid tissue inducer, *KIR* killer-cell immunoglobulin-like receptor

including humans, do not have bursa of Fabricius or an equivalent organ/tissue. Subsequently, it was found that B-cells develop and mature within the bone marrow. The major function of B-cells is to produce antibody. In comparison, T-cells arise from within the bone marrow, but they migrate to the thymus to undergo maturation (sometimes referred to as T-cell education). Subsets of B-cells include follicular B-cells, marginal B-cells, and B-1 cells. These B-cell subsets are localized in anatomically distinct regions within lymphoid tissues. The follicular B-cells make up the B-cell repertoire, representing diverse sets of antibodies which serve as the cell surface antigen receptor but with exquisite individual specificity. They also secrete important effector molecules. In contrast, marginal-zone B-cells and B-1 cells produce antibody but of limited diversity. Their anatomical locations are detailed elsewhere in this chapter (see “Lymph Nodes” and “Spleen”). Marginal-zone B-cells respond rapidly to blood-borne microbial antigens. They produce the antibody isotype, immunoglobulin (Ig) M, with limited diversity. Marginal-zone B-cells may represent a reservoir of memory B-cells [8]. In comparison, B-1 cells also have a limited repertoire but secrete IgM antibodies independent of infection that bind T-cell-independent antigens (reviewed in [9]). This is considered protective natural antibody. B-1 cells develop earlier during ontogeny than do conventional B-cells. Many B-1 cells express CD-5 (LY-1) molecules. They are found predominantly in peritoneal and pleural cavities.

T-cells: The two major subsets of T-cells are $CD4^+$ helper and CD8+ cytotoxic T-cells. These subsets express an antigen-specific αβ T-cell receptor (TCR) on their cell surfaces. They mediate cellular (or cell-mediated) immunity. Each individual $CD4^+$ and $CD8^+$ T-cell’s TCR recognizes only one foreign antigen in the context of self-MHC molecules. These T-cells make up the widely diverse T-cell repertoire, the mechanism by which these T-cells are activated and their effector functions are detailed elsewhere in this chapter (see “Adaptive Immunity” section). Another important subset of T-cells is regulatory T-cells (Tregs). They also express CD4 on their cell surfaces and express αβ TCRs, but they are distinguished from $CD4^+$ helper T-cells by the expression of CD25. Tregs function to inhibit immune responses. They contribute to the contraction of an immune response and to the return of homeostasis. The NKT cell subset also expresses αβ TCRs but is distinguished by its cell surface expression of CD16, which is the Fc receptor for IgG, and by expression of CD56. NKT cells can play a role in both suppressing as well as activating innate and adaptive immune responses. They recognize lipid antigens that are presented by CD1d which is a nonclassical MHC molecule. Upon activation, NKT cells produce interferon-γ, IL-4, and GM-CSF in large quantities as well as IL2 and TNFα. Another subset of T-cells is γδ T-cells which express the γδ heterodimer instead of the αβ heterodimer of the T-cell receptor (TCR). This subset has limited diversity and has helper and cytotoxic T-cell-like functions in innate immunity. They play a prominent role in lipid-antigen recognition. They do not recognize antigen peptides in the context of MHC molecules like αβ T-cells do [10]. They are mostly found expressed in epithelial barrier tissues.

Innate lymphoid cells: Innate lymphoid cells are derived from lymphoid lineage cells in the bone marrow that have similar effector functions to T-cells but lack T- or B-cell antigen receptors. ILCs play important roles in homeostasis regulation and the regulation of inflammation. ILCs are categorized into one of three types, ILC-1, ILC-2, or ILC-3, based upon what cytokines they secrete and on the growth factors that regulate their development and function [11]. These cells provide early defense against infectious agents to eliminate them. They also recognize and eliminate stressed or damaged tissues. ILCs also influence the subsequent adaptive immune response. The best characterized ILC type is the natural killer (NK) cell which is a member of the ILC-1 class. NK cells are cytotoxic effector cells that are similar to CTLs but act more quickly than CTLs. NK cells constitute approximately 15% of the lymphocytes in the blood. They play important roles in defense against viral infections and provide tumor surveillance. NK cells secrete interferon-γ. Other ILCs secrete IL-5, IL-13, IL-17, and IL-22. ILC-2 cells are involved

in allergic lung inflammation and helminth infections [12]. They produce IL-5, IL-13, IL-9, and IL-4 after stimulation. They are predominantly found in the skin, lungs, liver, and GI tract [13]. ILC-3 cells do not have cytotoxic effector functions, and they do not produce interferon-γ or TNF. They are predominantly found in mucosal tissues, particularly the GI tract. A subset of ILC-3 cells, called lymphocyte tissue inducer cells, express molecules needed for the development of lymphoid tissue and lymphoid organs.

Tissues of the Immune System

Introduction

Tissues of the immune system provide anatomically discrete environments in which cells of the immune system can develop as well as perform their functions efficiently by providing optimal environments for cellular interactions needed for antigen recognition and cellular activation. Tissues of the immune system include the bone marrow (in which hematopoiesis occurs after birth in humans) and lymphoid tissues. Lymphoid tissues can be categorized as central (also referred to generative or primary) and peripheral (or secondary) lymphoid organs. The bone marrow and thymus are considered central or generative, as they are the sites of B- and T-cell maturation, respectively. Generative lymphoid tissues create a nurturing environment for developing B- and T-cells that is provided by growth factors, cytokines, and other molecules. Generative tissues also provide an environment to present self-antigens for recognition, selection, and ultimately the elimination of maturing lymphocytes. In comparison, peripheral lymphoid tissues include lymph nodes, the spleen, as well as the immune system regions contained within cutaneous, mucosal, and connective tissues (e.g., Peyer's patches in the mucosa of the small intestine). Regardless of the location, the primary function of these peripheral lymphoid tissues is to create an environment that elicits the appropriate adaptive immune response from naïve lymphocytes in an efficient manner by providing locations in which antigens are delivered and presented to naïve lymphocytes and in which B- and T-cells can interact in a cooperative way.

Bone Marrow and the Hematopoietic Stem Cell Niche

The bone marrow is the site in which the vast majority of mature circulating blood cells are generated from birth in humans. It is also the site in which early B-cell maturation occurs [14].

Hematopoietic and progenitor stem cells reside within niches of the bone marrow [15]. Hematopoietic stem cell (HSC) niches are defined as the areas of bone marrow microenvironment in which HSCs' survival, renewal, and differentiation occur [16]. Networks involving cellular and molecular interactions within the bone marrow microenvironment along with normal stem cell trafficking to and from HSC niches maintain normal hematopoiesis. Disruption (e.g., dysfunction and/or dysregulation) of these networks leads to disease. The bone marrow stroma, chemokines, and molecules on the cell surface of the HSCs play important roles in HSC trafficking. The bone marrow stroma and extracellular matrix provide the scaffolding in which blood cell components develop and in which HSCs are maintained and renewed. Alterations of key cells or molecules within the bone marrow microenvironment lead to changes in homeostasis, i.e., disrupts it.

In homeostasis, bone marrow-derived HSCs exit their bone marrow niches and go to peripheral lymphoid tissues via the lymphatic system [17]. Figure 3.4 depicts a schematic diagram of mobilization and homing of HSCs [18]. After a period of time, these HSCs may reenter the bone marrow niche and remain there or repeat this migration pattern into the peripheral lymphoid tissues [19, 20]. Certain conditions, including inflammation, organ or tissue damage, and strenuous exercise, have been found to trigger a significant increase in the release of HSCs into the peripheral blood [21–24]. Mobilization is the term used to describe pharmacologically induced, forced egress of HSCs from the bone marrow into the peripheral blood. Once in the circulation, the HSCs can be removed from the peripheral blood by pheresis (see Chap. 8). The most common mobilizers of hematopoietic stem and progenitor cells are G-CSF, GM-CSF, cyclophosphamide, and, more recently, plerixafor. Table 3.3 summarizes these agents' mechanisms of action and kinetics. The commonly targeted

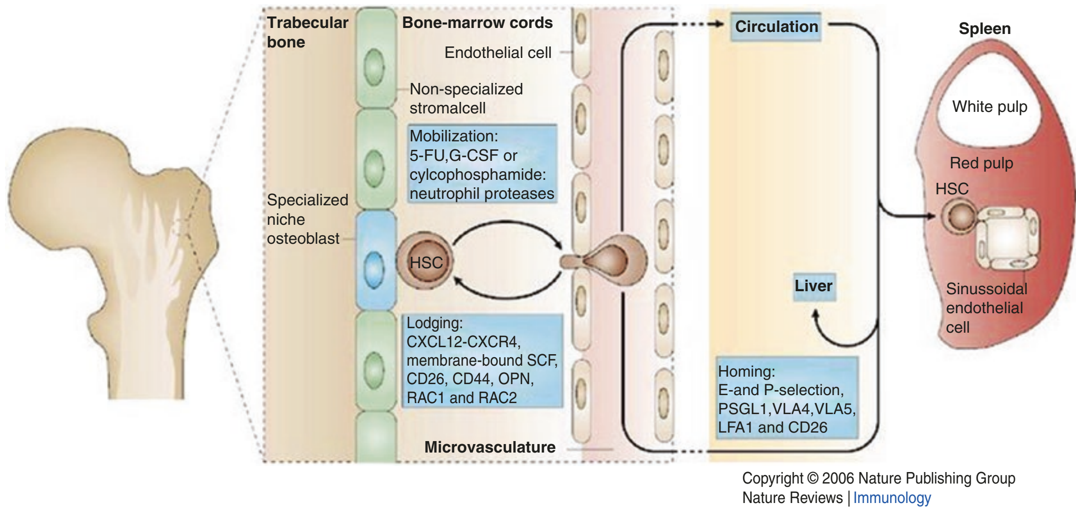

Fig. 3.4 Mobilization, homing, and lodging. Schematic diagram showing some of the factors implicated in each process. Hematopoietic stem cells (HSCs) bound to the bone-marrow niche are mobilized in response to granulocyte colony-stimulating factor (G-CSF) or cyclophosphamide or after peripheral myeloablation following treatment with 5-fluorouracil (5-FU). After extravasation from the bone-marrow cords into the microvasculature, HSCs enter the circulation and are distributed to peripheral tissues such as the spleen or liver. HSCs locate close to endothelial cells in the splenic red pulp. They home to the bone-marrow cords through the circulation, a process that is controlled by a number of adhesion molecules such as very late antigen 4 (VLA4), VLA5, lymphocyte function-associated antigen 1 (LFA1), or selectins. After entering the bone marrow, HSCs specifically lodge in the niche, a process requiring membrane-bound stem cell factor (SCF), CXC-chemokine ligand 12 (CXCL12), osteopontin (OPN), hyaluronic acid, and their corresponding receptors. CXCR4, CXC-chemokine receptor 4; E-selectin, endothelial cell selectin; P-selectin, platelet selectin; PSGL1, P-selectin glycoprotein ligand 1 (Reprinted by permission from Macmillan Publishers Ltd: Wilson A. and Trumpp A. Bone-marrow hematopoietic-stem-cell niches. Nature Reviews Immunology. 6:93-106, 2006 [18]). Copyright © 2006 Nature Publishing Group

Table 3.3 Summary of mobilization agents, the mechanism of action, and kinetics

Agent	Mechanism of action	Kinetics
• G-CSF	• Downregulates CXCL12 on bone marrow osteoblasts • Disrupts CXCL12/CXCR4 signaling • Releases proteases, such as MMP2 and MMP9 (minor mechanism) • Disrupts VCAM/VLA-4 axis	• Peak: 4–5 days
• GM-CSF	• Exact mechanism unknown – May involve alteration of adhesion molecules on HSCs	• Peak: 5–7 days
• Plerixafor	• Small molecule antagonist of CXCR4	• Peak: 3–4 h
• Cyclophosphamide	• Induces hypoxia within the central marrow that leads to loss of CXCL12 gradient and can increase VEGFA production resulting in increased protease production	–

pathway for HSC mobilization is CXCL12/CXCR4. G-CSF and cyclophosphamide interfere with CXCL12 by either disruption of CXCL12/CXCR4 signaling (G-CSF), downregulation of CXCL12 on bone marrow osteoblasts (G-CSF), or disruption of the CXCL12 gradient (cyclophosphamide) [25–27]. In comparison, plerixafor is a CXCR4 antagonist [28].

HSC niches: There are specialized areas within the bone marrow microenvironment termed HSC niches that provide a healthy environment in which HSCs reside. These niches are composed of cellular and noncellular components. The cellular components make up the bone marrow stroma which is composed of CXCL12-abundant reticular (CAR) cells [29], mesenchymal stem cells [30], adipocytes,

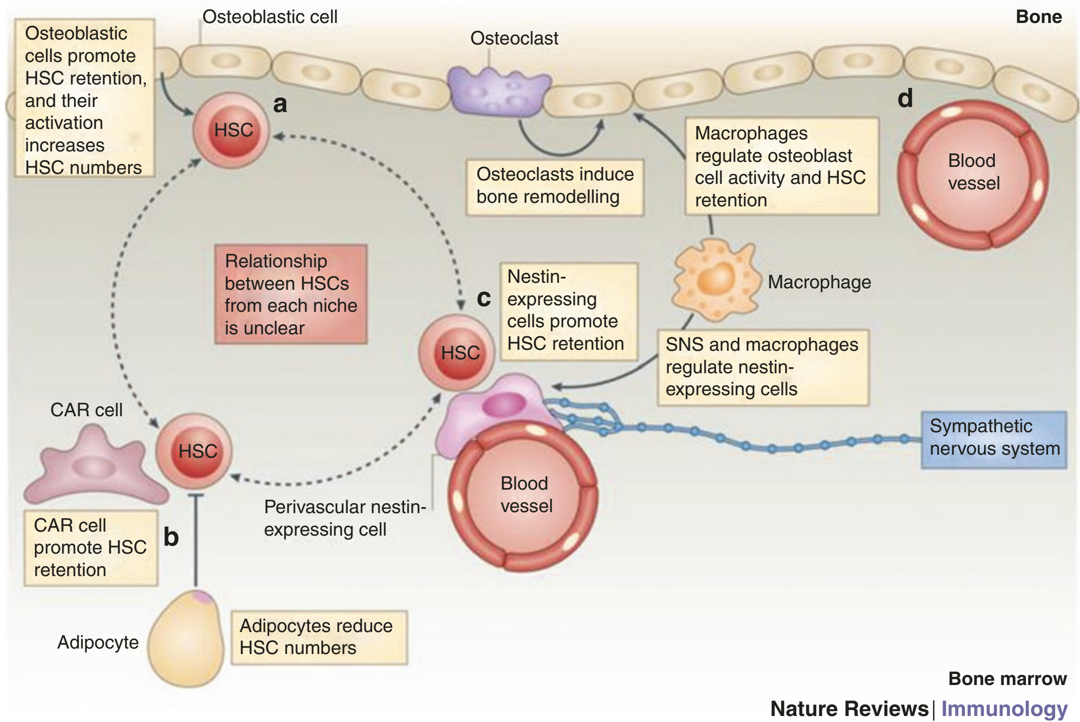

Fig. 3.5 Hematopoietic stem cell niches. In the bone marrow, hematopoietic stem cells (HSCs) can be found near the endosteal surface (**a**), in association with CXCL12-abundant reticular (CAR) cells (**b**), and in the periphery of sinusoids and perivascular nestin-expressing cells (**c**). Each niche is thought to provide signals that support HSC behavior, although the relationship between HSCs that are present in different niches is still unclear (*dotted arrows*). Likewise, blood vessels in the bone marrow are often in close association with the bone, although their interaction is still poorly understood (**d**). At the endosteal surface, osteoblastic cells express factors that participate in HSC retention; osteoclasts regulate osteoblastic cell function by inducing bone remodeling; and macrophages regulate osteoblastic cell activity and the retention of HSCs. In the bone marrow stroma, HSCs are associated with CAR cells, which express factors that promote HSC retention. Adipocytes negatively regulate HSCs in the steady state. In the perivascular area, HSCs are associated with nestin-expressing cells, which promote HSC retention and are regulated by macrophages and the sympathetic nervous system (SNS) (Reprinted by permission from Macmillan Publishers Ltd: Mercier FE, Ragu C, Scadden D. The bone marrow at the crossroads of blood and immunity. Nature Reviews Immunology. 12:49–60, 2011 [35])

macrophages [31], vascular endothelial cells [32], osteoblasts, osteoclasts, sympathetic neurons [33], Schwann cells, regulator T-cells (Tregs), megakaryocytes [34], and smooth muscle cells. Figure 3.5 illustrates the relationship between HSCs and the stromal cells of the HSC niche [35]. These stromal cells secrete fibronectin, hyaluronan, collagen, laminin, chondroitin sulfate, heparan sulfate, and glycosaminoglycans which are key components of the extracellular matrix (ECM) surrounding the HSC niche. They also secrete essential factors that promote HSC maintenance, including SCF and CXCL12 (see Fig. 3.6 for a schematic representation of the HSC niche and noncellular and cellular regulators [36]). They also express the nonessential factors thrombopoietin, angiopoietin-1, angiogenin, FGF1, TGFβ, osteopoietin, and Notch ligands. Tables 3.4 and 3.5 summarize the key cellular and noncellular components, respectively, of the HSC niche and the roles they play. The bone marrow stroma along with the ECM provides the scaffolding that permits a hospitable environment for HSC maintenance, development, renewal, and differentiation through direct cellular contact, adhesive molecules (such as hyaluronic acid), and cytokines. Cell-cell contact between HSCs and osteolineage cells is important for the regulation of the HSC niche. In vitro studies have shown that CD34^{+} HSCs make cell-cell contact with osteolineage cells and even exchange a portion

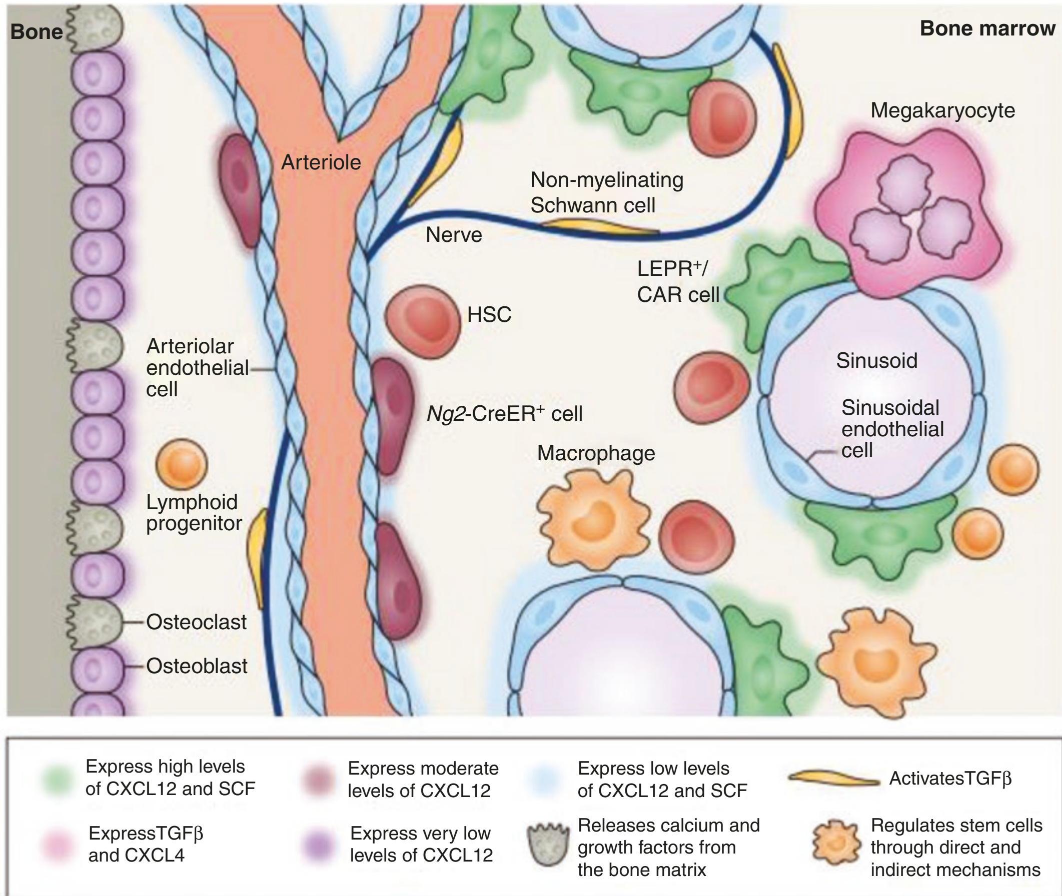

Fig. 3.6 A schematic of the HSC niche in adult bone marrow. Most hematopoietic stem cells (HSCs) localize adjacent to sinusoids, where they are in close contact with leptin receptor (LEPR)-expressing mesenchymal stromal cells (also known as CXC-chemokine ligand 12 (CXCL12)-abundant reticular (CAR) cells) and endothelial cells, both of which are necessary sources of the stem cell factor (SCF) and CXCL12 required for HSC maintenance. Approximately 10% of HSCs localize near to small-diameter arterioles, which are also associated with LEPR+ stromal cells, as well as rare Ng2-CreER+ cells (in which a tamoxifen-activated form of Cre recombinase is expressed from the Ng2 locus, which encodes neural-glial antigen 2) that may or may not be a source of the CXCL12 required for HSC maintenance. Nerve fibers, Schwann cells associated with nerve fibers, megakaryocytes, macrophages, and osteoclasts also regulate HSC maintenance through several mechanisms. Osteoblasts do not directly regulate HSC maintenance through any known mechanism, but they probably indirectly regulate HSC maintenance through cross talk with other cell types in the bone marrow, such as the cells comprising the vasculature. Osteoblasts promote the maintenance of a subset of early lymphoid progenitors by secreting very low levels of CXCL12, but other lymphoid progenitors reside in sinusoidal niches, where they depend on CXCL12 synthesized by LEPR+ cells. TGFβ, transforming growth factor-β (Reprinted by permission from Macmillan Publishers Ltd: Crane GM, Jeffrey E, Morrison SJ. Adult Haematopoietic Stem Cell Niches. Nature Reviews Immunology. Published online 12 June 2017, doi:https://doi.org/10.1038/nri.2017.53 [36])

of their membranes, creating a signaling endosome that causes stromal cells to downregulate Smad signaling and increase CXCL12 production which will lead to a more hospitable environment for HSCs. When irradiation damages HSC niches, HSCs secrete angiopoietin which helps to repair vascular leakiness and restores hematopoiesis [37]. Thus, HSCS can help create their own niches in the bone marrow. Bone marrow is highly innervated [38]. The nervous system regulates HSC retention in HSC niches via circadian rhythms [39]. β-Adrenergic stimulation leads to oscillating release of norepinephrine, CXCR4 expression, and CXCL12 production which results in a regular

Table 3.4 Cell types found in the hematopoietic stem cell niche and their roles

Cell type	Location	Role and/or mechanism
• CAR cell • LEPR stromal cell • Nestin + MSC	• Perivascular – Mainly perisinusoidal but also periarticular – Located adjacent to HSCs	• Subpopulation of mesenchymal stromal cells • Expresses high levels of CXCL12 • Produces SCF
• Osteoblast	• Lining of endosteum	• Expresses low levels of CXCL12 (1000-fold lower than CAR cells) • A decrease in cell number → decreased cellularity and increased extramedullary hematopoiesis • Changes in CXCL12 cell surface expression → HSC mobilization • Produces thrombopoietin
• Osteoclast	• Lining of endosteum	• Regulates bone resorption • Stress → increased osteoclasts increased HSCs in peripheral blood • A decrease in number → increased trabecular bone formation, decreased number of HSCs, decreased engraftment, and increased HSC mobilization
• Sinusoidal endothelial cell		• Produces SCF • Expresses CXCL12 but 100-fold lower than CAR cells
• Macrophage	• Scattered throughout bone marrow	• Promotes retention of HSCs/HPCs in the niche • A decrease in cell number → HSC mobilization via CXCL12 depletion
• Adipocyte		• A decrease in cell number → increased trabecular bone, increase HSC number, and increased HSC engraftment
• Sympathetic neuron – Specific nerve fibers	• Usually associated with arterioles in central bone marrow	• Regulates daily circadian rhythm of HSC mobilization • Required for hematopoietic recovery after chemotherapy • β-Adrenergic tone can regulate nestin + stromal cells • Inhibition → increased HSC mobilization
• Schwann cell	• Associated with nerve fibers	• Regulates proteolytic activation of latent TGFβ – TGFβ promotes HSC maintenance
• Skeletal stem cell	• Metaphysis	• Forms fibroblasts • Can undergo multilineage differentiation into osteoblasts, chondrocytes, and adipocytes
• Regulatory T-cell	• Widely dispersed – But commonly found near endosteal surfaces	
• Megakaryocyte	• Perisinusoidal • Close proximity to HSCs	• Secretes TGFβ1 which promotes HSC quiescence • Can promote regeneration of HSCs after myeloablation via production of FGF1

CAR cell CXCL12-abundant reticular cell, *MSC* mesenchymal stem cell, *CXCL12* CXC motif chemokine ligand 12, *SCF* stem cell factor, *HSCs* hematopoietic stem cells, *HPCs* hematopoietic progenitor stem cells, *TGFβ* transforming growth factor beta, *FGF1* fibroblast growth factor 1

transient egress of HSCs from the bone marrow niches [39, 40]. HSCs express β2-adrenergic and dopamine receptors, and these neurotransmitters are chemoattractants for HSCs [41]. Nestin which is normally restricted to expression in nerve cells is an intermediate filament protein that is also expressed on mesenchymal stem cells found in the bone marrow [42]. Repeated exposure of the HSC niche to cisplatin causes injury to the HSC niche presumably through damage to the nervous system within the bone marrow [33].

Many adhesive molecules that play a role in retaining HSCs in their niches have been identified. Interactions with N-cadherin is involved, but its exact mechanism remains unclear with contradictory evidence reported [43–45]. Other adhesion

Table 3.5 Noncellular components that regulate hematopoietic stem cells and the HSC niche

Component	Source	Effector cell	Role and/or mechanism
• Oxygen	–	–	• Hypoxia induces CXCL12 expression through HiF1-α
• Calcium	• Concentrated along the bone • Sensed through calcium channels on HSCs		• Deletion of the calcium-sensing receptor → reduced bone marrow cellularity with a decrease in HSC number and concurrent increased progenitor cell mobilization into the peripheral blood and spleen
• CXCL12 (SDF-1)	• CAR cells • Endothelial cells • Nestin + MSCs • Osteoblasts	• CXCR4-expressing cells (HSCs)	• Promotes HSC maintenance and retention in the bone marrow via gradient-mediated chemotaxis – Gradient is affected by G-CSF • Maintains HSC pool size • Homing, retention, and mobilization of HSCs • Deficiency of CXCL12 or its receptor, CXCR4, in mice → disruption of colonization of the bone marrow
• SCF	• CAR cells • Endothelial MSCs • Osteoblasts • Nestin + MSCs	• HSCs	• Binds to KIT receptor • Promotes HSC maintenance (LT-HSCs)
• Thrombopoietin	• Osteoblasts		• HSC quiescence and maintenance • Produced in the liver, kidney, and bone marrow stroma and by osteoblasts – May be transported from the blood to the bone marrow – Deficiency of thrombopoietin or its receptor (c-Mpl) → profound decrease in HSC number
• Angiopoietin-1	• Osteoblasts • Nestin + MSCs • LEPR+ cells • HSCs	• Endothelial cells	• HSC quiescence • Promotes the maintenance of quiescent HSCs (LT-HSCs) but *not* required • Regulates the regeneration of the HSC niche
• Notch ligands	• Endothelial cells • Osteoblasts	• Osteoblasts • HSCs • Endothelial cells	• Binds Notch in HSCs → quiescence • Notch 2 promotes HSC regeneration after myeloablation • Parathyroid hormone increase expression of Notch ligands on osteoblasts that results in an increase number of HSCs in the bone marrow
• Angiogenin	• MSCs • HSCs	• HSCs • LSK cells	• Promotes HSC quiescence and proliferation of myeloid progenitors • Promotes recovery of hematopoiesis after myeloablation
• FGF-1	• Megakaryocytes	• HSCs • Megakaryocytes	• Promotes regeneration of HSCs and megakaryocytes after myeloablation
• TGFβ	• Megakaryocytes • Schwann cells • Others	• HSCs • Others	• Promotes HSC quiescence and self-renewal
• Osteopontin	• Endosteum-lining cells	• HSCs	• Negatively regulates the number of HSCs • Mice deficient in osteopontin have increased number of HSCs in the bone marrow

CXCL12 CXC motif chemokine ligand 12, *CAR cell* CXCL12-abundant reticular cell, *CXCR4* CXC motif chemokine receptor 4, *HSCs* hematopoietic stem cells, *G-CSF* granulocyte colony-stimulating factor, *MSCs* mesenchymal stem cells, *SCF* stem cell factor, *LSK cells* lineage-SCA+, KIT+ myeloid progenitors, *FGF1* fibroblast growth factor 1, *TGFβ* transforming growth factor beta

molecules identified in contributing to HSC tethering to the HSC include markers of the integrin family (integrin α4β1, very late antigen-4 (VLA-4), α5β-very late antigen-5, α4β7 integrin, lymphocyte Peyer's patch adhesion molecule (LPAM)-1, the alpha 6 integrins (lamina), CD44, E-selectins, the angiopoietin receptor, calcium-sensing receptor, stromal cell-derived factor-1a, and osteopontin) [46–53]. Studies also show that transplanted HSCs localize and engraft to endothelial microdomains that express E-selectin and SDF-1α [54]. Furthermore, stromal cells expressing signaling lymphocytic activation molecules (SLAM) which were found adjacent to the bone marrow sinusoidal blood vessels were enriched for HSCs [55].

Taichman et al. [56, 57] showed that cells of the osteoid lineage secrete growth factors, such as G-CSF, GM-CSF, IL-1, IL06, and TGFB, that play significant roles in the regulation of hematopoiesis in the HSC niche. Others have found that the Notch ligand, Jagged 1, helps to regulate HSC self-renewal versus differentiation [58, 59]. However, other studies in which all Notch signaling was inhibited did not result in deficits in HSC function [60].

While the bone marrow has been classically divided into two anatomical niches (the perivascular and endosteal niches), location alone is not sufficient to provide an adequate environment for HSCs to flourish [45, 61, 62]. These niches need to be functional, and the relationship between the HSCs and their niches is dynamic and appears to rely on the maturation stage of the HSC. For example, more immature HSCs and those that respond to damage signals [63] are found near the endosteum whereas more mature, quiescent, long-term HSCs reside near the perivascular niche [64]. HSC entrance into the cell cycle and differentiation are highly regulated processes to prevent HSC exhaustion [65]. In healthy patients who are not physiologically stressed, the vast majority of HSCs are quiescent, residing in the perivascular niche. However, damage to the stroma/niches such as irradiation and chemotherapies used in myeloablative conditioning regimens results in approximately 50% of the HSCs recycling through the peripheral blood.

HSC homing to the bone marrow: Stromal cell-derived factor-1 (SDF-1) (also known as CXCL12) is a chemotactic factor that is expressed on the surface of bone marrow stromal cells, specifically, the CXCL12-abundant reticular (CAR) cells and, to a lesser extent, osteoblasts. Its ligand is CXCR4 and is expressed on circulating HSCs. Circulating HSCs gravitate toward the HSC niche via adhesion molecule interactions and by secreting metalloproteases that disrupt the extracellular matrix in order to move toward and settle into the HSC niches. High concentrations of CXCL12 are thought to promote HSC survival and quiescence. The expression of CXCR4 and CXCL12 (SDF-1) as well as other genes that regulate hematopoiesis and HSC fitness and stemness is regulated by HIF-1α transcription factor; hypoxia stabilizes HIF-1α [66, 67]. Decreased oxygen tension (i.e., hypoxia) induces CXCL12 through the action of HIF-1α and correlates with increased expression of CXCL12 on endothelial cells. The HSC niche has been found to be hypoperfused, creating a hypoxic environment [68, 69]. Another key molecule that plays an important role in HSC homing is stem cell factor (SCF). SCF can increase CXCR4 expression to promote homing of HSCs to the HSC niche [29]. When the bone undergoes osteoclast-mediated reabsorption, calcium is released into the bone marrow microenvironment. This extracellular calcium acts as a chemoattractant to HSCs that express calcium-sensing receptors on their cell surfaces [46].

Thymus

The thymus which is considered a central or generative lymphoid organ is the site of T-cell maturation. T-cells found in the thymus, even if they are located there transiently, are often referred to as thymocytes. The thymus is located in the anterior mediastinum. It is a bilobed organ. Each bilobe is divided into multiple lobes that are divided by fibrous septa. Each lobe is further divided into an outer cortex and inner medulla. The epithelial cells of the thymus which make up the infrastructure of the thymus are derived from the ectoderm that had invaginated to form branchial pouches. Once T-cells enter the thymus, their maturation (also referred to as T-cell education) begins in the cortex. As their maturation progresses, T-cells migrate

from the cortex to the medulla. Thus, the medulla contains the most mature, but naïve, T-cells. Most of the T-cells within the thymus are concentrated into the cortex whereas the medulla typically contains macrophages and dendritic cells with just a few T-cells. Also, cortical epithelial cells are scattered throughout the thymus and secrete IL-7 which is necessary for normal T-cell development. Medullary thymic epithelial cells are located exclusively in the medulla. They play an important role in T-cell education in that they present self-antigens to developing T-cells and induce apoptosis (i.e., programmed cell death) in the developing T-cells that react to self-antigens. Approximately 90–95% of all developing T-cells react to self-antigens and then end up being eliminated within the medulla. In the medulla, areas of packed epithelial cells and remnants of degenerated cells make up Hassall's corpuscles. The thymus is quite vascular, and efferent lymphatic vessels drain into the mediastinal lymph nodes.

Lymph Nodes

Lymph nodes are considered a part of the peripheral or secondary lymphoid organs. Lymph nodes are encapsulated, vascular tissues whose architecture is conducive for promoting the initiation of adaptive immune responses to presented non-self-antigens that are delivered to these sites via the lymphatics. The lymphatic system connects lymph nodes to each other. Lymphatic capillaries absorb fluid from spaces between tissues. This absorbed fluid is called lymph, and the lymph gets pumped into converging larger lymphatic vessels called the afferent lymphatics which drain into the lymph nodes. The afferent lymphatics deliver dendritic cells (which have captured and ingested microbes and other soluble antigens) as well as inflammatory mediators to naïve lymphocytes located in the lymph nodes. Lymph drains from the lymph nodes via the efferent lymphatics. Eventually, efferent lymphatics drain into the thoracic duct which, in turn, empties into the superior vena cava (SVC) which finally returns the lymph to the blood stream. Approximately 2 L of lymph circulates within the lymphatic system and returns to the peripheral blood circulation each day.

There are approximately 500 lymph nodes in each human. All lymph nodes are surrounded by a fibrous capsule. Beneath the capsule lies a sinus system that is lined with reticular cells. Collagen and other extracellular proteins make up fibrils that bridge across the sinuses. Lymph nodes are filled with lymph as well as a variety of cell types, the majority of which are APCs (i.e., macrophages and dendritic cells). The afferent lymphatics empty into the subcapsular (marginal) sinus. Lymph traverses the lymph node into the connected medullary sinus and then flows out of the lymph node via the efferent lymphatics. Just below the subcapsular sinus, the lymphocyte-rich cortex exists. Within the lymph node cortex, B- and T-cells are sequestered in their own distinct regions that consist of a unique collection of reticular fibers and stromal cells. In the outer portion of the cortex within the B-cell zones, collections of cells called follicles are found. They are organized around follicular dendritic cells (FDCs). Primary follicles predominantly contain naïve B-cells. The germinal centers arise in response to antigenic stimulation and are sites of substantial B-cell proliferation as well as selection of the B-cells that produce high-affinity antibodies and generate memory B-cells and plasma cells. Each germinal center contains a dark-staining zone that is packed with proliferating B-cells and a light-staining zone that contains B-cells that are no longer proliferating because they are destined to undergo further differentiation and become long-lived memory B-cells. Follicles with germinal centers are referred to as secondary follicles. The area surrounding the follicles are organized into cords and are made up of matrix proteins, fibers, lymphocytes, dendritic cells, and mononuclear phagocytes.

In contrast, naïve T-cells reside in the paracortical cords beneath the follicles and are often referred to as the paracortex. The paracortex contains fibroblastic reticular cell networks and forms fibroblastic reticular cell conduits. These conduits run from the subcapsular sinus to the medullary sinus lymphatics and to the cortical

blood vessels (called high endothelial venules (HEV)). T-cells enter the T-cell zone via the HEVs and are densely packed around the fibroblastic reticular cell conduits. Most of these T-cells are phenotypically $CD4^+$ helper T-cells with a few $CD8^+$ T-cells. Dendritic cells are found in this region as well.

The regional zones of lymph nodes are driven by chemokines secreted by the surrounding stromal cells. These chemokines direct the migration of naive T- and B-cells to their respective regions. All naïve lymphocytes enter lymph nodes by an artery and then enter into the lymph node stroma via HEVs. Chemokines that are secreted by the central stroma provide chemoattractants to direct B- and T-cells to their respective zones. The stromal cells that express CCL19 and CCL21 bind to CCR7 which is expressed on naïve T-cells and microbe-activated dendritic cells. Naïve B-cells express CXCR5 which binds to CXCL13 which is produced only by FDCs which specifically attracts naïve B-cells to follicles [70].

The development of lymph nodes during fetal life is stimulated by lymphoid tissue-induced cells, a subset of innate lymphoid cells. Proteins, such as lymphotoxin α and lymphotoxin β, which are produced by lymphoid tissue inducer cells, mediate lymph node development. Lymphotoxin β activates FDCs to produce CXCL13 which recruits B-cells to follicles. It also activates fibroblastic reticular cells to produce CCL19 and CCL21, resulting in the recruitment of T-cells and dendritic cells into the T-cell zone [71].

When T- and B-cells are activated after recognition of their specific antigen, they begin to express different chemokine receptors that result in their migration toward one another. Once activated, T-cells may migrate toward follicles to help B-cells or leave the lymph node and enter the circulation. After migration into germinal centers, activated B-cells differentiate into plasma cells and may exit the lymph node and home to the bone marrow.

Substances contained within lymph enter the lymph node at the subcapsular sinus where they are separated out by molecular size and then delivered to different cell types to elicit specific immune responses. The structure of the cortex permits APCs to enter the cortex but not soluble molecules contained within the lymph. Instead, microbes and high molecular weight antigens are ingested by resident macrophages and processed for antigen presentation to B-cells. Low molecular weight antigens exit the sinus via conduits and then are taken up by dendritic cells' processes that protrude into the lumen of the conduit for presentation to T-cells.

Spleen

The spleen is another peripheral (or secondary) lymphoid organ. Similarly to lymph nodes, one of the major functions of the spleen is to provide an environment in which adaptive immune responses are initiated in response to antigen presentation. The other major function of the spleen is the removal of aging and damaged red blood cells as well as immune complexes and opsonized microbes from the circulation. This highly vascularized organ is divided into red pulp and white pulp. The red pulp is composed of blood-filled vascular sinusoids whereas the white pulp is packed with lymphocytes. Blood flows into the spleen via the splenic artery, delivering blood at the hilum. Progressively smaller branches of the splenic artery eventually end in the vascular sinusoids. These sinusoids are filled with erythrocytes and lined with macrophages. The blood is then carried out of the spleen via the splenic vein and into the portal circulation. Macrophages located within the red pulp remove microbes, damaged cells, and opsonized (antibody-coated) cells and microbes. While the red pulp acts as an important filter of the blood, the white pulp houses the cells that mediate adaptive immune responses to blood-borne antigens. The white pulp is organized around central arteries that are distinct branches from the splenic artery. Smaller branches of the central artery pass through the white pulp regions, draining into a marginal sinus. An area that surrounds the marginal sinus (the marginal zone) forms the anatomical boundary between the white pulp and red pulp. The white pulp is further delineated into B-cell and T-cell zones, similarly to the

architecture of lymph nodes. T-cells are located in zones called periarteriolar lymphoid sheaths. B-cells are located in follicles located between the marginal sinus and the periarteriolar sheath. The marginal zone which is located just outside of the marginal sinus is a distinct region that contains B-cells and specialized macrophages. The B-cells located in the marginal zone (called marginal-zone B-cells) have distinct functions as compared to those residing in the follicles (follicular B-cells) in that they have a much more limited antigen recognition repertoire (and are described in more detail elsewhere in this chapter). Circulating dendritic cells deliver antigens from the blood to the marginal sinus. In addition, macrophages in the marginal zone present the antigens.

Again, segregation and interaction of T- and B-cells are highly regulated by cytokines and chemokines produced by the surrounding stromal cells of the spleen. The chemokines and cytokines are the same that regulate T- and B-cell interactions and localization in the lymph nodes. Lymphotoxin stimulates the production of stromal cell-derived chemokines (CXCL13 for B-cell migration and CCL19 and CCL21 for T-cell migration).

Immune Tissue Regions

The most important immune tissue regions are the skin, GI mucosa, and bronchial mucosa. Mucosal-associated lymphoid tissues are important in providing protection against ingested and inhaled pathogens and foreign antigens.

Immune Responses

Immunity and immune responses can be classified as innate or adaptive. Innate immunity provides initial defense against microbes whereas adaptive immunity acts later and is long-lasting. However, innate and adaptive immune responses are highly connected by many different mechanisms. Engagement of the innate immune system triggers the innate immune system to stimulate adaptive immune responses. It also influences the adaptive immune response that is produced. Conversely, the adaptive immune response can enhance the protective effects of innate immunity. Table 3.6 compares innate versus adaptive immunity in terms of specificity, diversity, and memory. The key components (i.e., cellular and chemical barriers, proteins, and cells) of innate

Table 3.6 Comparison of innate versus adaptive immunity

Feature	Innate immunity	Adaptive immunity
• Species	• Present in all living organisms from plants and insects to humans	• Vertebrates only
• Specificity	• Minimal – For structures shared by groups of related microbes	• For antigens of microbes and for nonmicrobial antigens
• Diversity	• Limited • Germline encoded	• Very large • Somatic recombination of gene segments
• Memory	• No	• Yes
• Nonreactivity to self	• Yes	• Yes
• Cellular barriers	• Skin • Mucosal epithelia	• Lymphocytes in epithelia
• Chemical barriers	• Antimicrobial molecules	–
• Proteins	• Complement	• Antibodies
• Cells	• Phagocytes – Macrophages – Neutrophils • Innate lymphoid cells – NK cells	• Lymphocytes – T-cells – B-cells

and adaptive immunity are compared as well. Both innate and adaptive immune responses are nonreactive to self-antigens, thus preventing autoimmune reactions.

Innate Immunity

Innate immunity is the first line of defense against microbes. It is also referred to as natural or native immunity. Innate immunity provides immune defense mechanisms against microbes that are in place even before encountering the infectious agent. It is poised to respond quickly and efficiently. Almost all multicellular organisms from plants and insects to humans have innate immunity mechanisms present. Generally, innate immunity is nonspecific: Innate immunity does not recognize subtle differences between different microbes per se. The response is essentially the same to repeated exposures. Innate immunity constitutes chemical and physical barriers, phagocytic cells that will ingest and kill pathogens, dendritic cells, NK cells, other innate lymphoid cells, the complement system, and mediators of inflammation. The mechanisms of innate responses occur within hours of the infection being detected, whereas adaptive immune responses take days to be fully deployed. Most innate immune responses take place at potential portals at which microbes can enter the body when the epithelial barriers have been breached. These sites include the skin, lungs, and GI tract. The two major responses of innate immunity are inflammation and antiviral defense. The process of inflammation consists of recruitment of leukocytes and proteins from the blood into the affected tissue(s) to eliminate the pathogen(s). Phagocytes, neutrophils, and monocytes are the most common cell types that are recruited to the sites of inflammation. NK cells identify and kill virally infected cells. If microbes are able to circumvent these mechanisms of immune defense at these sites and make it into the circulation, then components of the complement system are activated, resulting in proteolytic cleavage products. These products go on to mediate inflammatory responses, opsonize microbes for phagocytosis, and directly lyse the microbes. However, many pathogens have evolved mechanisms that evade innate immune responses and thus require adaptive immune responses to fully eradicate these pathogens.

Adaptive Immunity

Introduction: Also referred to as specific or acquired immunity, adaptive immunity provides later responses against pathogens via three main mechanisms: (1) secreted antibodies that bind to extracellular microbes, blocking their ability to infect host cells and promoting the ingestion and elimination by phagocytes; (2) phagocytosis that is enhanced by antibodies and helper T-cells; and (3) direct killing by cytotoxic T-cells of infected host cell that are otherwise inaccessible to antibody- or phagocytic-mediated destruction. Only vertebrates have adaptive immunity. Lymphocytes, APCs, and effector cells are the principal cellular components of adaptive immunity. Because of their ability to specifically recognize and respond to foreign antigens, lymphocytes can recognize and respond to a huge number of microbial and nonmicrobial antigens. It is thought that there are approximately 10^7–10^9 distinct antigen determinants that lymphocytes can recognize as foreign. B-cells recognize soluble and cell surface antigens whereas T-cells recognize and respond to antigens of intracellular microbes and not soluble antigen. Adaptive immunity is characterized by its exquisite sensitivity and specificity to distinguish different antigens and respond more vigorously and more efficiently with each subsequent exposures (referred to as "memory"). Specificity is due to the expression of membrane receptors on individual lymphocytes that can distinguish subtle structural differences among antigens. The ability of the T- and B-cell repertoire to be so diverse is due to the variability in the structures of the antigen-binding sites of the antigen receptor expressed on lymphocytes.

Historical perspective: Early experiments in mice demonstrated that protective immunity against microbes could be achieved by adoptive transfer of lymphocytes or molecules secreted by lymphocytes from immunized to naïve mice. Subsequent in vitro studies demonstrated that lymphocytes stimulated by antigens resulted in

immune responses, identifying lymphocytes as mediators of both humoral and cellular immunity. Shortly thereafter, the role of the bone marrow and thymus in the development of B- and T-cells, respectively, became evident. The properties of high diversity and exquisite specificity of the B- and T- cell repertoire were appreciated. In addition, the consequences of absence or dysfunction of these systems were becoming apparent. One of the first observations made that supports the importance of lymphocytes to adaptive immunity was that patients with congenital or acquired immunodeficiencies have a decreased number of lymphocytes in the peripheral blood and in lymphoid tissues.

In 1883, Elie Metchnikoff postulated the cellular (or cell-mediated) theory of immunity that was based on his observations of phagocytes surrounding and engulfing a thorn that was stuck in a translucent starfish. He concluded from this observation that cells (and not "humors" from the blood) were the principal mediators of the immunologic response to foreign substances. However, the debate between cell-mediated versus "humors"-mediated immunity continued on. However, in the 1950s, the cellular theory of immunity was corroborated when it was shown that the adoptive transfer of cells alone and not serum (i.e., "humors") could provide protective immunity to the intracellular bacterium, *Listeria monocytogenes*. It is now well established that both humoral and cell-mediated mechanisms play important roles in adaptive immunity.

The cardinal features of adaptive immunity: All humoral and cell-mediated immune responses to foreign antigens have a number of cardinal features that are related to specificity, diversity, memory, clonal expansion, specialization, contraction and homeostasis, and nonreactivity of self. These properties are summarized in Table 3.7. The adaptive immune response to a specific antigen peptide determinant is targeted only to the cells of pathogen that express that specific antigen. This mechanism provides the specificity of adaptive immunity. Another cardinal principal of adaptive immunity is its diversity: adaptive immunity is able to respond to all potential antigens that may be encountered over an individual's lifetime, this diversity arises from somatic recombinations of genes that encode for MHC Class I and Class II molecules, and these events appear to occur randomly. Furthermore, this diverse lymphocyte "repertoire" is preset *before* the naïve T-cells ever encounter antigens. Memory of an adaptive immune response is another fundamental principal of adaptive immunity. Memory provides further, robust protection against future encounters with the same foreign antigen. Once activated, lymphocytes undergo a burst of proliferation in order to mount an effective immune response to eliminate effectively the pathogens or infected cells. Although it may take

Table 3.7 The fundamental properties of adaptive immune responses

Characteristic	Significance
• Specificity	• An adaptive immune response to a specific antigen is targeted only to that antigen
• Diversity	• Adaptive immunity is able to respond to all potential antigens that may be encountered over an individual's lifetime, and this diversity is preset before encountering antigens
• Memory	• Subsequent encounters to the same antigen evoke an increased immune response more efficiently
• Clonal expansion	• When activated, the antigen-specific effector lymphocyte undergoes a burst of proliferation in order to mount an effective immune response to the targeted antigen-expressing pathogen
• Specialization	• The adaptive immune response is optimally tailored to offer appropriate defense against different types of pathogens
• Return to homeostasis	• Once an adaptive immune response has successfully provided adequate defense against a foreign antigen, the immune system "resets" or contracts in order to respond effectively to another newly encountered foreign antigen (homeostasis)
• Nonreactivity to self	• Injury to the host tissues by the immune system is prevented during a response to foreign antigens, termed tolerance

more time to achieve this potent immune response, adaptive immune responses are optimally tailored to the specific types of pathogens. Thus, adaptive immunity has specialization as another cardinal feature. The mechanisms of adaptive immune responses are programmed to contract down to the basal state once the pathogen or foreign antigen is adequately eliminated such that it no longer poses a threat to the individual. This basal state is termed homeostasis. Fortunately, mechanisms are in place to eliminate lymphocytes that express antigen receptors that recognize self-antigens (this occurs in the thymus) or to inhibit self-reacting lymphocytes that made it through the thymus and now in the circulation in secondary lymphoid tissues/organs. This nonreactivity to self is termed self-tolerance. The mechanism of self-tolerance is extremely important in order to avoid autoimmune disorders from arising. All of the above cardinal features of adaptive immunity are necessary in order for adaptive immunity to provide effective protection against foreign antigens that are long-lived while preserving the integrity of normal host cells, tissues, and organs.

Humoral and cell-mediated immune responses demonstrate these fundamental properties differently. Table 3.8 presents a comparison of humoral versus cell-mediated immunity in terms of the effector cell type, the pathogen(s) it responds to, and the effector cell mechanism and their functions, and these differences will be discussed later on in this chapter (see “Humoral Immunity” and “Cell-Mediated Immunity”).

Once a naïve lymphocyte (T- or B-cell) encounters its specific antigen (presented by APCs) in peripheral lymphoid organs (i.e., the spleen and lymph nodes), the lymphocyte becomes activated through a series of highly regulated steps that results in a burst of proliferation, termed clonal expansion (up to a 50,000-fold expansion of the antigen-specific T-cell and up to a 5000-fold expansion for the antigen-specific B-cell). Morphologically, both effector T- and B-cells significantly increase in size with an increase in cytoplasm to accommodate the increase in protein synthesis. Upon activation, effector T-cells begin to secrete IL-2 which stimulates the proliferation of T-cells that express the IL-2 receptor ($CD25^{+}$) on their cell surface. Concurrently, these activated T-cells upregulate the expression of IL-2 receptor on its cell surface, resulting in an increased proliferation in an autocrine fashion, driving clonal expansion. In contrast, naïve B-cells produce on their cell surface the Ig subsets, IgM and IgD. Upon activation, effector B-cells undergo isotype switching and produce IgG, IgA, or IgE subclasses, resulting in antibody secretion. The affinity of the antibody produced by the effector B-cells increases during the immune response because the effector B-cells with the highest affinity are preferentially selected for and expanded. The clonally expanded, activated lymphocytes also undergo differentiation to become effector cells whose function is to eliminate directly or indirectly cells and/or organisms that express the antigen specifically recognized by the expanded lymphocyte.

Table 3.8 Comparison of humoral versus cell-mediated immunity

Feature	Humoral immunity	Cell-mediated immunity	
• Effector cell type	• B-cells	• $CD4^{+}$ helper T-cells	• $CD8^{+}$ cytotoxic T-cells
• Pathogen	• Extracellular microbes	• Microbes that have been phagocytosed by macrophages	• Intracellular microbes replicating within infected cells (e.g., viruses)
• Effector mechanism	• Antibody secretion by activated B-cells into the serum	• T-cell activation	• T-cell activation
• Function	• Blockade of infections • Elimination of extracellular microbes	• Macrophage activation resulting in the killing of phagocytosed microbes	• Killing of infected cells • Elimination of infection reservoirs

Memory: In general, the life-span of the majority of effector lymphocytes is short. However, a small portion of these activated T- and B-cells go on to differentiate into memory cells whose function is to mediate a rapid, enhanced response to subsequent exposures to the same antigen. These responses are called secondary immune responses. Until they reencounter their specific antigen, memory lymphocytes remain dormant. Lymphocytes are termed resting because they are not actively dividing, but they are metabolically active. Memory lymphocytes can live for months to years in this quiescent state. They remain in this state until they subsequently encounter their specific antigen. Memory B-cells express different classes of membrane immunoglobulin (termed isotype). Different phenotypes of memory lymphocytes are dictated by the transcription factors driving gene expression patterns in each phenotype.

Two Types of Adaptive Immunity

Adaptive immunity has two types: humoral and cell-mediated (also called cellular) immunity. Each has different mechanisms to eliminate different types of pathogens/microbes. They consist of different cell types and molecular mediators, but humoral and cell-mediated immunities often cooperate to enhance the immediate and long-term immune response (see Table 3.8).

Humoral immunity: Humoral immunity is the main defense mechanism against extracellular microbes and their toxins. The key mediator of humoral immunity is antibody produced by activated B-cells. Humoral immunity was first demonstrated by Emil von Behring and Shibasoburo Kitasato in 1890. Their experiments showed that the infusion of serum from animals that had been immunized with an attenuated form of diphtheria toxin could make naïve animals specifically resistant to diphtheria infection. Around the same time, Paul Ehrlich termed these serum proteins that bound toxins *antibodies*. He also termed the substances that triggered the generation of these antibodies *antigens*. He postulated the theoretical framework of the specificity of the antigen-antibody reactions [72].

Effector B-cells include plasma cells. Plasma cells have an eccentric nucleus and have abundant cytoplasm with large quantities of rough endoplasmic reticulum and distinct perinuclear Golgi bodies to produce large quantities of antibody proteins. Secreted antibodies can bind to extracellular microbes and their toxins to promote the ingestion of microbes by phagocytes. Antibodies can also bind to and elicit the release of cellular inflammation mediators. In addition, antibodies are actively transported across lumens of mucosal organs and across the placenta to offer primary defense against microbes that have been inhaled or ingested and to provide immunity against infections in the newborn. Depending on the protein antigens encountered, B-cells produce different classes of immunoglobulin from a single clone of B-cells. This process is called class switching. Helper T-cells are required for class switching to occur. The production of antibodies with increased affinity is stimulated by helper T-cells and is termed affinity maturation. This affinity maturation enhances the humoral immune response. When antibodies bind to a microbe, it prevents the microbe from infecting cells, thus neutralizing them. IgG classes of antibody coat microbes which target them for phagocytosis because neutrophils and macrophages express receptors that recognize a portion of the IgG molecule. Generally, the half-life of antibodies is a few days with the exception of IgG isotypes which have half-lives of approximately 3 weeks.

Immunity to a previously encountered foreign antigen can be measured by assaying for the type of isotype present and by measuring the amount of antibody in the serum, called antibody titers. If specific antigen antibody titers are present in the serum, then that individual is considered to be sensitized to that antigen and is capable of evoking a protective immune response to that specific antigen-containing microbe. The type of isotype detected (i.e., IgM versus IgG) can indicate the timing of the exposure to the pathogen. The presence of IgM isotype indicates a recent infection whereas the presence of IgG isotypes suggests a

past infection. Also, the quantity of antibody titers measured from an individual's serum can reflect the timing of the exposure to the foreign antigen. Relatively high antibody titers are found in individuals who were recently infected whereas low IgG titers are indicative of a past infection.

Cell-mediated immunity: Cell-mediated immunity predominantly provokes defense against phagocytes that have ingested microbes, working to destroy these cells. T-cells are the cell type that mediates cell-mediated immunity. Also, T-cells can help to eliminate extracellular microbes by helping B-cells produce antibodies or by the recruitment of leukocytes that have the capacity to kill these pathogens. Activated effector T-cells secrete cytokines and preferentially migrate to inflamed or damaged tissues in order to either directly or indirectly eliminate the cells expressing the specific antigen that triggered this cascade of events initially. Effector cells of the T-cell lineage include $CD4^+$ helper T-cells and $CD8^+$ cytotoxic T-cells. $CD8^+$ effector T-cells directly kill the antigen-specific expressing cell. Their cytoplasm contains proteins in cytoplasmic granules that, when released, will destroy their respective antigen-expressing cells (e.g., virally infected and tumor cells). Effector $CD4^+$ helper T-cells operate indirectly to eliminate foreign antigens. Effector $CD4^+$ helper T-cells express CD40 ligand (CD154); they secrete cytokines that bind to macrophages and B-cells resulting in their activation. $CD4^+$ helper T-cells also stimulate the proliferation and differentiation of T-cells themselves.

Active Versus Passive Immunity

Active immunity refers to the process in which an immune response is evoked by the exposure to a foreign antigen; the individual plays an active role in responding to the antigen. Examples of active immunity include vaccination and exposures to viruses, bacteria, and other pathogens. However, protective immunity can be adoptively transferred by the transfer of T-cells or the delivery of antibody. This is termed passive immunity. Lymphocytes collected from an immunized individual can be transferred to newly infected individuals, making the recipient immune without being previously exposed to the antigen. Administration of serum obtained from an immune individual(s) (i.e., antibodies) to a previously uninfected individual who is now infected is also passive immunity. Examples of this type of passive immunity include the transfer of antibody from the mother to her fetus via the placenta, the administration of IVIG post-HSCT, and infusing Cytogam to treat an individual with CMV reactivation or infection. Delivery of antibody to provide immunity is fast and effective but has no memory.

Principles of Transplantation Immunology

Introduction

The primary function of the immune system is to provide an effective defense against pathogens first initiated by innate immunity mechanisms and then by adaptive immune responses. Innate and adaptive immune mechanisms work in conjunction to provide not only immediate protection against foreign antigens but also long-lived protection in order to prevent harm if and/or when the antigens from a previous exposure are encountered again. The infusion of an allogeneic HSC graft from one individual (donor) into a different individual (the host or recipient) creates a unique situation to which both the donor and the host immune cells react. These responses are referred to as alloimmune responses due to alloreactivity.

Alloreactivity, Alloantigens, Antigen Presentation, and Tolerance

Introduction: The graft from one individual infused into the same individual is termed *autologous*, whereas a graft from a genetically identical individual into another genetically identical individual (i.e., an identical twin-to-twin HSCT) is called *syngeneic*. If the transplanted graft is from a donor of the same species but genetically

nonidentical (a sibling, a parent, or an unrelated donor), it is called an *allogeneic* HSCT. A graft between two individuals of different species, such as bone marrow from a human that is transplanted into a mouse, is referred to as a *xenogenic* graft (or *xenograft*).

Historical perspective: The principles of alloreactivity and the allorejection (i.e., the rejection of an allogeneic graft) were first derived from experiments performed with skin grafts. When skin grafts were performed to replace damaged skin on burn patients, all of the skin grafts became necrotic and fell off in approximately 1–2 weeks. Medawar and colleagues determined that this process was due to an inflammatory reaction that they had termed rejection. Furthermore, it was found that while rejection occurred 10–14 days after transplantation, the time to rejection was much shorter (3–7 days) after transplantation from the same donor but not a different donor, demonstrating specificity and memory of the immune response. Further studies showed that the allograft rejection was mediated by lymphocytes, i.e., by an adaptive, cell-mediated immune response. These lymphocytes were considered to be alloreactive to these antigens. Some of these principles that were formulated from these early skin transplantation mouse studies generally apply to hematopoietic stem cell transplantation but not all. Any component of the immune system (i.e., T-cells, B-cells, antibodies) that recognizes an alloantigen is also thought to be alloreactive and cause allorejection, as seen in these mouse skin transplantation experiments (see Chap. 11, "Graft Failure", for a detailed discussion related to graft rejection).

Antigen presentation and alloantigens: Alloantigens are the molecules expressed on an allograft that lymphocytes and antibodies recognize as being foreign. Alloantigens can elicit both a humoral and a cell-mediated immune response, and alloantigen recognition utilizes similar mechanisms to recognize foreign antigens of microbes. MHC molecules are responsible for strong, rapid rejection reactions. The severity of the immune reaction to alloantigens depends upon the degree of MHC incompatibility (see Chaps. 7 and 11). Histocompatibility is related to polymorphic MHC (HLA) Class I and Class II glycoproteins located on cell surfaces. MHC is codominantly expressed. The MHC locus encodes both MHC Class I and MHC Class II molecules. Both MHC Class I and Class II complexes contain non-polymorphic regions of polypeptides as well as an extracellular, polymorphic peptide-binding domain that is specifically recognized by T-cells. These glycoproteins bind small antigen peptide fragments (called determinants) that are derived from degraded proteins (antigens). Because the number of T-cells that have the capacity to recognize any one specific antigen is small or if the antigen load is low, mechanisms have evolved to concentrate antigens and deliver them to collections of naïve T-cells to increase the chance of antigen recognition by naïve T-cells. APCs are specialized cells of the immune system that can capture foreign antigens and process these antigen proteins to produce these small antigen peptide fragments. Dendritic cells are located within epithelia and connective tissues where they can efficiently capture pathogens and process them. Dendritic cells then migrate to secondary lymphoid tissues (i.e., lymph nodes and the spleen) via lymphatics through which naïve T-cells continuously circulate. Thus, the likelihood that an APC will display a foreign antigen peptide to the complementary T-cell receptor expressed on naïve T-cells is greatly improved.

Depending upon the origin of the antigens, the small antigen peptide fragments are bound to either MHC Class I or MHC Class II molecules in the endoplasmic reticulum, and then this complex of antigen peptide determinant and self-MHC is presented by APCs to T-cells. The antigen T-cell receptor on T-cells interact with APCs and either bind or not bind to these complexes, depending upon the avidity (i.e., how tightly they fit together). The antigen T-cell receptor expressed on $CD8^+$ T-cells recognizes an antigen peptide only when bound for display by MHC Class I molecules, whereas $CD4^+$ T-cells only recognizes an antigen peptide when bound and displayed by MHC Class II molecules, i.e., class restriction. The T-cell will either "ignore" the interaction or become activated after co-

stimulation and initiate an immune response, seeing the alloantigen as foreign, and eliminate or deactivate cells expressing this "foreign" antigen. Thus, any remaining host T-cells will recognize the donor HSCs as foreign, and, conversely, donor T-cells will recognize recipient cells as foreign, resulting in graft versus host disease (GvHD) or graft versus malignancy (GVM) effect.

Graft Rejection and Graft Versus Host Disease

During T-cell maturation in the thymus, T-cells undergo positive selection, a process that promotes the survival of T-cells that bind to self-MHC with a low affinity and thus have an intrinsic weak reactivity (avidity) to self-MHC. Thus, these T-cells most likely will have a high binding affinity to an allogeneic MHC molecule regardless of the antigen peptide fragment specificity, resulting in activation of the T-cell. Furthermore, memory T-cells that are often present in an allogeneic HSC graft can cross-react with allogeneic host MHC, eliciting a rapid and robust adaptive immune response, much stronger than what naïve T-cells would elicit. If host T-cells are present when allogeneic HSCT takes place, these host T-cells will react to alloantigen peptide determinants bound to donor self-MHC just as they would do to microbial antigen-derived peptide determinants. This indirect presentation may result in $CD4^+$ T-cell activation because the donor T-cells are phagocytosed by host APCs and presented on the APC cell surface bound to host-derived MHC Class II molecules. Sometimes, antigens from the donor cells enter the MHC Class I pathway of antigen presentation, and the antigen peptide fragments end up being bound to MHC Class I molecules when displayed on APCs; they are then recognized by $CD8^+$ T-cells. Generally, it is thought that the effector functions of alloreactive T-cells cause acute graft rejection by two distinct mechanisms: (1) direct alloantigen recognition primarily mediated by $CD8^+$ cytotoxic T-cells kills the graft's HSCs directly, whereas (2) both $CD8^+$ cytotoxic and $CD4^+$ helper T-cells, generated by either the direct or indirect recognition of alloantigens, can cause damage to cells contained within the allogeneic graft that results in cytokine-induced inflammation.

Mixed lymphocyte reaction (MLR) is an assay that tests the reactivity of alloreactive T-cells from one individual against the MHC antigens on blood cells from another individual. This in vitro assay is performed by co-culturing the mononuclear lymphocytes (i.e., T-cells, B-cells, NK cells, mononuclear phagocytes, and dendritic cells) from one individual with the mononuclear leukocytes from another individual. If the two sets of mononuclear leukocytes express different MHC alleles, then a large number of the lymphocytes will proliferate in 4–7 days when they are mixed together; this setup is called a two-way MLR. In a one-way MLR, one of the two leukocyte populations is rendered incapable of proliferation by gamma irradiation or treatment with antimitotic drug co-culture with the other individual's leukocytes. This leukocyte population acts as the stimulators whereas the leukocytes from the other individual serve as the responders. With this setup, CD4+ versus CD8+ T-cell-mediated (i.e., Class II versus Class I) alloreactivity can be determined after the resultant effector cell function has been tested. In other words, CD8+ T-cells are mediating the alloreactive response if target cells are lysed, whereas the alloreactive response is CD4+ helper T-cell-mediated if the responder cells secrete significant amounts of cytokines in response to exposure to the inactivated stimulator leukocytes.

Antibodies can also mediate donor graft rejection. Most high-affinity alloantibodies are produced after $CD4^+$ helper T-cell activation of alloreactive B-cells. The antigens that are most commonly recognized by alloantibodies are donor MHC molecules (both Class I and Class II). This process is the same as that of naïve B-cell recognition of foreign antigens. Alloantibodies activate complement as well as neutrophils, macrophages, and NK cells by binding to the Fc receptor.

Regarding HSCT and allogeneic donor graft rejection, even an individual with minimal immunocompetency is able to reject donor stem cells

by mechanisms other than adaptive immune responses and appears to be NK cell-mediated. NK cells will react against cells that do not express MHC Class I, and HSCs do not express MHC Class I, thus leaving them vulnerable to NK cell-mediated elimination.

While donor HSCT rejection arises from the host immune responses to prevent the donor HSCs from engrafting, allogeneic T-cells contained within the allogeneic donor HSC graft product will react to host alloantigens that will result in GvHD if the host is immunocompromised and unable to mount an immune response to reject the donor cells. GvHD and graft rejection can arise even if the HSCT recipient and donor are "identically" matched because they are only "matched" at the MHC (HLA) loci. Thus, there are other polymorphic antigens that can mount an immune response in addition to MHC molecules. These include minor histocompatibility antigens. Minor histocompatibility antigen proteins are often processed and presented bound to self-MHC by APCs to T-cells just like any other protein antigen. Minor histocompatibility antigens often elicit a weaker or slower immune reaction. Minor histocompatibility antigens are thought to play a significant role in inducing graft rejection that elicits a weaker or slower rejection reaction. Minor histocompatibility antigens can also play a role in the development of GvHD, but, again, with lesser clinical consequences as compared to the response driven by MHC incompatibility. There are two classifications of GvHD, acute and chronic. Historically, the distinction between acute and chronic GvHD was based upon the histology and timing post-HSCT that the GvHD developed. However, GvHD is classified predominantly based upon histology and clinical manifestations with disregard to the timeline of its development. (Please see Chaps. 18 and 19 for in-depth discussions on acute and chronic GvHD, respectively.) It is thought that mature T-cells contained within the allogeneic donor HSC product initiates acute GvHD. Activated donor T-cells migrate to the skin, GI tract, and liver which express chemokines that attract T-cells to these tissues. Both $CD4^+$ and $CD8^+$ T-cells migrate to these areas and produce inflammatory cytokines (often referred to as a "cytokine storm") that result in further injury to these targeted tissues. However, the effector cells that result in host (recipient) epithelial tissue damage are not as well defined. NK cells have been found within dying epithelial cells as seen on biopsy and may play a role. In addition, $CD8^+$ cytotoxic T-cells and cytokines secreted by donor effector cells appear to be involved in the pathogenesis of acute GvHD.

The pathogenesis of chronic GvHD is less understood, but it is not just a continuation of acute GvHD. While donor T-cells are implicated in the pathogenesis of chronic GvHD with an infiltration of mononuclear cells causing an intensely inflammatory environment, no one single animal model has been able to account for all of the clinical features observed in humans with chronic GvHD. However, the resultant fibrosis associated with chronic GvHD suggests chronic antigen stimulation. Thymic damage from total body irradiation used as part of the conditioning regimen, acute GvHD, or immunosuppressive medications may lead to a lack of negative selection of donor-derived immature T-cells to occur [73]. The failure of negative selection to take place allows for alloreactive donor T-cells to mature and recognize host alloantigens. Another potential contributor to chronic GvHD is an inadequate production of donor-derived regulatory T-cells which can lead to autoimmune disease [74, 75]. Many of the clinical manifestations of chronic GvHD are reminiscent of autoimmune-like disorders.

Both acute and chronic GvHD are treated with intense immunosuppression, but patients may not respond as expected because these treatment strategies only target certain aspects of donor effector mechanisms. Therapeutic approaches for the treatment of acute and chronic GvHD are addressed in Chaps. 18 and 19, respectively.

Tolerance and Immune Reconstitution

Tolerance: Immune tolerance is classified as central or peripheral tolerance. Central tolerance occurs in generative lymphoid organs when immature lymphocytes are exposed to self-antigens presented in these organs (bone marrow for immature B-cells and thymus for immature T-cells). Immature B-cells that bind to self-antigens with high avidity can undergo a process called receptor editing that results in the acquisition of a new specificity recognized by the B-cell receptor which is not self-reactive. If this receptor editing fails, then the immature B-cell expressing a B-cell receptor that binds to self-antigens with high avidity often undergoes apoptosis in the bone marrow or spleen by a process termed negative selection [76]. In comparison, in the thymus, immature T-cells that bind to self-antigens with high affinity can be eliminated with high efficiency by negative selection in order to avoid alloreactivity. Alternatively, these immature T-cells that bind to self-antigens can develop into regulatory T-cells that contribute to peripheral tolerance.

In contrast, peripheral tolerance is induced in mature T-cells [77]. In peripheral tolerance, anergy occurs in $CD4^{+}$ T-cells that encounter their specific antigen, but the T-cells are not activated because they did not have adequate co-stimulation or they engaged inhibitory receptors (e.g., CTLA-4 or PD-1) which bind with a higher affinity. Often these anergic cells undergo apoptosis. Similarly, anergy and subsequent apoptosis occurs to B-cells that recognize self-antigens when located in the periphery.

Immune reconstitution is addressed in Chap. 26.

Immunodeficiency

Conditioning regimens that HSCT recipients need to undergo prior to the infusion of their allogeneic donor HSC graft adversely affects the recipients' lymphocyte repertoire as well as eliminates memory cells and plasma cells. It often takes a long time for these cells to regenerate, and HSCT recipients will need to undergo revaccination (see Chap. 26, "Immune Reconstitution After Hematopoietic Stem Cell Transplantation"). During this period of immune regeneration, post-HSCT patients are very vulnerable to infection, particularly viruses (see Chap. 17, "Infectious Complications and HSCT"). They are also susceptible to developing EBV-associated PTLD and B-cell lymphomas (see Chap. 17, "Infectious Complications and HSCT"). Passive immunity with IVIG and the use of prophylactic antibiotics and antiviral and antifungal agents can help prevent these life-threatening, opportunistic infections while awaiting immune reconstitution.

Key Points

- The immune system provides a vital network of defense mechanisms against pathogens and foreign substances that employ a combination of cellular and noncellular components that are highly integrated and regulated.
- Cluster of differentiation (CD) nomenclature provides a uniform naming system for cell surface markers that was originally devised to characterize cells of the immune system, but now this nomenclature is applied to all cell types.
- Hematopoiesis is the process by which all mature blood cells are produced. Hematopoiesis is a highly regulated process that begins as early as day 18 of gestation in humans in the yolk sac. By birth, hematopoiesis takes place in the bone marrow in humans.
- The hematopoietic stem cell (HSC) is a pluripotent stem cell that gives rise to all mature blood cells through a process called multilineage differentiation. HSCs have two main properties of reconstitution and self-renewal. The majority of HSCs are quiescent and reside in designated, hospitable regions in the bone marrow called HSC niches. These niches have a number of cell types and molecules

(cytokines, chemokines, and growth factors) that function in concert to maintain HSCs and the HSC niche.

- The most highly active sites poised to provide an immune response are those that are most likely to be portals of infection or foreign substances; these include the skin, the respiratory tract, and the GI tract.
- Organs of the immune system are functionally and anatomically organized to provide rapid, directed responses in defense of pathogens and foreign antigens. Generative or primary lymphoid organs are the bone marrow and thymus because they are the predominant sites of immune cell development. Secondary lymphoid organs include the spleen, lymph nodes, and lymphoid mucosal tissue.
- The key effector cell types that provide immunity are phagocytes, antigen-presenting cells, and lymphocytes, all of which have distinctive roles in providing innate and/or adaptive immune responses.
- In innate immunity, neutrophils, monocytes, and natural killer (NK) cells are the primary cells involved in the delivery of innate immune responses. Neutrophils are the most abundant immune cell type in the circulation, and they are recruited to sites of infection and tissue injury where neutrophils eliminate pathogens and damaged tissues by releasing their contents of their lysosomal granules or by phagocytosing them.
- Adaptive immunity cell types are B- and T-cells. The cardinal features of adaptive immunity are specificity, diversity, memory, clonal expansion, specialization, contraction, and nonreactivity to self.
- Both B- and T-cells have highly diverse repertoires and specific antigen receptors to provide adaptive immune responses which increase in effectiveness with each subsequent encounter with the specific antigen the lymphocyte is programmed to recognize.
- Adaptive immune responses are classified into two types: humoral and cell-mediated. Humoral immunity is mediated by antibodies secreted by activated B-cells whereas cell-mediated immunity is mediated by activated T-cells that act to directly or indirectly kill infected or tumor cells.
- The function of antigen-presenting cells (APCs) is to capture, process, and present antigen peptide fragments (also referred to as determinants) that are bound to MHC molecules for recognition and activation of lymphocytes to elicit a cell-mediated immune response.
- Alloreactivity is the state in which T-cells or antibodies recognize and react to an antigen (alloantigen) expressed on cells or tissues from another individual that can result in allorejection of the cells/tissues expressing the alloantigens. In HSCT, recipient alloreactive immune cells must be suppressed or eliminated; otherwise the donor HSC graft will be rejected, and graft failure will result. Conversely, suppression of donor alloreactive T-cells is necessary to prevent graft versus host disease from occurring.

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Part II

Topics Related to the Pre- HSCT Period

4 Pretransplantation: Indications and Timing

Alicia McFarren and Michael A. Pulsipher

Abstract

Hematopoietic stem cell transplantation (HSCT) is now indicated in over 100 disease types and subtypes. While autologous stem cell transplantation is mainly reserved for high-risk malignancy, allogeneic stem cell transplantation can be used in both malignant and nonmalignant disorders. The most common types of malignant disorders that undergo treatment with transplantation include leukemia, lymphoma, brain tumors, and neuroblastoma. The most common types of nonmalignant diseases that are treated with transplantation include severe aplastic anemia, inherited bone marrow failure syndromes, immunodeficiencies, hemoglobinopathies, and inherited metabolic disorders. For nearly all indications, chemotherapy is given prior to the transplant either as primary treatment for the disease and/or to allow space for the new stem cells through myeloablation. Toxicity from both the chemotherapy as well as infectious risks should be balanced with the existing clinical status of the patient prior to moving ahead with any transplant in order to choose the best approach. Because the number of pediatric patients undergoing transplantation is relatively small, it is strongly recommended that when available, patients be treated in the context of a clinical trial. Recommendations on whom and when to transplant are frequently being updated, so it is important that transplant physicians and referring physicians carefully review therapeutic options for each patient.

Introduction

Hematopoietic stem cell transplantation (HSCT) was first investigated in humans during the 1950s [1]. Its primary indications at the time were for cancer, aplastic anemia, and immunodeficiency [2]. Prior to the identification of human leukocyte antigen (HLA) typing, successful early trials used identical twin donors. Since the discovery of

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and later increased accuracy of molecular HLA typing, the ability to treat patients with transplant using either related or unrelated donors has rapidly expanded. In this chapter, we will address the disease indications for HSCT in children. We will discuss the indications of both autologous and allogeneic HSCT for malignancy as well as allogeneic transplantation for nonmalignant disorders.

Malignant Disorders

Acute Lymphoblastic Leukemia

Indications for HSCT of ALL in First Complete Remission (CR1)

Acute Lymphoblastic Leukemia (ALL) is the most common malignancy of childhood affecting approximately 2700 children in the United States per year. Most of these children will be cured with conventional chemotherapy. However, a small percentage will need an allogeneic HSCT (allo-HSCT) in order to obtain a sustained remission and/or cure. Once a child is diagnosed with either a B- or T-cell lymphoblastic leukemia, the first step is to obtain a remission. Those patients that do not obtain a morphological remission after induction therapy have traditionally been referred for allo-HSCT. Another major risk factor for relapse has been patients with >0.01% disease detected by flow cytometry after completion of consolidation therapy. The term minimal residual disease (MRD) has been utilized for this subset of patients [3–5]. European groups have routinely transplanted these patients, and many North American centers now consider this an indication once MRD negative or low status has been obtained with subsequent therapy. Additional information on this topic can be found in Chap. 5.

There are two other subsets of patients at high enough risk for relapse to consider HSCT in CR1. The first are those patients with hypodiploid features defined as <44 chromosomes on initial cytogenetics [6]. The second group are those with infant ALL with the following specific features: age <6

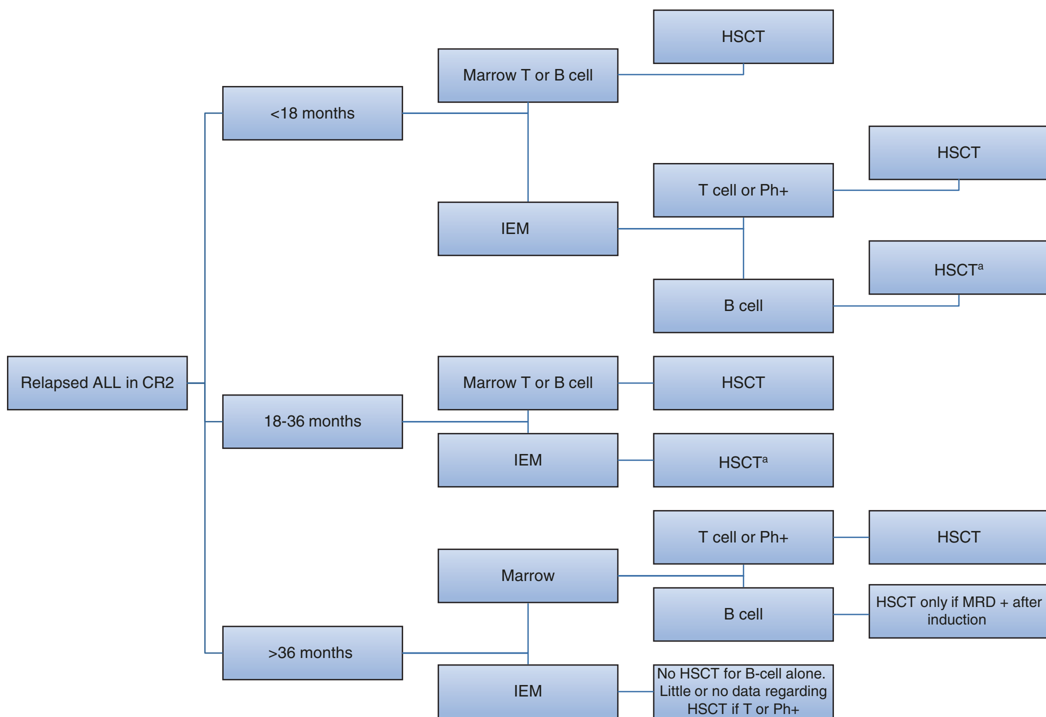

Fig. 4.1 Indications for transplant for ALL in CR2. IEM-isolated extramedullary relapse. These recommendations are based on outcomes of CNS IEM data. Little data exists for isolated testicular relapse. [a]HSCT is controversial. If evidence of MRD in BM of IEM patients, treat as a BM relapse

months at diagnosis, +MLL rearrangement on cytogenetics, and an initial white blood cell (WBC) count of >300,000 [7–9]. A number of genetic mutations have been assessed to choose candidates for HSCT in CR1 (iamp21, Ph+-like, Ph+); however; in general, with the use of intensive contemporary therapy, only patients in these categories with persistent MRD are at sufficient risk to merit a transplant approach as primary therapy. The remainder of the indications for transplantation of ALL is reserved for those with relapse.

Indications for Transplant of ALL in CR2: (See Fig. 4.1 for Algorithm)

For B-ALL, patients who relapse in the bone marrow (BM) within 18 months of diagnosis are at the highest risk for failure with subsequent therapy, followed by those with bone marrow involvement either in the first 36 months from diagnosis or within 6 months of completing maintenance therapy [3, 4, 10]. In addition to early B-ALL relapses, patients with relapse of T-cell leukemia or those who are Philadelphia chromosome positive (Ph+) should be referred to transplantation if they have marrow relapse at any time [3, 11–13]. Finally, late BM relapsing patients with B-ALL who fail to achieve MRD <0.1% after re-induction should be considered for allo-HSCT [14].

Transplantation for isolated extramedullary relapse (IEM) of ALL remains an ongoing topic of study. The majority of data focuses on isolated central nervous system (CNS) relapse with very limited data regarding the use of HSCT for isolated testicular disease or other locations of relapse. It is also known that minimal residual disease can often still be detected in the marrow when there is overt relapse in the CNS, and those with MRD levels >0.01% have a worse prognosis than those with no detectable MRD [15]. IEM relapse within 36 months from diagnosis of T-cell or Ph+ ALL is considered by most to be an indication for allo-HSCT. If the relapse occurs after 36 months in these subgroups, there are little data on outcomes of HSCT in these patients.

In standard-risk B-cell ALL, isolated CNS relapse at <18 months from diagnosis is considered to be a very early, high-risk relapse and thus an indication for allo-HSCT by many groups [16]. Transplantation for those with isolated CNS relapse of B-cell ALL between 18 and 36 months remains controversial. Isolated CNS relapse in B-cell ALL after 36 months can be cured with conventional chemotherapy and craniospinal radiation approximately 60% of the time, a rate similar to those who underwent matched sibling donor transplant as an alternative therapy [17].

Indications for Transplantation of ALL in CR3 and Beyond

Patients who have relapsed more than once should always be considered for HSCT. At this point it is safe to say that their disease is resistant to conventional chemotherapy and utilizing a graft-versus-leukemia (GVL) effect could be beneficial. An alternative to HSCT at this point would also be the experimental use of cellular or immunotherapy.

Relapse of ALL After HSCT

A second allo-HSCT for relapse after an initial allo-HSCT can be beneficial if the patient can achieve a complete remission prior to second transplant and enter it with adequate organ function and performance status (Lansky/Karnofsky ≥ 50). Outcomes depend on a number of risk factors including time to relapse and ability to achieve disease remission. Overall survival (OS) of these patients is in the range of 10–40% [18–21].

Acute Myeloid Leukemia

Allogeneic HSCT for acute myeloid leukemia (AML) is recommended for any relapsed disease including IEM relapse. In addition, primary induction failure, as defined by >5% blasts after one to two cycles of induction chemotherapy, should also be referred for allo-HSCT [22]. Children with relapsed or refractory AML have minimal chance of cure with chemotherapy alone [23, 24]. HSCT allows treatment intensification for such high-risk disease. Disease status at time of HSCT is a predictor of disease relapse and OS with 5-year OS of 47, 28, and 17% for patients who underwent HSCT in second CR, relapse, and primary induction failure [25]. Those patients who develop AML secondary to previous chemotherapy or radiation, as defined as treatment-related AML (t-AML), should also be referred for HSCT because this sub-

type of AML has been historically resistant to treatment with conventional chemotherapy alone [26]. With the advent of cytogenetic testing, specific markers have been associated with both high- and low-risk diseases. High risk is currently defined by evidence of monosomy 7, monosomy 5 (-7/7q- or -5/5q-), or FLT3-ITD mutations with an allelic ration >0.4 [27, 28]. If these are present on initial diagnosis, the patient should be referred to allo-HSCT in first remission regardless of the availability of a related donor. Those patients determined to be low risk by cytogenetics are those with core-binding factor (CBF) mutations, such as inv(16) or t(8;21), as well as those with mutations of NPM1 and CEBPA [29–31]. These patients only need to be referred for allo-HSCT in the case of relapsed or refractory disease.

The majority of newly diagnosed AML will not fall into the low or high-risk categories. Historically, patients who have fully matched related donors were referred to undergo HSCT. There are ongoing investigations looking to stratify these patients further based on MRD results after induction. At this time, results of these trials are not available, so the choice of referring standard-risk AML patients for transplant in CR1 with matched sibling donors is at the discretion of the transplant center.

Acute promyelocytic leukemia (APML) deserves special mention. Since the discovery of the PML-RARα translocation and its responsiveness to all-trans retinoic acid (ATRA), disease-free survival of newly diagnosed patients exceeds 75% [32]. Although the cure rate is high, there is still a percentage of patients who will relapse. Some of these patients may be salvaged with arsenic-based regimens, but HSCT is still a viable approach that may be considered. APML is unique in the fact that comparison trials of both autologous versus allogeneic HSCT have yielded similar overall survival (OS), whereas allo-HSCT has clearly shown to improve OS for all other subtypes of AML. A retrospective analysis of 32 APML patients receiving either autologous (n = 11) versus allogeneic (n = 21) HSCT showed similar OS; however, the autologous group had more relapse whereas the allogeneic group suffered from greater transplant-related mortality (TRM) [33, 34]. If an autologous HSCT is to be considered for APML, the patient should have a PML-RARα translocation, PCR-negative remission, and a PCR-negative stem cell product.

Myelodysplastic Syndrome

Much of the data in regards to risk stratification and treatment of myelodysplastic syndrome (MDS) pertains to adults [35]. MDS in the pediatric population should always be evaluated for allo-HSCT [36, 37]. However, determining the timing for HSCT is a more complicated topic. Patients without excess blasts and mild cytopenias can either be closely observed or move directly to transplant, as early treatment is associated with better outcomes. If a watchful strategy is employed, then a patient should move quickly to HSCT when they become persistently neutropenic and transfusion dependent or have increasing blasts [38]. Emerging data shows that patients with a marrow blast count of 5–20% do worse than with <5% blasts [39]. Because MDS in the pediatric population is rare and may be associated with genetic diseases, evaluation for genetic disorders associated with MDS such as Fanconi anemia (FA), GATA-2 mutations, dyskeratosis congenita (DC), Diamond-Blackfan anemia (DBA), Shwachman-Diamond syndrome (SDS), and severe congenital neutropenia (SCN) should be considered [40].

The European Working Group of MDS in Childhood (EWOG-MDS) 98 study reported the outcome of children with advanced MDS (n = 97) who received HSCT. Donor sources were matched sibling donor (MSD) (n = 39), matched unrelated donor (MUD) (n = 57), or alternate family donor (n = 1) with bone marrow (n = 69) or peripheral blood (n = 28) grafts. Median age at the time of HSCT was 11.1 years (range 1.4–19.0) with a median follow-up of 3.9 years (range 0.1–10.9). The 5-year probability of overall survival was 63%, while the 5-year cumulative incidence of transplantation-related mortality (TRM) and relapse was 21% each. Age at HSCT greater than 12 years, interval between diagnosis and HSCT longer than 4 months, and occurrence of acute or extensive chronic graft-versus-host disease were associated with increased TRM. The risk of relapse increased with more advanced disease. This study indicates that HSCT following a myeloablative preparative

regimen offers a high probability of survival for children with advanced MDS [41].

Juvenile Myelomonocytic Leukemia

The diagnosis of juvenile myelomonocytic leukemia (JMML) is a sufficient criterion for most oncologists to recommend allo-HSCT. It has been shown that chemotherapy alone is not adequate to eradicate the disease. A cure relies heavily on the newly transplanted immune system attacking residual leukemia cells, a concept referred to as graft-versus-leukemia (GVL) or graft-versus-malignancy (GVM). Locatelli et al. described 100 children transplanted for JMML with 5-year event-free survival of approximately 50% [42]. Spontaneous remission in the context of Noonan's syndrome with germline mutations of *PTPN-11* is expected, so these patients are usually not referred for HSCT. Future research into germline mutations of *N-RAS, K-RAS*, and *CBL* is warranted due to documented case reports of spontaneous remission in some of these children [43–45].

Chronic Myelogenous Leukemia

Chronic myelogenous leukemia (CML), characterized by a translocation between the BCR and ABL genes t(9;22), has historically been an indication for HSCT in adults. However, with the approval of imatinib mesylate and other tyrosine kinase inhibitors (TKIs), many adult patients can continue taking these medications indefinitely, treating CML as a chronic, indolent disease. Although CML is rare in the pediatric population, children have been shown to also respond well to TKIs [46]. There is little research on the long-term outcomes of chronic TKI use, particularly in patients who are still growing and developing. The timing of allo-HSCT in children with CML is controversial. It is difficult to successfully treat CML once it progresses to accelerated or blast phase, so patients must be followed very closely [47].

Offering CML patients allo-HSCT at diagnosis with well-matched unrelated or sibling donors is a reasonable option [48], although most pediatric centers wait for signs of progression or TKI resistance prior to pursuing HSCT.

A recent report from CIBMTR included CML patients (n = 499) with early disease receiving myeloablative HSCT which included analysis of the effect of pre-HSCT TKI in pediatric patients (age <18 years, n = 177) and young adults (age 18–29 years, n = 272) with the goal of identifying prognostic factors. In this study, post-HSCT probability rates of 5-year overall survival (OS) and leukemia-free survival (LFS) were 75% and 59%, respectively. Rates of OS and LFS were 76% and 57% in <18-year group and 74% and 60% in 18- to 29-year group, respectively. Five-year rates of OS for MSD and bone marrow stem cell source were 83% and 80%, respectively. In multivariate analysis, there was no effect of age (<18 versus 18–29) or pre-HSCT TKI therapy on OS, LFS, transplant-related mortality, or relapse. Favorable factors for OS were MSD (P < 0.001) and recent HSCT (2003–2010; P = 0.04). LFS was superior with MSD (P < 0.001), BM as graft source (P = 0.001), and performance scores >90 (P = 0.03) compared with unrelated or mismatched peripheral blood stem cells donors and recipients with lower performance scores. Older age was associated with increased incidence of chronic graft-versus-host disease (P = 0.0002). In the current era, HSCT outcomes are similar in young patients and children with early CML, and best outcomes are achieved with BM grafts and MSD. Although the curative effect of HSCT in CML traditionally relies on the GVL effect, in this CIBMTR report consistent with other reports, donor source/matching did not play a role in decreasing relapse or LFS [48].

Autologous Stem Cell Transplant

Autologous stem cell transplant (ASCT) is a treatment option for those malignancies requiring extremely high doses of chemotherapy with the major side effect of myeloablation. Stem cells are usually collected after one or two cycles of initial chemotherapy and frozen in liquid nitrogen until high-dose chemotherapy (HDCT) with ASCT is indicated. There is clear indication for this type of treatment in some childhood malignancies, such as relapsed lymphoma,

high-risk neuroblastoma, and a number of brain tumors. There have been some studies attempting to define possible benefits with this approach for other malignancies such as sarcomas, retinoblastoma, and Wilms tumor. We will discuss each of these in the categories below.

Lymphomas

Hodgkin Lymphoma

Hodgkin lymphoma (HL) is a type of lymphoma that affects both children and adults. In the pediatric population, it is most commonly found in adolescents. Current cure rates approach 90% with risk-based treatment options including chemotherapy and/or radiation therapy. For the minority of patients that suffer from relapse or refractory disease, there are a number of treatment options. High-dose chemotherapy with autologous stem cell transplant (HDCT/ASCT) is recommended for those patients with relapsed/refractory disease who are deemed to be high risk (relapse <12 months, extra-nodal disease, presence of B symptoms) [49–53]. The most favorable outcomes following HDCT/ASCT can be predicted based on response to previous salvage chemotherapy. Metzger et.al reported a 5-year OS of 97.2% following HDCT/ASCT for those patients who responded to salvage therapy versus only 17.9% for patients who had active disease at time of ASCT [54]. Another study by Moskowitz et al. found similar results despite giving intensified chemotherapy as salvage to those with poor initial response [55]. There are a number of novel therapies being tried for relapsed and refractory Hodgkin lymphoma that may change the way we treat a subset of these patients. Brentuximab vedotin (BV) is being used in many clinical trials for relapsed/refractory Hodgkin lymphoma. BV is an anti-CD30 antibody conjugated by a protease-cleavable linker to the microtubule-disrupting agent MMAE (monomethyl auristatin E). Targeted delivery of MMAE to CD30-expressing tumor cells is the primary mechanism of action. Additional mechanisms of tumor cell killing include antibody-dependent cellular phagocytosis, immunogenic cell death, and the bystander effect. Another type of novel antibody therapy is PD-1 and PD-L1 inhibitors. By blocking signaling of PD-1 or PD-L1, the immune system is essentially reactivated to fight the cancer. Both anti-CD30 antibodies and PD-1/PD-L1 inhibitors are being tried as either single agent or in conjunction with chemotherapy for relapsed and refractory Hodgkin's [56, 57]. If relapse occurs following HDCT/ASCT, patients should be considered for a clinical trial with novel agents. There are some data that an allogeneic stem cell transplant (allo-HSCT) with reduced intensity conditioning can be beneficial due to the graft-versus-lymphoma effect and is an acceptable approach for those with relapsed/refractory disease after an autologous transplant [58, 59].

Non-Hodgkin Lymphoma

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of malignancies found commonly in children, adolescents, and adults. The 5-year overall survival (OS) for NHL in children and adolescents is >80% [60]. The major subtypes found in pediatrics include:

- Mature B-cell lymphoma
  - Burkitt and Burkitt-like lymphoma/leukemia
  - Diffuse large B-cell lymphoma (DLBCL)
  - Primary mediastinal B-cell lymphoma
- Lymphoblastic lymphoma (LBL)
- Anaplastic large cell lymphoma (ALCL)

Treatment for Burkitt lymphoma and DLBCL is the same with risk-stratified upfront chemotherapy. HSCT is an option for those patients with relapsed/refractory disease [61, 62]. For those unable to achieve remission prior to transplant, results are dismal. Whether to treat with an autologous versus allogeneic transplant remains to be elucidated. There is some theoretical benefit to allo-HSCT due to a GVL effect, however, whether that benefit surpasses the transplant-related mortality is unclear. In some trials, patients have been treated with an ASCT followed by a reduced intensity allo-HSCT [63]. Novel therapies are needed in this subgroup of patients. With the advent of antibody and cellular therapy, these recommendations may change in the near future [64]. Primary mediastinal B-cell lymphoma in children is quite rare.

Current upfront therapy with dose-adjusted etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and rituximab (DA-EPOCH-R) has yielded a 5-year EFS >90%. Cases of relapsed/refractory disease should be treated in the context of a clinical trial when available [65].

Although there is no consensus on the standard of care for relapsed/refractory lymphoblastic lymphoma, Gross et al. showed a 5-year EFS of 40% following an allo-HSCT compared to only 4% following an auto-HSCT, and most HSCT physicians recommend allogeneic HSCT for this disease [61]. Similar to the other relapsed/refractory NHL, there is no consensus on optimal treatment for relapsed ALCL. Options vary from single-drug vinblastine to triple therapy with ifosfamide, carboplatin, and etoposide (ICE) to allogeneic HSCT [61, 66–68]. Novel therapies with crizotinib (an ALK inhibitor) or brentuximab vedotin (anti-CD30 mAb) are being investigated in the context of clinical trials [69, 70].

Neuroblastoma

Patients diagnosed with standard and intermediate risk neuroblastoma (NB) do well, with OS approaching 90% with chemotherapy and/or surgery. However, those classified as high-risk disease historically have had poor survival, with some improvement in outcomes noted with the addition of high-dose chemotherapy (HDCT) followed by ASCT [71–74] and immunotherapy. The Children's Oncology Group (COG) recently tested a single vs. two transplants in tandem followed by immune therapy in high-risk patients and noted improvement in survival with two transplants [75]. In that study, high risk was defined by:

- Stages II–IV with MYCN amplification
- Stage III if >18 months with unfavorable pathology, regardless of MYCN status
- Stage IV if >18 months or >12 months if additional unfavorable features, regardless of MNYC

Ongoing studies adding iodine-131 metaiodobenzylguanidine (^{131}I-MIBG) to consolidation therapy as part of the preparative regimen for ASCT are being performed (see Chap. 6 for more details). For further definitions of disease stage, refer to the International Neuroblastoma Staging System (INSS) as well as the International Neuroblastoma Risk Group Staging System (INRG) [76, 77].

Brain Tumors

Brain tumors are the second most common malignancy of childhood and the most common solid tumor in pediatrics. Standard treatment for all patients begins with a gross-total resection (GTC) when feasible. Historically, in cases of high-grade tumors or residual disease, radiation therapy has been given. The blood-brain barrier makes it difficult to achieve high levels of chemotherapy in the brain. Unfortunately, irradiating the brains of young children causes numerous long-term side effects specifically with neurocognitive and endocrine impairments [78, 79]. It is unclear at what age craniospinal radiation becomes "safe." Many studies over the last 25 years have attempted to minimize or forgo radiation therapy in favor of HDCT/ASCT in order to achieve sufficient doses of chemotherapy in the brain. Initially, this therapy was reserved for relapsed or refractory disease; however, the favorable results have led to HDCT/ASCT as upfront therapies for some tumor types.

Embryonal Brain Tumors

Embryonal brain tumors include medulloblastoma, atypical teratoid/rhabdoid tumor (AT/RT), and a category previously known as primitive neuroectodermal tumors (PNET). This subgroup of tumors is the most common solid tumor in children less than 4 years of age and has been an important subgroup to study the benefits of HDCT/ASCT as treatment in place of, or in conjunction with, radiation therapy (RT). A series of protocols titled "Head Start" have been conducted on these patients, with Head Start I commencing in 1991 to the most recent, Head Start 4, commencing in 2016. Outcomes of Head Start I and II on the treatment of non-metastatic medulloblastoma in children <3 years showed promising 5-year OS (70%) and EFS (50%) without the use of radiation [80]. Since these results are comparable to

the use of radiation in the older subset of patients and believed to yield fewer long-term effects, the use of HDCT/ASCT as upfront therapy for this subgroup has become an accepted approach. Optimal treatment for recurrent medulloblastoma has mainly consisted of additional radiation. Some studies have added HDCT/ASCT to RT as salvage, but it is unclear if it has offered additional benefit [81, 82].

AT/RT has proven exceedingly difficult to treat particularly in the youngest age group (<3 years) with OS typically at <30%. The use of RT and/or HDCT/ASCT is still being evaluated. A review of 31 patients treated at St. Judes Research Hospital between 1984 and 2003 showed that the 2-year EFS and OS of children aged 3 years or older was significantly better as compared to the results of children under the age of 3 years (EFS, 78% + 14% versus 11% ± 6%, $P = 0.009$; OS, 89% ± 11% versus OS, 17% ± 8%), $P = 0.0001$, respectively). The majority of patients aged 3 years or older received postoperative craniospinal radiation [83]. In comparison a study from Canada did not confer a benefit to upfront RT but did find benefit to HDCT/ASCT, as HDCT led to a 2-year OS of 47.9% ± 12.1% versus 27.3% ± 9.5% for the conventional chemotherapy group ($P = 0.036$). Many of these patients received triple tandem transplants with carboplatin and thiotepa [84]. Results of the treatment of patients with AT/RT on Head Start I–III have been dismal [85, 86].

Primitive neuroectodermal tumors (PNET) have undergone revised nomenclature by the World Health Organization in 2016. Many of these tumors display amplification of the C19MC region on chromosome 19 (19q13.42). C19MC-amplified tumors include the lesions previously known as ETANTR (embryonal tumors with abundant neuropil and true rosettes, but also referred to as embryonal tumors with multilayered rosettes), ependymoblastoma, and, in some cases, medulloepithelioma [87]. In the interest of referencing previous studies on the role of ASCT, we will continue to refer to this tumor subtype as PNET.

Supratentorial PNET (sPNET) is often treated on protocols with medulloblastoma but consistently yields worse outcomes. Head Start I and Head Start II showed that the 5-year EFS and OS were 39% (95%CI: 24%, 53%) and 49% (95%CI: 33%, 62%), respectively, with improved outcomes in patients with non-pineal sPNETs [88]. At this time, HDCT/ASCT can be considered as a treatment option for young patients with relapsed non-pineal sPNET; however, due to the rarity of these tumors and <50% EFS, these patients should be treated in the context of a clinical trial when available.

Ependymoma

The mainstay of treatment for ependymoma is surgical resection followed by RT. Because it is often an isolated tumor location, focal RT versus craniospinal RT can be used, limiting many of the long-term toxicities. Koshy et al. even found a significantly improved OS survival in children <3 years who received postoperative RT, compared to those who did not (81% vs. 56%, respectively, $P = 0.005$) [89]. Multiple attempts have been made using HDCT/ASCT for both initial and recurrent disease, but none have demonstrated improved survival [90–92].

Germ Cell Tumors

Malignant germ cell tumors (GCT) are made up of two subgroups, pure germinomas or nongerminomatous germ cell tumors (NGGCT). Pure germ cell tumors have a more favorable prognosis and respond well to RT. NGGCT, on the other hand, require the combination of surgery, RT, and chemotherapy for treatment. Upfront HDCT/ASCT for patients with poor prognosis (i.e., metastatic germ cell tumors) did not show an improved OS as compared to standard treatment [93]. However, a number of studies have shown improvement in EFS and OS using HDCT/ASCT as salvage therapy in relapsed disease [94, 95].

Malignant gliomas: Patients with high-grade, malignant gliomas have a dismal prognoses. Since the majority of these tumors are found in adult patients, the standard treatment has been gross-total resection followed by RT. The Head Start trials evaluated RT-sparing treatment for anaplastic astrocytomas and glioblastoma multiforme in those <6 years of age. In the most recent published data of this patient population, they found a 5-year EFS and OS for all patients on the trial of 25% ± 8% and 36% ± 9%, respectively. When broken down by age group, the younger population (<36 months) fared much better than

those >72 months of age (OS 63% ± 17% vs. 13% ± 12%, respectively) [96]. Diffuse intrinsic pontine glioma (DIPG) has shown even worse OS, approaching 0%, with any current therapy. Attempts at using HDCT/ASCT did not show any improvement and is currently not recommended for this type of tumor [97, 98].

Additional solid tumors that could be considered for ASCT include sarcomas, kidney tumors (rhabdoid and Wilms), retinoblastoma, and desmoplastic small round cell tumor (DSRCT) that are refractory to or have relapsed after being treated with conventional chemotherapeutic approaches [99–104]. A summary of transplantable malignant diseases can be found in Table 4.1.

Table 4.1 Indications for transplant of malignant disease

Malignant diseases treated with allogeneic HSCT
• Acute lymphoblastic leukemia—relapsed/refractory
• Acute myelogenous leukemia—relapsed/refractory
• Myelodysplastic syndrome
• Juvenile myelomonocytic leukemia
• Chronic myelogenous leukemia[a]
• Lymphoblastic lymphoma—relapsed/refractory
Malignant diseases treated with autologous HSCT
• Hodgkin's lymphoma—relapsed/refractory
• High-risk neuroblastoma
• Acute promyelocytic leukemia—relapsed
• Brain tumors
– Medulloblastoma <3 years of age
– Atypical teratoid/rhabdoid tumor
– ETANTR—non-pineal[b]
– Non-germinomatous germ cell tumors—relapsed
– Malignant gliomas <6 years of age
• Sarcomas
– Ewing's sarcoma—relapsed/refractory[c]
• Kidney tumors
– Wilms—relapsed/refractory[c]
– Rhabdoid—relapsed/refractory[c]
• Retinoblastoma—relapsed/refractory[c]
• Desmoplastic small round cell tumor—relapsed/refractory[c]

[a]See full text section as it relates to a subset of patients with CML

[b]ETANTR—embryonal tumors with abundant neuropil and true rosettes. Formerly categorized as primitive neuroectodermal tumors (PNET)

[c]True benefit still being investigated and should only be done in the context of a clinical trial

Nonmalignant Disorders

There are numerous nonmalignant diseases that can be cured with an allogeneic HSCT. The majority of these diseases affect one or all of the cell lines derived from the bone marrow. Subsets of these diseases fall into the categories of bone marrow failure, immunodeficiency, and hemoglobinopathy. In addition, some inherited metabolic disorders (IMD) can be treated with allo-HSCT. Timing of transplant for each of these diseases will vary, but the process of the allo-HSCT is similar to that used when treating malignant disease with a few exceptions. The first exception is that there is no benefit to any chemotherapy administered in the preparative regimen on the disease, aside from immunosuppression to decrease rejection and allowing "space" for the new, transplanted cells to grow through myeloablation. In addition there is no benefit to graft-versus-host disease (GVHD), as there is in decreasing relapse for malignant disorders. GVHD can actually be extremely toxic and negate the benefit of HSCT for patients with nonmalignant disorders. Therefore, in some cases, only a well-matched donor would be appropriate for HSCT. In others, where quick HSCT is necessary, significant efforts at GVHD prevention should be employed. The remainder of this chapter will outline each disease category and define which patients would be acceptable candidates for allo-HSCT.

Severe Aplastic Anemia

Acquired severe aplastic anemia (SAA) is most often due to autoimmune destruction of marrow elements, and it is defined by deficient or nonexistent hematopoiesis occurring when all other bone marrow failure syndromes have been ruled out. Current standard of care is to move directly to allo-HSCT if there is a matched sibling donor available. However, if one is not available, then a trial with immunosuppressive therapy (IST) consisting of horse anti-thymocyte globulin (hATG) and cyclosporine for 6 months is the standard of care. If a remission is not reached at

the end of 4–6 months, and there is a matched unrelated donor (MUD) available, then a patient should move forward with allo-HSCT [105]. There is emerging evidence to suggest that delaying HSCT in cases with a suitable MUD will have worse outcomes than if these patients moved directly to allo-HSCT without a trial of IST first. Dufour et al. retrospectively described 29 pediatric patients who underwent MUD HSCT without prior IST and compared them to historical controls of MSD HSCT, IST alone, and IST followed by MUD transplant. They found improved OS of those receiving a MUD HSCT as upfront therapy compared to IST followed by MUD HSCT. OS was equal among the other groups [106]. Studies comparing this in a prospective fashion are ongoing [107].

Inherited Bone Marrow Failure Syndromes

The classification of inherited bone marrow failure syndromes (IBMFSs) generally refers to any congenital syndrome leading to inadequate production of one or more cell lines produced by the bone marrow to include white blood cells, red blood cells, or platelets. For many of these syndromes, there is now genetic testing that can confirm a diagnosis; however, in some cases, the diagnosis remains purely clinical. Not all BMF syndromes require transplant upon diagnosis. Factors to consider include availability of a suitably matched donor, either related or unrelated, transfusion dependence, and/or frequency and severity of infections. In addition, a number of these syndromes have a predisposition for myeloid malignancies, which need to be screened for regularly. Table 4.2 outlines the most common bone marrow failure syndromes [108].

General rules of when to consider transplantation of patients with bone marrow failure syndromes include non-response to available therapies (i.e., steroids for Diamond-Blackfan anemia (DBA), G-CSF for severe congenital neutropenia (SCN), and eculizumab for paroxysmal nocturnal hemoglobinuria (PNH)), transfusion dependence, frequent infections, or progression to MDS/AML [109–111]. Both Fanconi anemia and dyskeratosis congenita patients are particularly sensitive to radiation therapy and alkylating agents, and many new reduced-intensity chemotherapy preparative regimens are being utilized in clinical trials [112].

Table 4.2 Indications for transplant of bone marrow failure syndromes

Syndrome	Lab testing/features	Cancer predisposition
Fanconi anemia	DEB or MMC chromosomal breakage, genetic testing available	MDS/AML, squamous cell carcinoma
Shwachman-Diamond syndrome (SDS)	Pancreatic function, genetic testing available	MDS/AML
Dyskeratosis congenita (DC)	Telomere length, genetic testing available	MDS/AML, squamous cell carcinoma [105]
Diamond-Blackfan anemia (DBA)	Marrow with pure red cell aplasia, eADA activity, high MCV, genetic testing available	MDS/AML, carcinomas, sarcomas [106]
Severe congenital neutropenia (SCN)	Isolated neutropenia, genetic testing available	MDS/AML
Paroxysmal nocturnal hemoglobinuria (PNH)	Complement mediated hemolysis. Flow for CD55/59. Predisposition for thromboembolism	Unknown
Amegakaryocytic thrombocytopenia	Thrombocytopenia, genetic testing available	Unknown
Thrombocytopenia-absent radii (TAR) syndrome	Thrombocytopenia, absent radii, unknown genetic marker	Unknown

DEB diepoxybutane, *MMC* mitomycin C, *MDS* myelodysplastic syndrome, *AML* acute myelogenous leukemia, *eADA* erythrocyte adenosine deaminase activity, *MCV* mean corpuscular volume

Primary Immunodeficiency

Severe deficiencies of the immune system can lead to life-threatening infections and are a general indication for allo-HSCT [113]. Immune deficiencies can be subclassified into lymphoid versus myeloid diseases.

Lymphoid-related immunodeficiencies: The major form of lymphoid deficiency is severe combined immunodeficiency (SCID). There are at least 18 different genetic mutations leading to the clinical phenotype classified as SCID, which leads to a failure of T-cell development or function.

X-linked SCID is the most common type leading to a γ-chain defect in the interleukin 2 receptor gene (IL2RG) which plays a major role in signaling the growth and development of lymphoid cells, specifically T cells. The IL2Rγ chain also plays a role in other interleukin receptor formation including that of IL4, IL7, IL11, IL15, and IL21 [114]. Other mutations that can lead to a similar clinical picture involve autosomal recessive mutations in Janus kinase 3 (JAK3) or in the IL7 receptor alpha chain (IL7Rα). The next most common type of SCID, which accounts for approximately 17% of cases, is caused by a deficiency of adenosine deaminase (ADA). This deficiency leads to lymphoid apoptosis. In addition patients with ADA deficiency suffer from skeletal complications as well as sensorineural hearing deficits that are not rectified with HSCT [115].

Recombinase-activating gene deficiencies (RAG-1 or RAG-2) are another form of autosomal recessive SCID. These mutations result in the inability to form antigen receptors involving any V, D, or J gene rearrangements. A variant of this allows for only partial impairment of genetic rearrangement resulting in a clinical phenotype called Omenn's syndrome. Omenn's syndrome is characterized by desquamation, diarrhea, hypereosinophilia, hepatosplenomegaly, and increased IgE with few to absent additional immunoglobulin subtypes. Circulating T cells are present but ineffective [116]. Additional indications for HSCT that affect the lymphoid population of cells are outlined in Table 4.3.

Table 4.3 Indications for transplant of primary immune deficiencies

Primary immune deficiencies treated with allogeneic HSCT
Lymphoid deficiencies
T-cell predominantly affected SCID
X-linked SCID
γ-Chain defect of IL2RG
Autosomal recessive variants of SCID
JAK3 deficiency
IL7Rα deficiency
ADA deficiency
Purine nucleoside phosphorylase deficiency
RAG1 or RAG2 deficiency
Omenn's syndrome
Artemis mutation
DNA ligase IV mutation
CD3 chain mutation
CD45 deficiency
Zap70 mutation
Mixed T and B cells affected
Ataxia telangiectasia
MHC II (bare lymphocyte syndrome)
B cells primarily affected
X-linked infantile hypogammaglobulinemia
X-linked hyper IgM
Mixed immunodeficiency
Wiskott-Aldrich syndrome
Immunodeficiency affecting myeloid cells
Chronic granulomatous disease
Chediak-Higashi syndrome
Leukocyte adhesion deficiency
Griscelli syndrome

HSCT for SCID is considered a medical emergency. Outcomes for allo-HSCT are vastly improved when HSCT occurs within the first few months of life prior to any infections [117–119].

Wiskott-Aldrich syndrome (WAS) is an X-linked disorder caused by mutations in the WASP gene. It is characterized by microthrombocytopenia, recurrent infections, and eczema. These patients also have an increased predisposition to leukemia and lymphoma. Although symptoms can be managed for some time with platelet transfusions and prophylactic antimicrobials, the ultimate treatment is allo-HSCT. Improved outcomes have been shown if allo-HSCT takes place prior to 5 years of age [120].

Myeloid-related immunodeficiencies: Chediak-Higashi syndrome is an autosomal recessive disorder affecting lysosomal trafficking. Its symptoms manifest as oculocutaneous albinism, neurologic abnormalities, and immune defects such as failure of phagolysosome formation and absent secretory granules by cytotoxic T cells. If untreated patients develop an "accelerated" form of the disease with symptoms mimicking macrophage-activating syndrome, treatment with allo-HSCT can ameliorate the immune defects; however, it remains to be determined if long-term neurologic sequelae persist in later years after HSCT [121].

Chronic granulomatous disease (CGD) is caused by a defect in the enzyme phagocyte NADPH oxidase (PHOX). NADPH oxidase is essential to form reactive oxygen species used in the phagocyte killing of bacteria. It is either inherited as X-linked or autosomal recessive pattern. Allo-HSCT is the only curative treatment. As with other immune disorders, the best outcomes from allo-HSCT result when HSCT is performed early on in the course of CGD and prior to severe infectious complications or diffuse granuloma formation [122–124].

Hemophagocytic Lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (HLH) is a severe syndrome of immune dysregulation in which there is uncontrolled activation of T-lymphocytes and macrophages. There are variants inherited in an autosomal recessive fashion and acquired variants which are sometime referred to as macrophage activation syndrome (MAS). For the familial or genetically inherited variants, the only cure is with allo-HSCT. Allo-HSCT for acquired types of HLH is mainly reserved for progressive or refractory disease despite therapy and for CNS involvement [125]. Best outcomes are in patients with well-controlled disease with a matched related (unaffected) donor (74% ± 16%), matched unrelated donor (MUD) (76% ± 12%), followed by mismatched unrelated donor (mMUD) (61% ± 23%), followed by haploidentical donor (43% ± 21%) [126].

Hemoglobinopathy

Beta-thalassemia major (BT) and sickle cell disease (SCD) are the two most common types of hemoglobinopathy referred for transplant.

Beta-thalassemia major: Beta-thalassemia major is caused by mutations affecting both copies of the beta-globin genes and is inherited in an autosomal recessive pattern. This results in the inability to make hemoglobin A, the predominant type of hemoglobin in adults. Patients with beta-thalassemia major become transfusion dependent at a young age. While red cell transfusions have become safer and chelation therapy for iron overload has improved, the only definitive cure is with allo-HSCT. If an unaffected, matched, related donor is identified, it is reasonable to offer allogeneic HSCT prior to becoming transfusion dependent. OS and disease-free survival (DFS) at 5 years from this type of transplant approach is 98% and 90%, respectively [127]. When a matched related donor is not available or the patient has undergone an extensive amount of transfusions, the risks and benefits must be carefully weighed [128, 129]. Risk stratification based on level of iron overload, hepatomegaly, and portal fibrosis, known as the Pesaro criteria, was developed to help correlate with outcomes of allo-HSCT in beta-thalassemia major [130].

Sickle cell disease: Indications for allo-HSCT in patients with SCD are based mainly on two criteria: availability of a hematopoietic stem cell source and severity of disease manifestations. Allo-HSCT for patients with matched sibling donors over the last 10 years has shown OS and EFS of greater than 90% [131–136]. Unfortunately, only about 15–20% of patients will have an unaffected matched sibling available as a donor. For those patients without a matched related donor, availability of an unrelated donor in the National Marrow Donor Program (NMDP) registry is also low due to the underrepresentation of minorities and less homogenous gene pool.

Historically, only those patients with severe types of sickle cell are referred for an unrelated donor transplant. Criteria for severe

disease include frequent vaso-occlusive crises (≥3 over 2 years), history of stroke (silent or overt) requiring chronic transfusions, elevated transcranial Doppler velocities, multiple episodes of acute chest syndrome (≤3 in a lifetime), pulmonary hypertension, and red cell alloimmunization [137]. Because the risks of graft failure, graft-versus-host disease, and transplant mortality are much higher with an unrelated donor, it is currently recommended that these HSCTs only occur in the setting of a clinical trial. Previous trials using unrelated umbilical cord blood as a hematopoietic stem cell source showed unacceptably high rates of graft rejection [138]. There are current studies looking at the use of haploidentical related donors for sickle cell disease in order to be able to offer allo-HSCT to a greater number of patients [139, 140].

Inherited Metabolic Disorders

Inherited metabolic disorders are a diverse group of diseases that affect the function of lysosomal enzymes or peroxisomes. When left untreated, toxic substances can build up, leading to impairments in neurocognitive function, growth, and damage to other organs including the heart, liver, and spleen. Enzyme replacement has been developed as treatment for a number of these disorders, but the infused enzyme is not able to cross the blood-brain barrier. The process of allo-HSCT for these patients provides them with non-diseased hematopoietic stem cells that produce the enzyme for which they are deficient [141]. Those hematopoietic stem cells have the unique ability to migrate across the blood-brain barrier thereby stabilizing the disease process. It is crucial to transplant these patients early in the disease process, since much of the damage is irreversible even with transplant. Some rapidly progressing metabolic disorders do not seem to be improved with transplant. Current standard of care is to offer transplant for Hurler syndrome, X-linked cerebral adrenoleukodystrophy (cALD), juvenile metachromatic leukodystrophy (MLD), and infantile Krabbe [142–146]. Of note, treating infantile Krabbe with allo-HSCT should take place immediately after birth because delaying treatment by even a month can lead to severe neurologic deterioration. It is crucial for prenatal diagnosis to occur in order to expedite the transplant process. Caution should be used in patients with advanced forms of these diseases. Thorough neurocognitive testing by experienced personnel should be done, and patients going to transplant should have a functional IQ score of at least 70 and a Lansky performance status of 80 or above [147, 148]. In the case of cALD, a Loes score should be given on the brain MRI. Best practice is that this score should be <10 in order for transplanted patients to have an acceptable quality of life [149]. Allo-HSCT is currently not indicated for adrenomyeloneuropathy (AMN), late infantile MLD, Alexander syndrome, Morquio syndrome (MPS IV), vanishing white matter disease, Zellweger syndrome, cerebrotendinous xanthomatosis, Fabry syndrome, Canavan syndrome, and cystinosis [150, 151]. For the numerous other inherited metabolic disorders, it is important to check for new enzyme replacement therapies as well as updated information as to the outcomes of allo-HSCT in those patients.

Osteopetrosis

Osteopetrosis is a heterogeneous, autosomal recessive disease affecting the activity of osteoclasts leading to excess bone formation and extramedullary hematopoiesis. Osteoclasts are derived from hematopoietic stem cells, and therefore the use of an allo-HSCT can cure the disease [152]. HSCT is indicated in the majority of subtypes except the neuronopathic form caused by a mutation in the OSTM1 gene [153]. As with metabolic disorders, the best outcomes following HSCT are dictated by getting to transplant early in the disease process with good overall function and a well-matched, preferably related donor. Delay in transplantation can lead to blindness and deafness in these patients, as lack of ability to remodel bone in these patients leads to progressive damage of nerves passing through cranial foraminae.

Key Points

- HSCT for malignant diseases can be either autologous or allogeneic.
- Allogeneic HSCT for malignant disorders is mainly reserved for diseases in the bone marrow such as leukemia and myelodysplasia.
- Autologous HSCT is mainly utilized in solid tumors and in some relapsed or refractory lymphomas.
- Allogeneic HSCT can also be utilized to cure many nonmalignant diseases. These diseases can be broken down into five categories:
  - Acquired severe aplastic anemia
  - Inherited bone marrow failure syndromes
  - Primary immune deficiencies
  - Hemoglobinopathies
  - Inherited metabolic disorders
- If possible, all pediatric patients undergoing a transplant should be enrolled on a clinical trial.

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5 Spotlight on Minimal Residual Disease (MRD): Impact of MRD on HSCT Outcomes for Pediatric Leukemia

Hisham Abdel-Azim and Michael A. Pulsipher

Abstract

In leukemia, morphologic complete remission is defined as less than 5% lymphoblasts (i.e., leukemia cells) detected in the bone marrow by standard light microscopy. However, the level of detection of lymphoblasts by light microscopy is only approximately 1 in 100. As more sensitive techniques to detect lymphoblasts were perfected [such as multichannel flow cytometry and antigen receptor or translocation amplification by polymerase chain reaction (PCR)], it became clear that patients in "morphologic remission" may in fact harbor up to 10^{10} leukemia cells. The presence of this "minimal residual disease" (MRD) at the end of the induction or consolidation phases of treatment of newly diagnosed ALL patients has been found to be an independent prognostic factor. Furthermore, the presence of MRD before HSCT strongly correlates with risk of relapse both in ALL and AML. At the defined levels of MRD (e.g., ≥0.1% or $\geq 10^{-3}$ for ALL and ≥0.1% for AML), there is a significant worsening in outcome.

Introduction

Morphological complete remission (CR) of leukemia is defined as less than 5% blasts by standard microscopic examination of the bone marrow aspirate. Evaluation by light microscopy often cannot distinguish leukemic blast cells from normal hematopoietic progenitor blast cells. Patients with leukemia in remission may have variable numbers of leukemic cells that are not detectable by microscopy. It is estimated that patients who are in morphological complete remission can harbor up to 10^{10} leukemic cells [1]. When patients in morphological CR have residual leukemic cells noted by more sensitive detection techniques than microscopy, this is referred to as minimal residual disease (MRD) positivity.

In the modern era of risk-adapted therapy, long-term survival can be achieved in up to 80–90% [2, 3] and 60–70% [4, 5] of patients diagnosed with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), respectively. Application of risk-adapted therapy requires accurate identification of risk factors

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that allow escalation or de-escalation of therapy. Persistence of MRD after induction and/or consolidation chemotherapy courses has become an important risk factor for both ALL and AML.

Earlier studies for MRD detection in pediatric patients with newly diagnosed ALL showed that MRD at the end of induction or consolidation is an independent prognostic factor; these measurements are now used to define higher-risk groups that receive more intensive chemotherapy treatment regimens [6]. This applies to both low- and high-risk patients as defined by traditional parameters and is often used in combinations with other known risk factors (e.g., age, cytogenetics) to determine therapy [7]. Persistence of MRD after reinduction has also been noted to be prognostic in patients with relapsed ALL [8].

Although the role of the level of MRD after initial therapy leading to determination of high or low intensity of therapy in pediatric AML is less defined, similar MRD-based risk determination of therapy is being prospectively investigated, and an adverse impact of MRD on relapse risk in AML has been observed [9, 10].

Allogeneic hematopoietic stem cell transplantation (HSCT) is used to treat patients noted to be at very high risk for relapse based on genetic mutations or persistence of MRD after initial therapy or who have relapsed after initial chemotherapy treatment. In spite of the intensity of this therapy, a significant number of patients relapse after allogeneic HSCT. Several studies have shown that MRD pre-HSCT is predictive of relapse and survival; children who are MRD negative in the bone marrow before undergoing HSCT have improved relapse-free survival compared to MRD-positive patients [11–13]. Because the presence of MRD pre-HSCT predicts relapse risk, centers are modifying their therapies either to lower MRD pre-HSCT or offer treatment post-HSCT to prevent relapse. Additionally, monitoring MRD after HSCT allows identification of candidates who may benefit from early intervention to prevent relapse [14, 15].

Approaches for MRD Assessment

Table 5.1 summarizes differences between various methods used for MRD assessment.

Multichannel flow cytometry (MFC). Multichannel flow cytometry is a highly sensitive, readily available, and rapid method for the detection of the abnormal phenotypes associated with leukemia cells, allowing low-cost analysis in large number of patients. Performing MFC-MRD is considered the standard of care in North America and has recently been standardized at a large number of centers throughout the country in order to facilitate participation in Children's Oncology Group (COG) protocols.

Normal lymphoid and myeloid precursors exhibit a reproducible surface protein expression patterns through various stages of maturation, while leukemic cells express antigen patterns not found in normal cell differentiation (e.g., over- or underexpression of antigens expressed in cell differentiation, expression of antigens not expressed on normal progenitors, or simultaneous expression of antigens usually expressed at different stages of normal cell development and deviation from normal maturation patterns) [16]. These patterns allow recognition of distinct leukemic cells at very low numbers by MFC.

Polymerase chain reaction (PCR). This includes antigen receptor PCR and translocation PCR. B- and T-cell leukemias have the unique property of having undergone a unique rearrangement of their immunoglobulin heavy chain (IgH,

Table 5.1 Summary of differences between various methods used for MRD assessment

Method	MFC	Translocation PCR	Antigen receptor PCR	NGS
Sensitivity	10^{-4}	10^{-4}–10^{-6}	10^{-5}	10^{-7}
Utility (% of informative cases)	>95	<50	90	95%
Need for pretreatment sample	Preferred (not essential)	None	Essential	Essential
Use universal reagents	Yes	Yes	No	Yes
Turnaround time	Same day (h)	2–3 days	Weeks	1 week

MFC multichannel flow cytometry, *PCR* polymerase chain reaction, *NGS* next-generation sequence

for B-cells) or T-cell receptor (TCR) genes prior to the cell becoming a cancer clone. When cancer occurs, these unique rearrangements are located in all of its progeny, and testing for these rearrangements has become a widely accepted method to measure MRD in pediatric ALL [17, 18].

Real-time quantitative (RT-Q) PCR can also be used for detection of MRD in AML in cases that have chimeric fusion genes generated by chromosomal rearrangements/translocations (e.g., PML-RARA/t(15;17), RUNX1-RUNX1T1/t(8;21), CBFB-MYH11/(inv(16)/t(16;16), t(11q23)/MLL fusions); this method allows MRD monitoring of approximately 60% of AML cases in children [19, 20]. High WT1 expression has been used for MRD monitoring for AML in some cases as well [21, 22].

Because of the universal nature of IgH and TCR rearrangements in ALL, groups in Europe and other areas have adopted molecular approaches as a preferred method of MRD monitoring. However, molecular methods are much more challenging for AML due to the lack of general applicability, expense, and other issues, and these methods have not been widely adopted. RT-QPCR for BCR-ABL can be used to monitor MRD in CML and Ph+ ALL [15].

Next-generation sequence (NGS)-MRD. NGS offers the possibility for detection and follow-up of a wide number of genetic abnormalities. NGS is being explored for MRD detection in ALL because it can easily measure immunoglobulin heavy chain (IgH)–variable, diversity, and joining (V[D]J) or T-cell receptor clonal rearrangements [23], similar to the PCR methods mentioned above. This approach increases the sensitivity of MRD detection from 1 blast cell in 10^4 to 10^5 cells offered by flow cytometry and PCR, respectively, to as high as 1 in 10^7 cells and has been shown to be predictive of relapse in children with ALL receiving standard chemotherapy [24, 25] as well as better predicting relapse or lack of relapse after HSCT [26].

NGS-MRD is being explored in AML [27]. In AML, there is a significant heterogeneity with associated genetic mutations making standardization and need for mutation-specific PCR probes challenging. Therefore, NGS may have advantages for evaluation of MRD in AML. The current major challenge to standardizing this approach for MRD detection in AML is the base error rate leading to decreased sensitivity below the other methods. Current research is underway to overcome these technical issues [28].

ALL

Table 5.2 (A, B, C, D) summarizes various studies of MRD in ALL.

MRD is an independent prognostic factor for newly diagnosed and relapsed ALL.

In COG/CCG/POG 9900 series (n = 2143), MRD was measured by MFC in BM and PB in children newly diagnosed with BP-ALL. In multivariate analysis, end of induction (EOI) MRD was the most significant (HR 4.31, $P < 0.001$) independent predictor of survival across all risk groups. Patients with undetectable MRD (≤0.01%) had a 5-year EFS of 88% compared to 30% for patients with high MRD (>1%) [7].

In COG AALL01P2 study (n = 124), MRD was measured by MFC in BM in children with relapsed ALL. MRD was measured after each block of the four blocks of therapy. The 1-year EFS was 80% in the EOI MRD-negative (≤0.01%) and 58% in the MRD-positive (>0.01%) group ($P < 0.0005$). Four patterns for MRD response kinetics were observed. R1 patients included those negative at all time points tested; R2 included those who became negative after either block 2 or block 3; R3 included those patients who decreased but still had detectable disease at the end of the final block of therapy; and R4 included patients who showed no decline or rising MRD. The 12-month EFSs were 86% ± 8%, 73% ± 8%, 70% ± 16%, and 19% ± 10%, respectively. Patients who are MRD positive at EOI (block 1) or consolidation (block 2) have inferior outcomes compared to patients with negative MRD, and patients who are MRD+ at the end of delayed intensification (block 3) have exceptionally poor outcomes and are unlikely to be cured with the same regimen/line of treatment [8].

Given the proven predictive value of MRD, several studies have used MRD to define candidates for HSCT in CR1 and CR2. In St. Jude (total XV) study, newly diagnosed patients with end of induction (EOI) MRD ≥1% received additional chemotherapy

Table 5.2 Summary of various studies of MRD in ALL

Study group/treatment protocol	MRD method	MRD+ cutoff or sensitivity	MRD measurement time points	Results
A: Newly diagnosed ALL				
COG/P9900 [7]	MFC	≥0.01%	D8 PB, EOI and EOC BM PB-ALL	• EOI-MRD is an independent prognostic factor and a strong predictor of outcome across all risk groups • EOI-MRD is a strong prognostic factor in early and late relapse • Increasing MRD levels associated with a progressively poorer outcome • MRD identifies a subgroup of patients with excellent outcome on minimal therapy • EOC-MRD identifies patients with very high risk of relapse • MRD is a powerful tool for risk stratification and for risk adapted therapy
St. Jude/Total XIII [29]	MFC	≥0.01%	EOI, Wks 14, 32 and 56 BM	• Sequential monitoring of MRD is an independent prognostic factor, higher MRD is associated with relapse
AIEOP/AIEOP-BFM ALL 2000 [30]	MFC	≥0.1%	D15 and EOI BM	• D15 BM MRD by MFC was the most important prognostic factor for risk of relapse • MRD ≥ 0.1% was associated with 2–5 folds increased risk of relapse
CHLA/BFM-based therapy [31]	MFC	≥0.01%	EOI BM T-ALL	• Persistent EOI MRD *alone* is not an indication to alter therapy or relapse risk in pediatric T-ALL
DFCI/DFCI 95-01 [32]	RT-QPCR	≥0.1%	EOI BM BP-ALL	• Higher (>0.1%) EOI MRD is associated with 10.5 fold of increased risk of relapse • There is a linear relation between MRD level and risk of relapse
BFM/AIEOP-BFM ALL 2000 [33]	RT-QPCR	≥0.01%	EOI (D33) and EOC (D78) BM BP-ALL	• MRD is an independent prognostic factor of white blood cell count, age, early response to prednisone, and genotype • MRD response at 2 predefined time points is highly predictive for relapse and risk stratification
BFM/AIEOP-BFM ALL 2000 [34]	RT-QPCR	≥0.01%	EOI (D33) and EOC (D78) BM T-ALL	• EOC MRD (>0.1%) is the most predictive factor for relapse • Early (EOI) MRD level has no prognostic significance if late (EOC) MRD is negative
Belarus/ ALL-MB-2002/2008 [35]	RT-QPCR	≥0.01%	D15, EOI (D36), before and after maintenance therapy BM	• Relapse-free survival revealed to be significantly associated with MRD levels at different time points • Unfavorable prognosis is associated with MRD ≥ 0.1% on EOI, and any positive MRD before and after maintenance therapy

Table 5.2 (continued)

Study group/treatment protocol	MRD method	MRD+ cutoff or sensitivity	MRD measurement time points	Results
B: Relapsed ALL				
St. Jude/R11 and R15 [36]	MFC	≥0.01%	EOI (D36) BM	• EOI MRD ≥0.01% is associated with subsequent risk of relapse • EOI MRD was a significant predictor of outcome for patients whose first relapse occurred late (i.e., after completion of primary therapy) • Early relapse (i.e., while still receiving primary ALL therapy) is associated with subsequent relapse regardless of EOI MRD status • EOI MRD is a useful determinant for HSCT indication
COG/AALL01P2 [8]	MFC	>0.01%	Blocks 1, 2, and 3 BM	• Patients who are MRD+ at EOI (block 1) or consolidation (block 2) have inferior outcomes compared to patients with negative MRD • Patients who are MRD+ at the end of delayed intensification (block 3) have exceptionally poor outcomes and unlikely to be cured with the same regimen/line of treatment
BFM/ALL-REZ BFM 90, 95, and 96 [37, 38]	RT-QPCR	≥0.01%	EOI (D36) BM	• EOI MRD < 0.1% is associated with EFS of 86%, compared with 0% when it is ≥0.1% (retrospective study intermediate risk group) • EOI MRD < 0.1% is associated with EFS of 76%, compared with 18% when it is ≥0.1% at 10-year follow-up (prospective study intermediate risk group) • Patients with EOI MRD < 0.1% have excellent outcome with chemo-/radiotherapy, whereas patients with EOI MRD ≥ 0.1% have poor prognosis • EOI MRD can be used to allocate patients to conventional post-induction therapy vs. allogeneic HSCT
BFM/ALL-REZ BFM 2002 [39]	RT-QPCR	≥0.01%	EOI (D36) BM	• HSCT markedly improved (EFS 64%) the prognosis of patients with intermediate risk of relapse ALL and EOI MRD ≥ 0.1% • Patients with EOI MRD <0.1% has similar outcome with HSCT from a sibling donor (EFS 80%) compared to salvage chemotherapy (EFS 66%)
CCLG-ANZCHOG/UK ALL R3 relapse study [40]	RT-QPCR	≥0.01%	EOI (D35) BM	• Patients with EOI MRD ≥ 0.01%, from the high-risk and intermediate risk groups, received HSCT • Patients who received mitoxantrone reinduction therapy have superior (64.6%) EFS compared to idarubicin, with no difference in the kinetics of MRD clearance

(continued)

Table 5.2 (continued)

Study group/treatment protocol	MRD method	MRD+ cutoff or sensitivity	MRD measurement time points	Results
C: Pre-HSCT MRD for ALL				
Royal Hospital for Sick Children, Bristol, UK [41]	PCR	0.001–0.1%	Pre-HSCT BM	• EFS for patients with high, low, or negative MRD was 0%, 36%, and 74%, respectively • Pre-HSCT MRD allow identification of patients with resistant leukemia and of those with more responsive disease
University Hospital Rotterdam, The Netherlands [42]	RT-QPCR	≥0.01%	Pre-HSCT BM	• Relapse-free survival was 80% in the MRD-negative compared to 33% in the MRD-positive groups • Presence of detectable MRD pre-HSCT is associated with high chance of relapse
University Children's Hospital of Tubingen, Germany [43]	PCR	0.001–0.1%	Pre-HSCT BM	• EFS for patients with high, low, or negative MRD was 23%, 48%, and 78%, respectively • MRD status pre-HSCT is a powerful predictor for post-HSCT outcome in children with ALL
Pre-BMT MRD Study Group [44]	PCR and RT-QPCR	0.001–0.1%	Pre-HSCT BM	• EFS for patients with high, low or negative MRD was 21.4%, 40.7% and 75.2%, respectively • EFS of pre-HSCT MRD-positive (high and low) group was 29.8% • Pre-HSCT MRD is an independent factor to influence EFS post-HSCT
Huddinge University Hospital, Sweden [45]	PCR	0.001–0.1%	Pre-HSCT BM	• Patients are pre-HSCT MRD+ are more likely to relapse than MRD patients • No difference in relapse rates between the high-(10^{-2} to 10^{-3}) and low-(10^{-4} to 10^{-5}) level MRD groups • Combined acute and chronic GVHD disease is associated with lower risk of relapse in MRD+ patients
BFM/ALL-REZ BFM 96 or 2002 [11]	RT-QPCR	≥0.01%	Pre-HSCT BM	• Included children with relapsed ALL treated according to the ALL-REZ BFM 96 or 2002 protocols and receiving stem-cell transplantation in ≥ second remission • Patients with pre-HSCT MRD level $< 10^{-4}$ had 60% EFS compared to 27% in patients with MRD level of $\geq 10^{-4}$ • A significant prognostic impact of pre-HSCT MRD was observed intermediate risk patients transplanted in CR2 (EFS 68% vs. 20% in the MRD-negative vs. MRD-positive group) and in HR patients transplanted in second or third remission • MRD as the only independent parameter predictive for EFS for post-transplantation outcome

Table 5.2 (continued)

Study group/treatment protocol	MRD method	MRD+ cutoff or sensitivity	MRD measurement time points	Results
Clinica Pediatrica, Monza, Italy [46]	RT-QPCR	≥0.01%	Pre-HSCT BM	• Pre-HSCT MRD of <10^{-4} compared to ≥10^{-4} was associated EFS and CIR of 77.7% and 11.4% vs. 30.8% ($P < 0.001$) and 61.5% ($P < 0.001$), respectively • Pre-transplant MRD ≥ ×10^{-4} compared to ≥10^{-4} was associated with a 9.2-fold risk of relapse ($P < 0.001$) • Pre-HSCT chemotherapy to reduce MRD level was associated with a 5-fold reduction of risk of failure (HR 0.19, $P = 0.01$)
French minimal residual disease-guided protocol study, France [47]	RT-QPCR	≥0.01%	Pre-HSCT BM	• The association between pre-HSCT MRD and CIR did not differ according to CR status • Pre-transplant MRD is a predictor of outcome for ALL and is associated with OS • OS was 72.3% of patients with pre-HSCT MRD < 10^{-3} and 40.4% of those with MRD ≥10^{-3}
COG/PBMTC [12]	MFC	≥0.01%	Pre-HSCT BM	• Pre-HSCT MRD of ≥0.1% was associated with higher relapse risk (HR 3.3) and decreased EFS (HR 2.2) • Grades 1–3 aGVHD are associated with increased EFS (HR 0.4)
COG/PBMTC [26]	NGS	≥0.00001%	Pre-HSCT BM	• Pre-HSCT MRD with NGS predicted relapse and survival more accurately than MFC ($P < 0.0001$), especially in the MRD-negative group (relapse, 0% vs. 16%; $P = 0.02$; 2-year overall survival, 96% vs. 77%; $P = 0.003$) • aGVHD defined the relapse risk of pre-HCT NGS-MRD-positive patients; 2-year relapse probabilities were 73% for patients with no aGVHD by day +55 and 17% for those who experienced aGVHD by day +55 ($P = 0.02$)
D: Post-HSCT MRD for ALL				
Royal Hospital for Sick Children, Bristol, UK [48]	PCR	0.001–0.1%	Post-HSCT: 1–4, 6, 9, 12, 18, and 24 months BM	• Any positive post-HSCT MRD is a poor prognostic factor; 88% post-HSCT MRD-positive patients relapsed • Of the patients who remained in CR, 22% showed intermittent low level of MRD positivity (0.01–0.001%) up to 9 months post-HSCT, and most of them had aGVHD grade I–II raising the possibility of a GVL effect and benefit from immune intervention
Czech Pediatric Hematology Group [49]	RT-QPCR	0.01–0.1%	Post-HSCT: 1–3, 6, 9, 12, 18, and 24 months BM and PB	• All patients with detectable post-HSCT MRD eventually relapsed • Immune modulation and DLI did not prevent relapse

(continued)

Table 5.2 (continued)

Study group/treatment protocol	MRD method	MRD+ cutoff or sensitivity	MRD measurement time points	Results
Clinica Pediatrica, Monza, Italy [46]	RT-QPCR	≥0.01%	Post-HSCT: 1, 3, 6, 9, and 12 months BM	• Patients who were post-HSCT MRD-positive post-transplant had 40.3% EFS; and a 2.5-fold risk of failure ($P = 0{\cdot}04$) if any MRD was detected in the first 100 days, which increased to 7.8-fold ($P = 0{\cdot}002$) if detected after 6 months • Post-HSCT MRD positivity was not always associated with relapse (EFS 40.3%), but was associated with a 2.5-fold risk of failure ($P = 0.04$) if any MRD was detected in the first 100 D, and 7.8-fold ($P = 0.002$) if detected after 6 months • Immunosuppression modulation or DLI based on MRD improved outcome • Post-HSCT MRD $\geq 10^{-3}$ was always associated with relapsed, despite immunosuppression modulation or DLI
BFM/ALL-REZ BFM 2003 [14]	RT-QPCR	≥0.01%	Post-HSCT: 1, 2, 3, 6, and 12 months BM	• Post-HSCT MRD was inversely correlated with EFS ($P < 0.004$) and positively correlated with CIR ($P < 0.01$) • Post-HSCT MRD $\geq 10^{-4}$ was associated with lower EFS ($P < 0.003$) • Post-HSCT MRD predictive power of leukemic relapse at 1, 3, 6, and 9 months was >96%, >87%, >71%, and >61%, respectively
COG/PBMTC [26]	MFC NGS	≥0.01% ≥0.00001%	Post-HSCT: peri-engraftment, 3-month, and 8-month BM	• Post-HSCT NGS-MRD predicted relapse than MFC-MRD ($P < 0.0001$), especially early after HSCT (day 30 MFC-MRD-positive relapse rate, 35%; NGS-MRD-positive relapse rate, 67%; $P = 0.004$) • Any post-HSCT NGS positivity increased the relapse risk (HR 7.7; $P = 0.05$), independent of pre-HSCT MRD and aGVHD

ALL Acute lymphoblastic leukemia, *MRD* minimal residual disease, *BP* B-precursor, *COG* Children's Oncology Group, *MFC* multichannel flow cytometry, *D* day, *EOI* end of induction, *EOC* end of consolidation, *BM* bone marrow, *Wks* weeks, *CHLA* Children's Hospital Los Angeles, *RT-QPCR* real-time quantitative polymerase chain reaction, *DFCI* Dana–Farber Cancer Institute, *AIEOP* Associazione Italiana Ematologia Oncologia Pediatrica, *BFM* Berlin–Frankfurt–Muenster, *EFS* event-free survival, *HSCT* hematopoietic stem cell transplantation, *CCLG* Children's Cancer and Leukaemia Group in the UK and Ireland, *ANZCHOG* Australian and New Zealand Children's Haematology/Oncology Group, *CR* complete remission, *HR* high risk, *CIR* cumulative incidence of relapse, *OS* overall survival, *PBMTC*

with the goal of reducing MRD prior to allogeneic HSCT. In this study, patients received HSCT due to high MRD ($n = 26$; 21 at EOI and 5 for persistent MRD at week 16) resulting in 5-year EFS of 79.5% compared to 43% in historical controls [50]. COG study AALL 0232 for high-risk B-precursor (BP)-ALL with high EOI MRD (>0.1 to <1%) demonstrated that additional intensification of chemotherapy did not prevent relapse and death with no improvement in 5-year OS and EFS. Subjects (9.2%, $n = 37$) that were withdrawn from the study in CR1 to pursue HSCT had no relapses prior to or

during the follow-up period [51]. In the AIEOP-BFM ALL 2000 study, patients (21%) with high level ($\geq 10^{-3}$) of MRD at day 78 of chemotherapy had a 7-year EFS of 40.5% when they received HSCT in CR1 demonstrating lower rate of relapse after HSCT (13 of 55 patients) as compared to patients who did not receive HSCT (23 relapses over 42 patients) [34]. These results collectively support the role of HSCT in CR1 based on MRD.

The BFM study group showed that MRD status can be used as an indication to allocate patients in CR2 to HSCT. HSCT improved EFS of patients with intermediate-risk relapsed ALL with high EOI MRD (week 5) to achieve similar outcome to low MRD patients treated with chemotherapy only [37–39].

On MRD blinded results, patients who were MRD-negative ($<10^{-3}$) as compared to MRD-positive patients ($\geq 10^{-3}$) had 10-year EFS of 76% and 18% and cumulative incidence of relapse of 21% and 61%, respectively [38]. Subsequently, patients with intermediate-risk relapse and a high EOI MRD level ($\geq 10^{-3}$, $n = 99$) were assigned to receive HSCT, while those with low MRD levels continued chemo- and radiotherapy ($n = 109$). This MRD-based treatment allocation to HSCT improved the EFS of patients with intermediate-risk relapsed ALL and high EOI MRD from 18 to 64%, which was similar to patients with low EOI MRD (70%) [39].

St. Jude R11 and R15 studies for ALL in CR2 demonstrated similar results to the BFM group. Multivariate analysis showed that positive ($\geq 0.01\%$) EOI MRD (day 36) was independently associated with subsequent relapse with high incidence among patients treated with chemotherapy alone who had positive MRD ($n = 12$) compared to those who became MRD negative ($<0.01\%$) [$n = 13$] with cumulative incidence of relapse of 81% vs. 25% ($P = 0.004$). Therefore, EOI MRD is a useful determinant for HSCT indication [36].

Multiple studies showed that pre-HSCT MRD level of (10^{-2}–10^{-3}) and (10^{-5}–10^{-7} to undetectable) was associated with up to 80% and 0–30% relapse probability, respectively [11, 12, 26, 42–47]. Pre-HSCT MRD identified patients that are at high risk for relapse post-HSCT.

A study from the BFM group examined the prognostic value of pre-HSCT MRD in children ($n = 91$). Patients with pre-HSCT MRD ($n = 46$) $<10^{-4}$ had an EFS of 60% and cumulative incidence of relapse of 13% compared to an EFS of 27% and cumulative incidence of relapse of 57% in patients with MRD level of $\geq 10^{-4}$ ($n = 45$) (with $P = 0.004$ for EFS and $P < 0.001$ for cumulative incidence of relapse). Although the pre-HSCT MRD strongly impacted the EFS post-HSCT, this information indicated that patients with persistent pre-HSCT MRD may still benefit from HSCT as this group had an EFS of 27% [11].

A study ($n = 146$) from COG/PBMTC showed increased risk of relapse in children with pre-HSCT MRD $\geq 0.1\%$ (HR 3.3; $P = 0.01$), whereas acute GVHD grades I–III were associated with lower relapse risk (HR 0.4; $P = 0.04$) [12]. Another study by the same group showed that pre-HSCT NGS-MRD in patients with BP-ALL ($n = 56$) predicted relapse and survival more accurately than MFC ($P < 0.0001$), especially in the MRD-negative group (relapse, 0% vs. 16%; $P = 0.02$; 2-year overall survival, 96% vs. 77%; $P = 0.003$), and that aGVHD defined the relapse risk of pre-HCT NGS-MRD-positive patients; 2-year relapse probabilities were 73% for patients with no aGVHD by day +55 and 17% for those who experienced aGVHD by day +55 ($P = 0.02$) [26].

Several studies have shown that detectable post-HSCT MRD is associated with relapse [14, 26, 46, 48, 49]. A recent BFM study ($n = 113$) showed that positive post-HSCT MRD predicts relapse. The level of MRD was inversely correlated with EFS ($P < 0.004$), and MRD $\geq 10^{-4}$ was associated with inferior EFS ($P < 0.003$). The ability of MRD to predict relapse after 1, 3, 6, and 9 months was >96%, >87%, >71%, and >61%, respectively [14]. Similarly, a study from COG/PBMTC showed that any post-HSCT NGS-MRD positivity increased risk of relapse (HR 7.7; $P = 0.05$). Additionally, post-HSCT NGS-MRD detection was better at predicting relapse than MFC-MRD ($P < 0.0001$), especially early after HSCT (day 30 MFC-MRD-positive relapse rate, 35%; NGS-MRD-positive relapse rate, 67%; $P = 0.004$) [26].

Post-HSCT MRD positivity increased the relapse risk (HR = 4.5, $P = 0.01$). An optimal window to initiate intervention to prevent relapse is between day +55 and +200 after HSCT [52]. MRD assessment pre- and post-HSCT identifies

a group of patients who are at a particularly high risk or with impending relapse post-HSCT. This group of patients may benefit from immune intervention including faster withdrawal of immunosuppression and use of donor lymphocyte infusions and post-HSCT leukemia therapy.

COG/PBMTC studies [12, 26] showed that aGVHD post-HSCT is associated with decreased relapse in patients with positive pre-HSCT MRD, supporting the role of immune intervention. In a study from Sweden, patients with detectable pre-HSCT MRD (n = 25), 2 of 11 patients with both acute and chronic GVHD relapsed, as compared with 11 of 14 patients with no GVHD or only acute or chronic GVHD (P = 0.005). The combination of acute and chronic GVHD was associated with lower risk of relapse (odds ratio 0.07; P = 0.014) [45]. A Dutch study showed that, in patients with pre-HSCT MRD $\geq 10^{-4}$ (n = 18), preemptive immune intervention (early cyclosporine tapering (n = 13)) followed by consecutive, incremental DLI (n = 6) was associated with aGVHD grade II in 23% of patients, and EFS in the intervention group was 19% [53].

In an Italian study, patients who were MRD positive posttransplantation had an EFS of 40.3%. While post-HSCT MRD positivity was not always associated with relapse, it was associated with a 2.5-fold risk of failure (P = 0.04) if any MRD was detected in the first 100 days and 7.8-fold (P = 0.002) if detected after 6 months. Immunosuppression modulation or DLI based on MRD improved outcome. However, post-HSCT MRD $\geq 10^{-3}$ was always associated with relapse, despite immunosuppression modulation or DLI [46].

AML

The role of MRD in the management of AML is less defined. St. Jude AML02 study showed that patients with MRD positivity (≥0.1%) at the end of induction I are more likely to relapse than MRD-negative patients (39% vs. 17%) [10]. A British/Dutch study showed similar results, where patients who were MRD positive at the end of induction I had lower relapse-free survival compared to patients who were MRD negative (85% vs. 44%) [54]. COG showed that 16% of patients (n = 252) considered responsive to induction therapy had detectable disease (residual leukemia ≥0.5%), predicting a 4.8 and 3.1 times increased risk of relapse and death, respectively [55]. In another study, COG showed that end of induction I MRD in AML was the only factor that remained prognostic when compared with cytogenetic and molecular risk groups [9]. The BFM group reported that while MRD positivity in AML correlated with poorer outcomes, it did not contribute to overall risk stratification [56].

A retrospective study in adults (n = 99) examined the effect of pre-HSCT MRD in AML in CR1. Pre-HSCT MRD was associated with increased risk of relapse and death after myeloablative HSCT after controlling for other risk factors. Pre-HSCT MRD was positive (<0.01% in two patients, 0.01–0.1% in 8 patients, and >0.1% in 14 patients [range, 0.007–3%; median, 0.29%]) in 24 patients. The 2-year estimates of overall survival were 30.2% (range, 13.1–49.3%) and 76.6% (range, 64.4–85.1%) for MRD-positive and MRD-negative patients; 2-year estimates of relapse were 64.9% (range, 42.0–80.6%) and 17.6% (range, 9.5–27.9%). After adjustment for all or a subset of cytogenetic risk, secondary disease, incomplete blood count recovery, and abnormal karyotype, pre-HSCT MRD positivity was associated with increased overall mortality (HR 4.05; $P < 0.001$) and relapse (HR, 8.49; $P < 0.001$) relative to negative pre-HSCT MRD [58].

In a different study, the same group examined the effect of pre-HSCT MRD AML in CR1 (n = 183) or CR2 (n = 70) in adults demonstrating similar negative impact of pre-HSCT MRD positivity. The 3-year OS was 73% and 32% for MRD-negative and MRD-positive CR1 patients, respectively, and 73% and 44% for MRD-negative and MRD-positive CR2 patients, respectively. Similar estimates of relapse were 21% and 58% for MRD-negative and MRD-positive CR1 patients, respectively, and 19% and 68% for MRD-negative and MRD-positive CR2 patients, respectively. Risks of death and relapse were 2.61 times and 4.90 times higher for MRD-positive patients ($P < 0.001$). Together, these findings indicate that the negative impact of pre-HCT MRD is similar for AML in CR1 and CR2 with even minute levels (≤0.1%) as being associated with adverse outcome [13].

In a more recent study of adult patients with AML (n = 359), who underwent myeloablative HSCT from a peripheral blood or bone marrow grafts, the 3-year relapse estimates were 67% in 76 patients in MRD-positive morphologic remission and 65% in 48 patients with active AML compared with 22% in 235 patients in MRD-negative remission. The 3-year overall survival estimates were 26%, 23%, and 73% in these three groups, respectively. MRD-negative remission status was associated with longer overall and progression-free survival as well as lower risk of relapse compared with MRD-positive morphologic remission status or having an active disease, with similar outcomes between the latter two groups [58].

A recent study examined the effect of pre-HSCT MRD status on outcomes of HSCT, in AML, after either myeloablative (MAC) or reduced intensity (RIC) conditioning regimens in 203 adult patients (MAC, n = 80, and RIC, n = 123) with no morphologic evidence of disease pre-HSCT. The graft sources included 130 umbilical cord blood (UCB) and 73 sibling donors. MFC (sensitivity 0.1%) was used to evaluate MRD. Twenty-five patients were MRD+, including 15 (18.7%) receiving MAC and 10 (8.1%) RIC HSCT. Among RIC patients, MRD+ was associated with significantly inferior relapse, DFS, and OS (multiple regression HR, 3.8; $P < 0.01$ for relapse; HR, 2.9; $P < 0.01$ for DFS; and HR, 3.4; $P < 0.01$ for OS). In contrast, MRD+ status was not associated with relapse or decreased OS after MAC. These data suggest that MAC, but not RIC, overcomes the negative effect of pre-HSCT MRD+ after sibling or UCB HSCT [59].

In a St. Jude study, in children with very-high-risk ALL (n = 64) or AML (n = 58), MRD was an independent prognostic factor (P = 0.0035), and higher MRD levels pre-HSCT predicted a poorer survival after HCT (P = 0.0019). However, the increase in risk of death associated with a similar increment of MRD was greater in ALL than in AML, suggesting that a pre-HSCT reduction of leukemic burden would have a higher impact in ALL. At any given MRD level, survival rates were higher for patients treated in recent protocols: the 5-year overall survival for patients with ALL was 49% if MRD was detectable (66.7% if MRD was <0.1% and 42.9% if it was 0.1 to <5.0%) and 88% if it was not, and the corresponding rates for patients with AML were 67% and 80%, respectively. Although pre-HSCT MRD is a strong prognostic factor, it should not be regarded as a contraindication for HSCT [60].

Key Points

- Detection of MRD before HSCT strongly correlates with the risk of relapse both in ALL and AML.
- At the defined levels of MRD (e.g., ALL ≥0.1% [12] or ≥10^{-3} [38, 39]; AML ≥0.1% [13, 57, 58, 60]), there is a big falloff in outcome. With levels above that, outcomes are not zero but are much worse.

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6 HSCT Recipient Pretransplantation Evaluation

Carrie Eichelberger and Valerie I. Brown

Abstract

All patients being considered for hematopoietic stem cell transplantation (HSCT) are required by federal (e.g., the FDA) and other regulatory agencies [e.g., Foundation for Accreditation of Cellular Therapy (FACT)] to undergo a pre-HSCT evaluation. The purpose of this evaluation is to ensure that the HSCT candidate is adequately "fit" enough to tolerate HSCT and to determine if the potential benefit from HSCT (i.e., a cure from a malignancy, halting the progression of a disorder, or replacement of a non- or dysfunctional immune system) outweighs the potential risks including death and short- and long-term debilitating side effects. To this end, organ function (e.g., liver, kidneys, heart, and lungs) and performance status are determined. Because HSCT recipients are profoundly immunocompromised and thus susceptible to life-threatening and opportunistic infections as well as reactivation of latent infections, all HSCT candidates undergo a thorough infection evaluation *prior* to the start of the conditioning regimen. All potential HSCT recipients also undergo restaging or reassessment of their disease state. The results of this evaluation along with the results of the other pre-HSCT testing are used to customize the patient's conditioning regimen and supportive care plan in order to maximize the benefits while minimizing the morbidity of HSCT. This chapter details the components of this pre-HSCT recipient evaluation.

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Introduction

To ensure that HSCT recipients are able to tolerate hematopoietic stem cell transplantation (HSCT), an abundance of testing is performed prior to initiation of the conditioning regimen. Timing is of the essence when it comes to pre-transplantation workup and testing. The majority should be undertaken after all treatment for the patient's underlying condition has been completed, and the patient has sufficiently recovered from this treatment.

The official website of the National Donor Marrow Program (https://bethematch.org) is an invaluable resource for HSCT-related information for both health-care professionals and the patients and their families. Table 6.1 lists the required testing that is performed on all potential HSCT recipients prior to transplant.

Disease Assessment and Restaging

The selection of testing to be performed pre-HSCT is based upon the potential HSCT patient's underlying disease, and this testing must be completed and results finalized prior to the start of the conditioning regimen. The degree of disease response may influence the choice of conditioning regimen, the type of transplant, and if HSCT is the appropriate choice of treatment modality at that time. Table 6.2 summarizes the restaging workup by disease type.

Confirmatory HLA Typing

Human leukocyte antigen (HLA) typing of the potential HSCT recipient is performed at diagnosis or at time of decision to proceed to an allogeneic HSCT. Prior to moving forward with

Table 6.1 Required pre-HSCT recipient testing

Other	Organ function evaluation	Laboratory evaluation	Infection evaluation
• Disease assessment and restaging	• MUGA • Echocardiogram • EKG	• 24-h urine collection for creatinine clearance	• CT scan of the sinuses, chest, abdomen, and pelvis
• Confirmatory HLA typing for allogeneic HSCT	• Pulmonary function testing	• ABO • Rh • RBC antibody screen • Direct coombs	• Respiratory viral swab – Influenzas A, B – Respiratory syncytial virus (RSV) – Parainfluenzas 1, 2, 3 – Adenovirus – Metapneumovirus – Rhinovirus
	• Dental clearance	• Bun • Creatinine • Electrolytes • Glucose • Calcium • Magnesium • Phosphorus • LDH	• Antibody titers for: – CMV – Epstein-Barr virus – Herpes simplex virus – Toxoplasmosis – Varicella zoster – HIV-1 and HIV-2 – HTLV-1 and HTLV-2 – Syphilis
–	• Ophthalmologic exam	• Liver function tests	–
–	• Neuropsychological testing	• Total protein • Albumin	–
–	• Audiogram	• CBC with differential • Reticulocyte count	–
–	• Nutrition evaluation	• PT/INR/PTT	–
–	–	• Beta-HCG, if applicable	–
–	–	• Serum immunoglobulin G (IgG) level	–

Table 6.2 Summary of restaging workup by disease type

Disease	Restaging
• Leukemia: – Acute myelogenous leukemia – Acute lymphoblastic leukemia – Chronic myelogenous leukemia – Juvenile myelomonocytic leukemia	• Bone marrow biopsy and aspirate: – Cytogenetics – Flow cytometry – Minimal residual disease evaluation • Lumbar puncture: – Dependent upon disease type and history of CNS disease
• Lymphoma: – Hodgkin lymphoma – Non-Hodgkin lymphoma	• PET/CT or CT scan of the chest, abdomen, and pelvis • Bone marrow biopsy: – If history of marrow involvement
• Myelodysplastic syndromes • Myeloproliferative disorders	• Bone marrow biopsy and aspirate: – Cytogenetics – Flow cytometry – FISH
• Bone marrow failure syndromes: – Severe aplastic anemia – Fanconi anemia – Paroxysmal nocturnal hemoglobinuria – Pure red cell aplasia – Amegakaryocytosis – Congenital thrombocytopenia – Diamond-Blackfan syndrome	• Bone marrow biopsy and aspirate – Cytogenetics – Flow cytometry
• Inherited primary immune system disorders: – Severe combined immunodeficiency (SCID) – Combined variable immunodeficiency (CVID) – Wiskott-Aldrich syndrome	• Confirmation of inherited disorder
• Hemoglobinopathies: – Beta thalassemia major – Sickle cell disease	• Hemoglobin electrophoresis • Iron overload evaluation of the liver and heart • % hgb S for patients with sickle cell disease
• Inherited metabolic disorders: – Krabbe disease – Hurler syndrome – Adrenoleukodystrophy – Metachromatic leukodystrophy	• Enzyme levels • MRI of the brain
• Neuroblastoma	• MRI/CT scan • MIBG scan • Bone scan • Urine catecholamines (VMA and HVA) • Bone marrow biopsy and aspirate
• Brain tumors	• MRI of the brain and spine • LP for CSF cytology
• Germ cell tumors	• CT of the chest, abdomen, and pelvis • Tumor markers: – AFP – Beta-HCG

HSCT, transplant centers are required to have this typing completed twice. This confirmatory typing can be completed at any time, but it must be performed on a sample obtained from a different blood draw from the original specimen, and the results must be finalized prior to the start of conditioning.

Organ Function Evaluation

Cardiac Evaluation: Every patient being considered for HSCT undergoes a cardiac evaluation [1, 2]. A complete echocardiogram (EHCO) is performed before the conditioning regimen is begun in order to assess cardiac function and to

evaluate for any heart anomalies. A shortening fraction (SF) of 28% or greater is required before undergoing myeloablative conditioning. If decreased heart function or any other abnormality is detected, then a cardiology consultation may be needed to intervene and optimize the patient's cardiac function prior to undergoing HSCT. A multiple-gated acquisition (MUGA) scan can also be performed, but an echocardiogram is usually sufficient and better tolerated by pediatric patients. An electrocardiogram (ECG) is also performed in order to assess the HSCT candidate for the presence of arrhythmias and to provide a baseline ECG prior to the initiation of the conditioning regimen.

Pulmonary Evaluation: The pulmonary function of a HSCT candidate needs to be evaluated. Pulmonary function testing (PFT), including a diffusing capacity of the lungs for carbon monoxide (DLCO) corrected for hemoglobin, is performed to assess lung function prior to HSCT. DLCO should be >66% [2, 3]. If not, then a pediatric pulmonary specialist should be consulted. Generally, younger children may not be able to cooperate sufficiently when undergoing pulmonary function testing, and thus the results may not be reliable. However, oxygen saturation and symptomatology which are also assessed can be used as a surrogate of pulmonary function for children who are unable to correctly perform PFTs.

Dental Evaluation: Pediatric patients who are preparing to undergo HSCT (both allogeneic and autologous) should be up-to-date on their every 6-month dental maintenance. If not, then a dental assessment and a gentle cleaning should be performed prior to the start of the conditioning regimen. All dental caries need to be adequately addressed before undergoing HSCT. Any signs or symptoms of infection should be reported and completely treated prior to starting any conditioning regimen.

Ophthalmologic Examination: A baseline eye exam is completed on all anticipated allogeneic HSCT recipients prior to conditioning therapy.

Neuropsychological Testing: Children and adolescents whose planned conditioning regimen contains total body irradiation (TBI) should undergo a baseline neuropsychological assessment. This evaluation includes behavioral observations, intellectual ability, academic achievement, adaptive behavior, executive functioning, memory and learning, visual perceptual, behavioral and emotional status, and attention and response regulation.

Hearing Evaluation: An audiogram is performed for those recipients who will receive ototoxic conditioning regimens (e.g., carboplatin). Many of these children have had a baseline audiogram at diagnosis and have had serial tests done throughout therapy. Chemotherapy adjustments in dosing and/or selection can be made if there has been a considerable loss in hearing. This is particularly important in children whose language development is at its peak or earlier (usually <5 years of age).

Nutritional Status: Patients who undergo HSCT (both allogeneic and autologous), particularly when conditioned with a myeloablative regimen, are in a catabolic state, and thus their caloric demand is great. Thus, a nutritional assessment prior to starting the conditioning regimen is important. This evaluation, preferably performed by a registered dietician, should include a diet history, the need for prior supplementation (e.g., oral or nasogastric tube feedings and total parenteral nutrition), and laboratory evaluation including serum albumin and total protein. Every effort to improve a patient's nutritional status should be made prior to HSCT because the majority of patients will require enteral or parenteral supplemental nutrition during the immediate post-HSCT phase.

Laboratory Evaluation

24 h Urine for Creatinine Clearance: To help assess renal function, urine is collected for 24 h, and a creatinine clearance is calculated from this urine collection prior to the start of the conditioning regimen. This evaluation is necessary to ensure medication dosing accuracy and is particularly important when known nephrotoxic agents are part of the planned conditioning regimen or part of the supportive care plan.

ABO/Rh/RBC Antibody Screen/Direct Coombs: Prior to HSCT, a patient's blood type (ABO, Rh) is confirmed, and blood is screened for the presence of red blood cell (RBC) antibodies.

Blood Chemistries and Electrolytes: In addition to checking baseline electrolytes and LDH, BUN and creatinine are checked for baseline renal function. Similarly, baseline liver function tests (LFTs) (transaminases and bilirubin) are obtained prior to HSCT.

Hematopoietic Function: A complete blood cell count (CBC) with differential and reticulocyte count is performed as a baseline.

Coagulation Status: Prothrombin time and partial prothrombin time (PT/PTT) are measured as a baseline prior to HSCT.

Pregnancy Status: Pregnancy is contraindicated for undergoing HSCT. Thus, a beta-HCG test, if applicable, is performed prior to the start of the conditioning regimen.

Serum Immunoglobulin (IgG) Level: A serum IgG level is checked prior to HSCT. IF the IgG level is <400–500, then intravenous immunoglobulins (IVIg) is infused prior to the start of the conditioning regimen, and then continued replacement as per institutional guidelines, but is typically replaced every 2 weeks during the peri- and early post-HSCT period.

Infection Evaluation

CT Scans of Sinus/Chest/Abdomen/Pelvis: CT scans are done pretransplant for a multitude of reasons. While often done as part of the patient's disease restaging, CT scans of the sinuses, chest, abdomen, and pelvis are primarily performed in an anticipated allogeneic HSCT recipient to assess for any underlying infection prior to the start of the conditioning regimen [1, 2]. Any suspicious lesions (particularly of an infectious etiology) need to be investigated further prior to undergoing HSCT. A biopsy of the suspicious lesion may be warranted to establish a diagnosis. The presence of any active infection is an absolute contraindication for undergoing HSCT. The infection must be adequately treated before undergoing HSCT.

Respiratory Viral Swabs: A deep nasal pharyngeal swab is completed on both autologous and allogeneic HSCT candidates within 7 days of starting the conditioning regimen. This specimen is done to detect the presence of influenzas A and B; respiratory syncytial virus (RSV); parainfluenzas 1, 2, and 3; adenovirus; human metapneumovirus; and rhinovirus. The decision to delay HSCT is based upon type of virus isolated and/or symptomatology.

Infectious Disease Titers: It is imperative to evaluate all potential HSCT patients for active and prior infections, particularly latent infections that may be reactivated, while the patient is profoundly myelo- and immunocompromised during the peri- and post-HSCT periods. Infectious disease titers tested prior to starting any conditioning regimens include antibody titers for cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), toxoplasmosis, varicella zoster virus (VZV), HIV-1 and HIV-2, HTLV-1 and HTLV-2, and syphilis.

Fertility Preservation

Fertility should be discussed with parents and with patients who have reached puberty (Tanner stage III) prior to receiving high-dose chemotherapy and/or total body irradiation (TBI). TBI and many of the drugs used in conditioning regimens can cause gonadal failure and premature ovarian failure resulting in permanent infertility. Table 6.3 lists the commonly used drugs that can lead to male infertility and their corresponding cumulative doses. Sperm cryopreservation is highly recommended prior to the start of the conditioning regimen if the patient has not done so already [4]. Similarly, high-dose chemotherapy and TBI are likely to cause permanent ovarian failure in female patients. Fertility

Table 6.3 Commonly used drugs with their cumulative doses that are associated with causing male infertility

Drug	Cumulative dose
• Cyclophosphamide	• 7 g/m^2
• Ifosfamide	• 42–60 g/m^2
• Nitrosoureas-BCNU, CCNU	• 1 g/m^2, 500 mg/m^2
• Melphalan	• 140 mg/m^2
• Busulfan	• 600 mg/m^2
• Procarbazine	• 4 g/m^2
• Cisplatin	• 500 mg/m^2

cryopreservation in female patients may be more difficult due to timing issues and the general invasiveness of the necessary procedures. Ovarian stimulation prior to oocyte procurement is required, and oocyte procurement requires an invasive procedure and is difficult to preserve. An experience reproductive endocrinologist needs to be consulted.

Key Points

- A systematic "checklist" is completed for all HSCT candidates to assess their "fitness" and "appropriateness" to undergo HSCT.
- The pre-HSCT assessment includes an organ function evaluation of the liver, kidneys, heart, and lungs, laboratory evaluation, infection status evaluation, and performance status as well as restaging of the patient's underlying disease or disorder.
- The results of the pre-HSCT assessment are used to customize the HSCT candidate's conditioning regimen.
- If not previously addressed, fertility preservation is discussed with the HSCT candidate and their family as part of the pre-HSCT evaluation.

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7 How to Select a Donor and Hematopoietic Stem Cell Source: Related Versus Unrelated Donors for Allogeneic HSCT

Malika Kapadia and Robert Greiner

Abstract

The selection of the most suitable donor and stem cell source is a critical component of hematopoietic stem cell transplantation (HSCT). The factors that contribute to this selection are many, making the process complex. The most important contributing factor of donor selection and stem source is based on the inherent genetic makeup of the donor as it relates to the HSCT recipient. The cluster of genes that compared for compatibility (termed histocompatibility) contain human leukocyte antigens (HLA) genes located in the short arm of chromosome 6 in humans. These genes are inherited together (i.e., linked), and the most important determinants are HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, and HLA-DPB1. Because a person inherits one HLA cluster (termed locus) from each parent, siblings with the same parents have a 25% chance to be a "match" with the potential HSCT recipient. The process of evaluating histocompatibility is termed HLA matching and can be performed at the antigen and allele level. The allele level is more accurate and thus is termed "high-resolution" typing. Because increased HLA disparity between the donor and recipient increases post-HSCT morbidity and mortality, every effort is made to select the most suitable donor in terms of HLA histocompatibility. Potential hematopoietic stem cell (HSC) sources include bone marrow, peripheral blood after cytokine mobilization, and umbilical cord blood. Each HSC source is commonly used, with bone marrow as the most common source for pediatric allogeneic HSCT. However, umbilical cord blood is used frequently as an alternative source when no suitable related or unrelated matched sibling donor is available. Because of the

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nature of the naïve T cells contained within umbilical cord blood, greater HLA disparity between umbilical cord blood donor source and the potential HSCT recipient is better tolerated than with bone marrow or peripheral blood HSCs. Haploidentical related donors as HSC source is being used more frequently but is still considered investigational in the pediatric population.

Introduction

Hematopoietic stem cells (HSCs) are multipotent cells that give rise to all other blood cell types [1]. Their precursors arise from the mesoderm. Functional hematopoietic stem cells first appear in the human embryonal yolk sac, but, as the fetus grows, the HSCs migrate to the liver and undergo a burst of proliferation [1]. Throughout the remainder of the pregnancy, hematopoietic stem cells are produced in the liver. However, the production transitions to the bone marrow shortly after birth [1].

Hematopoietic stem cells are the main component in the hematopoietic stem cell transplantation (HSCT) product (the HSCT product is also referred to as "graft"). The selection of an appropriate donor source for the recipient is a critical and a complex process that takes into account multiple factors. HSCs can be collected from bone marrow (BM), peripheral blood (PB) after cytokine mobilization, and umbilical vein cord blood (UCB) after delivery [1, 2]. Selection of an HSC donor is unique to the patient's specific disease, the suitability of the donor-recipient match, and the HSC source(s) available. It is crucial to know the advantages and disadvantages as well as the relative risks of each HSC source, both related and unrelated. This chapter addresses how the suitability of a donor is determined and how well "matched" the donor-recipient pair impacts the outcome of the transplant. The impact of HSC source selection, i.e., bone marrow versus peripheral blood versus umbilical vein cord blood, is addressed in Chap. 8.

Human Leukocyte Antigen Nomenclature and Typing

HLA Nomenclature

In order for allogeneic hematopoietic stem cell transplantation (HSCT) to be successful, the donor's immune system must closely resemble (or "match") the recipient's immune system. Otherwise, the recipient will reject the donor's HSCs, blocking repopulation of the recipient's bone marrow, and, conversely, the donor's mature T cells will recognize the recipient as "foreign" and attack the recipient's tissues, causing graft-versus-host disease (GvHD) [2]. Selection of the most suitable donor for an allogeneic HSCT is primarily based on how closely matched the human leukocyte antigens (HLA) are between the donor and the recipient. Thus, HLA typing must be performed for both the recipient and the donor. HLA typing and matching are imperative in HSCT, since disparity in HLA antigens and alleles can result in graft rejection and/or significant morbidity and mortality due to GvHD [2]. The genes encoding these antigens in humans are located on the short arm of chromosome 6 (6p21.3) and consist of approximately 3600 kb of DNA called the major histocompatibility complex (MHC) [2–4]. (*Note:* MHC is another name for HLA; the two are interchangeable.) Figure 7.1 illustrates the relative location of the HLA genes on the short arm of chromosome 6.

Although there are more than 300 genes that contribute to immune response within the HLA system, there are only two major classes that govern the acceptance and/or rejection of a donor HSC graft. The first is MHC Class I whose key members include HLA-A, HLA-B, and HLA-C. The second is MHC Class II whose key members are HLA-DR, HLA-DQ, and HLA-DP [3–7]. MCH Class I and II alleles are highly polymorphic. They are antigen-presenting proteins that display degraded normal intracellular proteins (Class I) as well as foreign proteins derived from bacteria, viruses, and foreign allogeneic cells to T cells (Class II). The presentation of these antigens within the context of "self" results in T-cell recognition, activation, and elimination [4, 8]. In the context of allogeneic HSCT, these T

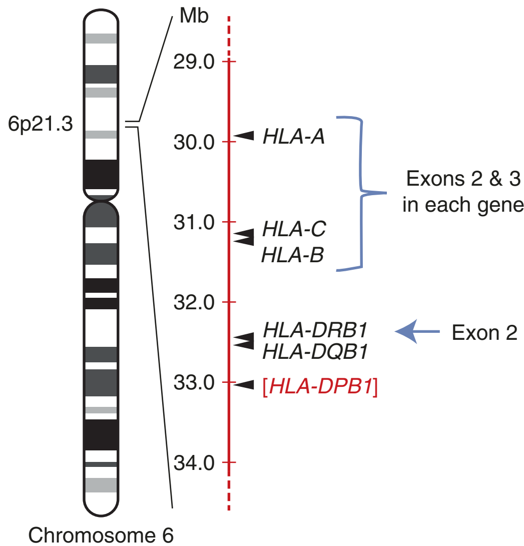

Fig. 7.1 Chromosome 6 and its inheritance of HLA are essential to HSCT and "matching" recipient to their donor. The relative position of the *HLA-A*, *HLA-B*, *HLA-C*, and *HLA-DRB1* genes in the MHC on the short arm of chromosome 6 at band 6p21.3 is indicated. These specific exons within each gene are routinely sequenced in the process of unrelated donor selection for allogeneic HCT. Most transplantation centers do not perform routine sequencing of exon 2 in the *HLA-DQB1* gene, but donor/recipient matching for sequences in this exon is typically performed with medium resolution molecular techniques. The relative position of the *HLA-DPB1* gene (in *red*), which plays a role in histocompatibility in the allogeneic HCT setting but at this time is not routinely sequenced during the donor selection process, is also indicated. This research was originally published in *Blood*. Edus H. Warren et al, Effect of MHC and non-MHC donor/recipient genetic disparity on the outcome of allogenic HCT. Blood. 2012;Vol:120. © the American Society of Hematology. Used with permission

cells provide alloimmunity, i.e., immune response to nonself-antigens, and are the basis for GvHD or graft failure [9].

The HLA Class I and II antigens are heterodimers and are structurally similar molecules. They differ in the polymorphism of their protein-/peptide-presenting domains. The amino acid variation within the antigen-presenting region changes the conformation within the domain, thereby altering peptide-binding specificity [4, 8]. The HLA gene system has the highest number of polymorphisms in humans [4, 6]. Thus far, more than 1300 alleles have been identified among the 12 expressed loci of HLA Class I and II [8]. The alleles may differ by as little as one amino acid caused by a missense mutation. For example, HLA-B has more than 400 known alleles [8]. In order to maintain diversity and create a unique but comprehensive T- and B-cell repertoire for each individual, one needs to maintain this diversity in the population. This uniqueness ensures that the immune system will be able to discern all potentially encountered foreign antigens from self and, therefore, be protected against foreign pathogens. The role of Class I ($CD8^+$) and Class II ($CD4^+$) molecules is to bind to self and/or nonself peptides and present them to $CD8^+$ cytotoxic T cells or $CD4^+$ helper T cells, respectively [8]. During T- and B-cell development, the body will eliminate cells that demonstrate higher affinity to self to avoid autoimmune dysregulation.

Individuals inherit half of their HLA alleles as a single linked locus from each parent, making each child haploidentical to the parent. Figure 7.2 illustrates an example of such an inheritance pattern, demonstrating that each full sibling has a 25% chance being a "match" to the patient at the antigen level. The loci of these HLA alleles are very close together, and, as a result, they are linked and are inherited together in a cluster as a single locus. However, there is a 1% chance of a crossover event to occur between the two HLA loci. Thus, a potential sibling donor matched at the antigen level may not match at the allele level which may have clinical relevance because allele-level mismatches negatively affect survival and increase the risk of developing GvHD. HLA allele frequency is also dependent of ethnic variation. Different ethnic groups often share various yet specific alleles when compared to the general population. As a result, one is more likely to match within the same ethnic group [4, 6, 8, 10]. The alleles are codominant, i.e., both alleles are equally expressed [4, 8, 11]. The genes encoding MHC Class I proteins are telomeric and are expressed on the cell surface of nearly all cells with the exception of erythrocytes (red blood cells) and corneal endothelium [8]. They bind and display peptides derived from cytosolic proteins. Conversely, the genes encoding MHC Class II are centromeric and are only expressed on antigen-presenting cells such as macrophages,

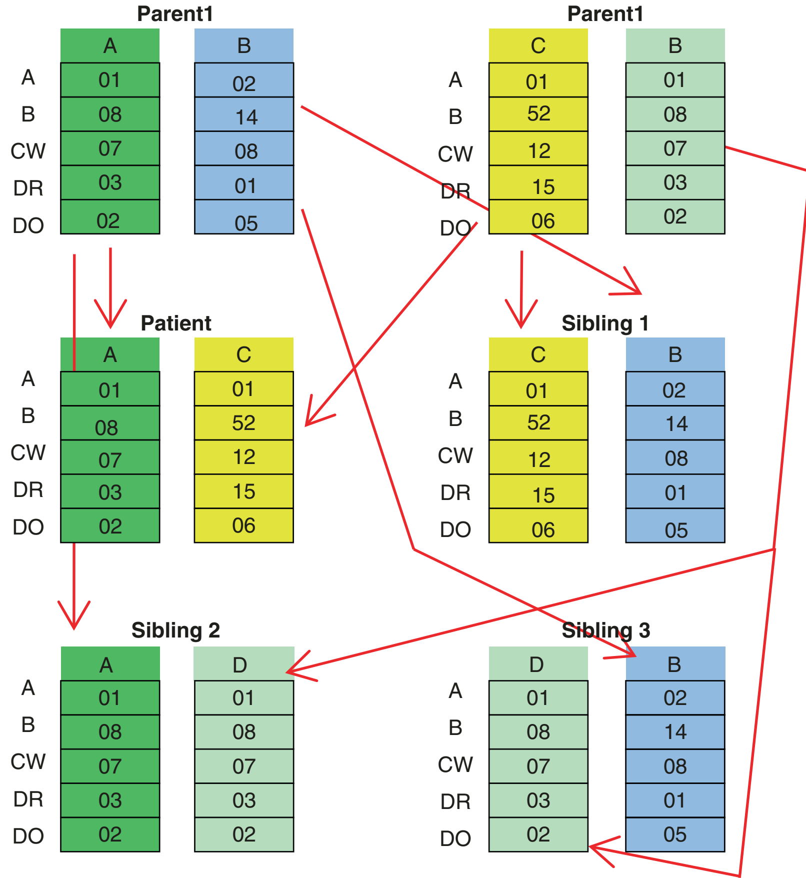

Fig. 7.2 Representation of inheritance pattern of HLA loci. Everyone inherits a cluster of HLA genes, each individual is a haploidentical to their parent

dendritic cells, Langerhans cells, monocytes, and B cells [3, 5, 6, 8]. They present peptides derived from endocytosed proteins from bacteria, viruses, and other foreign molecules.

HLA Typing

The HLA nomenclature was first established in 1968 by the World Health Organization (WHO) Nomenclature Committee [3, 7, 8]. This nomenclature was structured in a way to accommodate expandability, ambiguity, and uniqueness of HLA typing as more genes and alleles are discovered. HLA nomenclature provides information regarding its locus, allele family, amino acid difference, noncoding variations, intron variation, and level of expression [7, 8]. HLA typing was first performed by serology using antiserum that was obtained from pregnant women who were sensitized to paternal HLA haplotypes [8]. The first two digits in the nomenclature are serologically derived. The next two digits (digits three and four) specify the allele sequence. The fifth digit is used when needed to account for any silent polymorphisms. This is followed by the sixth and seventh digits which record any variations identified outside of the coding region [8]. Figure 7.3 represents an example of this nomenclature as it stands today.

Traditionally, HLA nomenclature has been categorized at three different levels of specificity: low, intermediate, and high resolution. The lowest level, which is the least specific, specifies the HLA locus and the antigen family only, e.g., HLA-A*02 [6, 7]. Low-resolution HLA typing is performed by serologic cytotoxicity. Lymphocytes are added to Terasaki plates which contain individual wells with specific antibodies [8, 11]. These antibodies can be synthesized or collected form maternal sera after pregnancy as mentioned above [8]. The best cells to

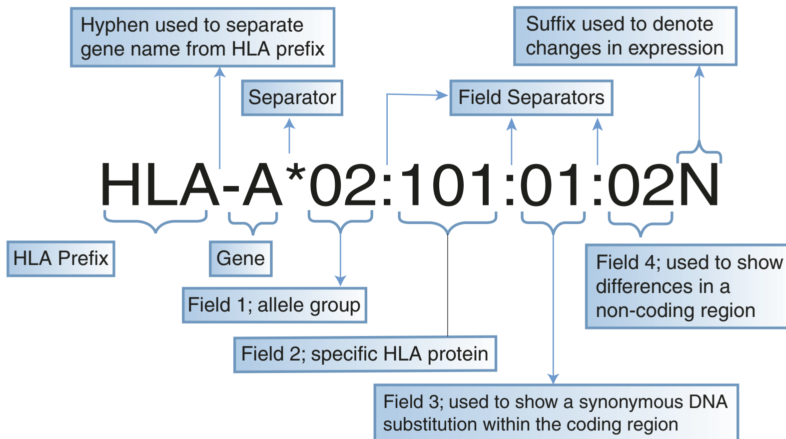

Fig. 7.3 HLA nomenclature. HLA-A*02:101:01:02:N: HLA-A is the locus, separated by an asterisk. The letter at the end is used to denote changes in the allele's expression. This research was originally published in Blood. (Eduardo Nunes et al. Blood 2011;118:e180–e183) Reprinted with permission: Eduardo Nunes et al. Definitions of histocompatibility typing terms. This figure was originally published in Blood. Definitions of histocompatibility typing terms. Blood. 2011; Vol 1:118. © the American Society of Hematology. Used with permission

type Class II are B cells. Class I can be typed with any type of lymphocyte [7, 8, 11]. The reaction between the antibody and the lymphocytes causes cell death, and it is this pattern that identifies the specific HLA antigens that are present for that individual [8, 11]. Intermediate-resolution typing specifies alleles within the HLA family, e.g., HLA-A*02:101 [6, 7, 11]. This typing is performed by flow cytometry whereby lymphocytes isolated from the patient's blood is mixed with fluorescently labeled monoclonal antibodies against HLA antigens. This process tags cells that express a specific HLA antigen that is recognized by the specific anti-HLA antibody [6, 11]. Lastly, high-resolution typing is the most specific and detailed method of HLA typing because it encompasses complete nucleotide sequencing of the HLA gene cluster [4, 6, 7, 8, 11]. This is required for hematopoietic stem cell transplantation, whereas low resolution is sufficient for organ transplantation [8]. High-resolution typing, also known as molecular typing, entails DNA extraction and amplification by polymerase chain reaction (PCR) of the genes that encode HLA-A, HLA-B, HLA-C, and HLA-DRB1 [4, 8, 11].

There are over 72 registries preserving more than 20 million unrelated donor samples that are used for high-resolution typing when indicated. These registries include samples from potential donors of both unrelated bone marrow and umbilical cord blood (UCB) [4, 7]. Low to intermediate typing is performed for all preliminary searches for unrelated donors, whereas high-resolution sequencing is typically reserved once potential donors are identified [4, 8].

Prior to 2002, all donor searches were performed with low to intermediate resolution [9]. From 1988 to 2002, there were multiple improvements in HLA typing methods and its technology [4, 9]. From 1988 to 1992, low-resolution HLA typing using serology was performed with antisera as described above [4, 6, 8, 9, 11]. Sequence-specific primer (SSP), which is high-resolution typing with DNA extraction performed by PCR technology, was introduced in 1993 for HLA Class II typing. In 1996, low-resolution sequence-specific primer (SSP) was introduced for HLA Class I typing [4, 6, 8, 9, 11]. By 2000, high-resolution (HR) HLA SSP typing was used for Class II, and, by 2002, HR HLA typing by SSP and sequence-based typing was available for both HLA Class I and Class II [4, 6, 8, 9, 11]. High-resolution typing for HLA Class I lagged behind HLA Class II due to the genetic complexity of the former (e.g., the presence of double exons that require more probes and

primers proving to be more difficult to perform as compared to HLA Class II) [4]. After 2002, high-resolution (HR) typing was routinely performed for HLA Class I and Class II. With high-resolution HLA typing, serologically indistinguishable but functionally distinct HLA subtypes in MHC Class I and Class II can be distinguished [4, 11]. This transition from low-/intermediate-resolution to high-resolution typing was shown to be the main factor that led to improvement of the overall survival by approximately 25% of recipients of matched unrelated donor (MUD) HSCTs after 2002 (Fig. 7.4) [4, 9]. The donor and recipient that were considered "matched" prior to 2002 often were not a match when retrospectively typed with high resolution [5, 9]. These results illustrate how subtle differences between the donor's and recipient's immune systems can impact outcome.

In general, the current standard for bone marrow and peripheral blood HSC HLA typing is to evaluate the degree of histocompatibility (i.e., match) at five different genetic loci (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1) with ten determinants. However, the practice at many institutions is to use matching at only four genetic loci (HLA-A, HLA-B, HLA-C, HLA-DRB1) with eight determinants, eliminating DQB1 [3, 4]. With UCB, the MHC histocompatibility between the donor and recipient can be more permissive as there is a lower incidence of GvHD due to the immunologic naivety (i.e., tolerance) of the lymphocytes within umbilical cord blood product. Thus, matching at only three genetic loci (HLA-A, HLA-B, HLA-DRB1) with six determinants is considered sufficient [3, 4, 12].

Donor Selection: Matched Sibling Donors Versus Alternative Donors

Once a suitable HLA-matched donor has been identified, then the donor must undergo a complete medical evaluation prior to donation. The evaluation of a suitable identified donor is detailed in Chap. 8.

An HLA-matched sibling donor is considered the optimal HSC donor for allogeneic HSCT, as it is associated with the lowest risk of GvHD and graft rejection [13, 14, 15]. However, only 25% of patients in need of an allogeneic HSCT will have a matched sibling donor (MSD). With the increasing numbers of "blended" families, the likelihood of a patient having an MSD decreases further. As a result, the majority of HSCTs utilize alternative donors including mismatched sibling donor (mMSD) with mismatch at 1 allele,

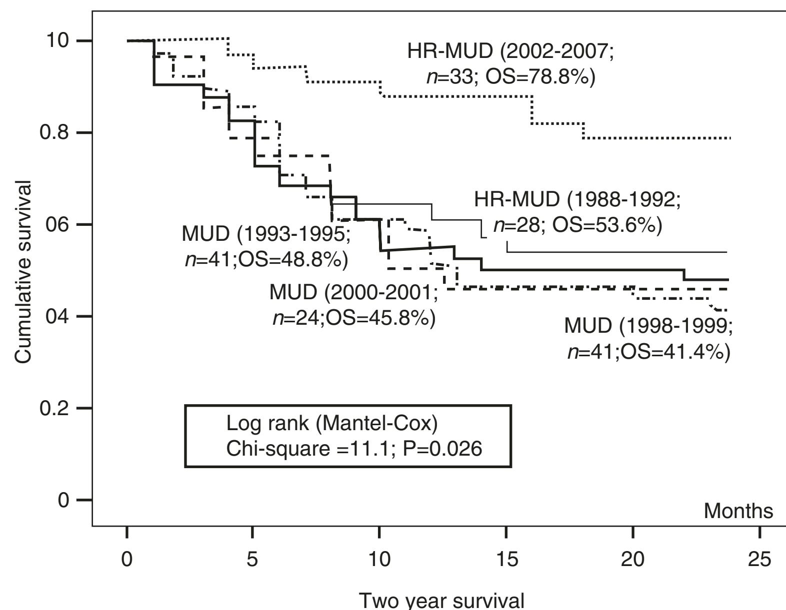

Fig. 7.4 Comparison of overall survival for matched unrelated donor HSCT *over time*. The analysis is over five HLA typing epochs spanning 20 years at a single transplant center for pediatric ALL patients. HR-MUD HSCT OS improved by >25% after 2002, $p = 0.026$. Reprinted by permission from Macmillan Publishers Ltd: Bone Marrow Transplantation (1294–1300; doi:10.1038/bmt.2012.8), copyright 20 February 2012

matched unrelated donor (MUD), mismatched unrelated donor (mMUD) with mismatch at 1 allele, unrelated umbilical cord blood (UCB) (matched versus 1–2 mismatches), and lastly haploidentical donor from a parent or a sibling (see Fig. 7.5: Algorithm for Donor Selection) [4, 5, 13, 14, 15]. These alternative donor HSCTs have been investigated for feasibility and efficiency of engraftment. With advances in immunosuppression and better supportive care to prevent and/or decrease morbidity and mortality related to GvHD, alternative donor HSCT has better success rates now than in the past. Figure 7.6 shows an algorithm used for the selection of an alternative donor when an MSD is unavailable [13–20].

Another important factor regarding donor selection for optimal outcomes in HSCT is the disease state of the recipient [16]. Regarding malignant conditions, if the recipient is not in remission at the time of transplant, then, regardless of HLA-matched donor or type of donor cells, the outcome is inferior as compared to HSCT recipients with no detectable disease at the time of HSCT. As a result, HSCT at an optimal time (i.e., when the recipient is in remission/has no detectable disease) may restrict availability of certain types of donors and/or donor HSC sources. For example, if the recipient does not have a matched sibling, it typically takes at least 2–4 months to identify and work up a matched unrelated donor. This may not be feasible for patients with high-risk disease, as the disease can progress while trying to finalize a donor. As a result, the availability of alternative sources such as UCB should be investigated since this HSC source is readily available: UCB is typed prior to cryopreservation and is available for use typically within 2–3 weeks and sometimes sooner [19–21]. Alternatively, haploidentical donors are more readily available than both MUD and

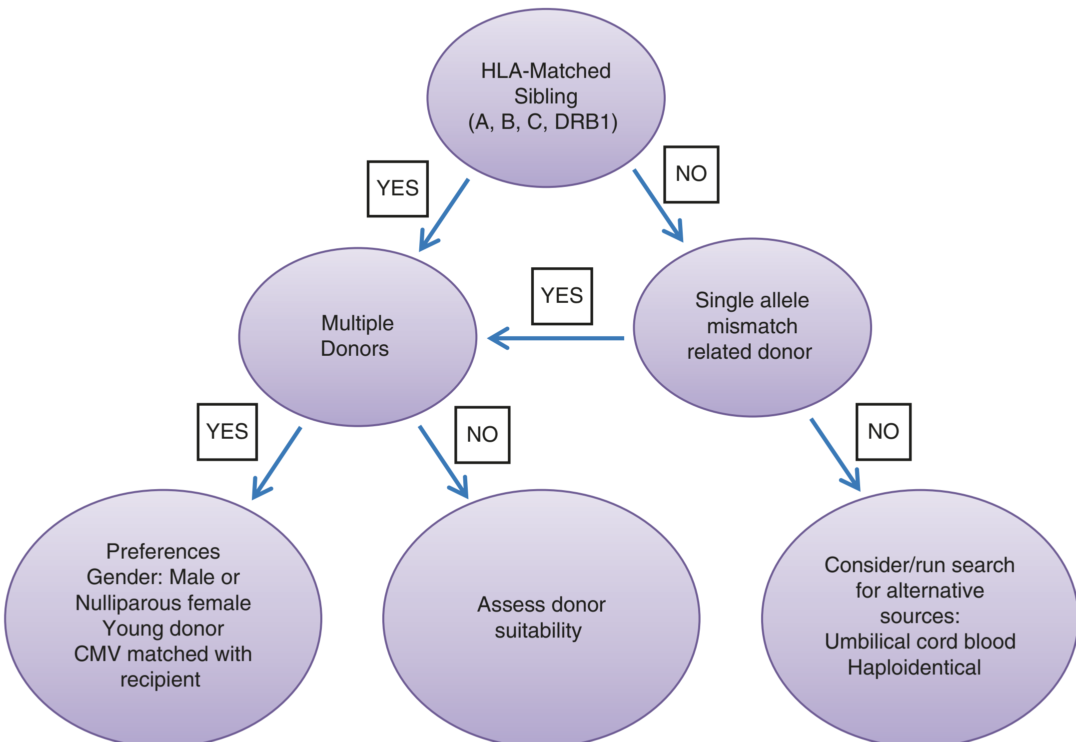

Fig. 7.5 Algorithm for donor selection. This figure illustrates the steps used to identify the most suitable donor. It is usually useful to conduct a search for an unrelated donor at the same time to prevent delay in case an appropriate well-matched related donor is not available

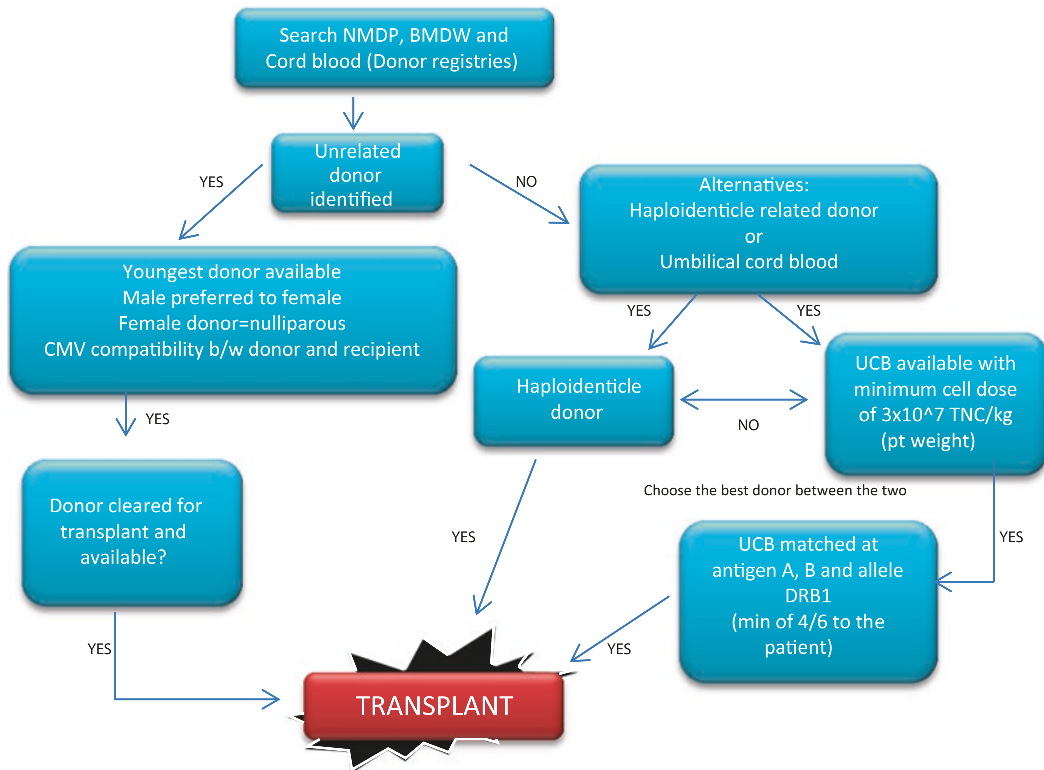

Fig. 7.6 General algorithm for selection of alternative HSC donors if matched sibling not identified or unavailable for donation

UCB but have historically been associated with unacceptable rates of GvHD and/or graft rejection and still considered investigational in the pediatric setting.

Donor Availability

The National Marrow Donor program (NMDP) was first established in 1986. Over the years, it has grown to 10.5 million adult donors and about two hundred thousand cord blood units banked [10]. Over the past few decades, the registered number of HSCTs facilitated by the NMDP has increased due to the vast donor pool. In 2002, the NMDP assisted in six thousand unrelated transplants compared to fifteen hundred in the decades past [10].

Despite the exponential growth in the donor pool, the availability and the likelihood of finding a matched unrelated donor are largely dependent on the ethnicity/race of the recipient [10, 12, 22]. For example, a potential HSCT patient of northern European descent has a much higher probability of having a suitably matched unrelated donor compared to other ethnic groups such as African American or Hispanic due to the underrepresentation of these populations in the donor pool [12, 22]. As seen in Table 7.1, a Caucasian recipient of northern European ethnicity has a 75% chance of having an 8 out of 8 HLA-matched donor and a 97% chance of having a 7 out of 8 HLA-matched donor [10]. On the other hand, a patient that is African American has only a 16–19% chance of having an eight out of eight HLA-matched donor and a 66–76% chance of having a 7 out of 8 HLA-matched donor [10]. This is also applicable to umbilical cord blood units available (e.g., 87% chance for a five out of six matched UCB donor available for a patient of Northern European descent versus 56–58% chance for a 5 out of 6 matched

Table 7.1 Summary of unrelated adult donor and umbilical cord blood availability by ethnicity/race and degree of histocompatibility

US racial or ethnic group	Likelihood of identifying an adult donor		Likelihood of identifying a cord blood unit for patients <20 years old		
	8/8 HLA match	≥7/8 HLA match	6/6 HLA match	≥5/6 HLA match	≥4/6 HLA match
White European	75	97	38	87	99
Middle Eastern or N. African	46	90	18	75	98
Native American (North, Central or South, Caribbean, Alaskan)	32–52	77–91	14–26	66–86	97–98
Asian and Pan Pacific (Chinese, Korean, Filipino, South Asian, Vietnamese)	33–42	83–88	12–20	70–77	97–98
Hispanic, Mexican	34–40	80–87	17–19	71–75	98
Southeast Asian	27	76	12	70	98
African American, Black South or Central American, Black Caribbean	16–19	66–76	5–7	56–58	95–96

Adapted from Gragert et al., NEJM, 2014, 371:339–348

UCB available for a patient of African American descent) [10]. The likelihoods of finding a suitable MUD for patients of other ethnicities and races, including Native American, Asian, Southeast Asian, and Hispanic, fall in between those of Northern European and African American descent. As ethnic and racial diversity increases, the donor pool becomes more limited. The issue of HSCT for underrepresented ethnicities and races goes beyond just the identification of a potentially suitable donor because not all identified potential donors will actually end up donating HSCs. Identified potential donors need to be contacted and have confirmatory testing done if they are still willing to donate. Once HLA typing has been confirmed, then the potential needs to be medically cleared for donation. Finally, the HSCs are then collected. Attrition occurs at every step of this process from the identification to actual donation of HSCs (see Fig. 7.7), and this attrition rate varies by ethnicity and race at almost every step of this process (see Table 7.2). The attrition rate is highest for donors of African American descent which results in the lowest overall availability of only 23%. As such, it is imperative to have alternative strategies and donor sources identified sooner rather than later and to encourage underrepresented minorities to register as donors with the NMDP and to donate UCB [12, 22].

Once a donor is selected and HSCs are infused, engraftment is what defines a successful HSCT (see Chap. 10). Briefly, engraftment is defined by neutrophil and platelet recovery, whereas immune reconstitution refers to T- and B-cell function recovery. Engraftment is critical for patient survival. Without it, survival is dismal due to death from infectious complications or uncontrolled bleeding. If there is graft failure, then patients frequently must undergo an emergent second HSCT in order to provide a working immune system and a chance at both cure and survival. Graft failure is a rare complication in HSCT and is discussed in detail in Chap. 11. One can have primary graft failure which is the lack of initial engraftment and/or autologous stem cell recovery without evidence of donor engraftment, or one can have secondary graft failure which is usually due to an infection insult (e.g., CMV, HHV6, adenovirus, parvovirus) resulting in the loss of initial graft and possible autologous recovery [18, 23–25]. As HLA disparity increases, the probability of graft failure also increases. The overall chance of graft failure is approximately 5–20%, and the probability is higher after an UCBT than an MSD or MUD (e.g., 10–20% versus 2% versus 9–12.3%, respectively) [13, 14, 17, 18, 23–25].

In general, platelet recovery lags behind neutrophil recovery by at least a week. Compared to

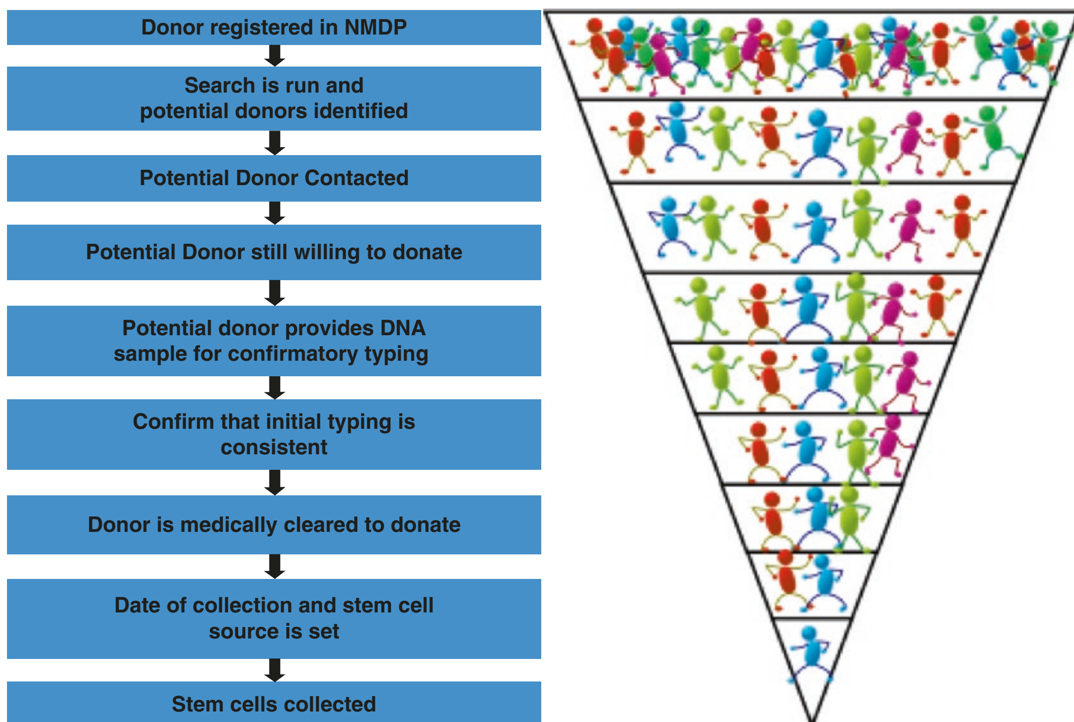

Fig. 7.7 Donor availability and attrition of actual unrelated donors. This figure demonstrates how a donor is selected for the recipient, which also represents the attrition down to the selection of the "most suitable" unrelated donor

Table 7.2 Summary of attrition of actual adult donors by race and ethnicity

Broad US racial and ethnic groups	% available for confirmatory HLA typing	% HLA typing NOT discrepant	% medically cleared and agree to donate	% overall availability
White	62	98	83	51
Black	36	95	69	23
Asian or Pacific Islander	42	97	73	29
Hispanic	44	96	68	29
Native American	45	98	98	28

Adapted from Gragert et al., NEJM, 2014, 371:339–348

MSD and MUD HSCT, UCB transplantation is associated with a slower recovery of both neutrophils and platelets [15, 18–22, 26–31]. This delay is likely due to limited cellularity/lower $CD34^+$ cell count (hematopoietic stem cell marker) and the immaturity of cord blood cells as compared to BM or PBSC used in MSD and MUD [18, 30, 32]. However, it is this naivety that allows for increased HLA disparity between the donor and recipient without worsening morbidity and mortality from GvHD, thus increasing the donor pool for under-represented ethnic and racial populations as mentioned above [3, 4, 10, 18, 22, 32]. On average, patients who undergo MSD HSCT have faster neutrophil engraftment (defined as a sustained ANC > 500) as compared to MUD HSCT (17 days versus 22 days) [15, 18, 27, 33]. Platelet recovery occurs on average 22 days after MSD HSCT versus 32 days after MUD HSCT (see Table 7.3) [15, 18, 27, 33]. Ninety-eight percent of HLA MSD HSCTs show neutrophil recovery by day 42 post-HSCT, as compared to 97% with MUD and 83% with UCB HSCT [13, 15, 17]. Similarly, platelet recovery by 100 days post-HSCT is 90%, 80%, and 65%, for MSD, MUD, and UCB HSCTs, respectively [13, 15, 17] (see Table 7.3). Delayed

Table 7.3 Timing from HSCT to engraftment by hematopoietic stem cell source

	MSD (days)	MUD (days)	UCB (days)
Neutrophil recovery (ANC > 500/μL)	16–18	22–24	25–28
Platelet recovery (>20,000/μL)	20–22	30–34	50–54

engraftment certainly increases the risk for treatment-related morbidity and mortality (TRM) with increased risk of infection and graft rejection [13, 14, 26]. However, given that most patients will not have a matched sibling donor, alternative donor stem cell sources (often mismatched) are frequently used because it is the sole option available, and thus, the risks of graft rejection and/or TRM supersede the option of not undergoing HSCT altogether for patients who will otherwise die emergently from their underlying disease. This is especially true for minority and mixed ethnicity patients who are underrepresented in the unrelated donor and UCB registries [19].

The incidence of grade II to IV acute GvHD at 100 days post-allogenic HSCT is less frequent in recipients of an MSD HSCT as compared to haploidentical and MUD HSCT [13, 14, 16, 17, 34, 35]. The incidence of severe, life-threatening acute GvHD is typically worse in haploidentical and MUD as compared to MSD HSCT [13, 14, 16, 17, 34, 35]. UCB transplantation, even in the cases with higher HLA disparity, is associated with a lower incidence and severity of both acute and chronic GvHD as compared to MUD and haploidentical HSCT [19, 20, 22, 26, 28, 29]. Similar to other sources, the incidence of GvHD in UCB recipients increases as HLA disparity increases. However, this is typically less frequent and less severe as compared to MUD, PBSC, haploidentical, and MSD HSCTs [19, 20, 22, 26, 28, 29].

Severe GvHD accounts for much of the morbidity and mortality during the post-HSCT period. However, GvHD has also shown improved survival due to underlying graft versus leukemia (GVL) effect. GvHD is thought to promote natural killer cell alloreactivity that induces GVL, thereby increasing the likelihood of preventing post-HSCT relapse [16, 17, 34, 36]. The goal is to balance the risk of GvHD with the benefit of GVL.

HLA mismatch directionality significantly impacts the likelihood of an HSCT recipient of developing GvHD and graft rejection/failure. For example, HLA DRB1 mismatch is associated with an increased incidence of GvHD, whereas HLA-C mismatch is associated with increased incidence of graft failure. Although any mismatch increases the risk of morbidity and mortality posttransplant, these two associations have been seen consistently in several past studies [37–40]. The two potential directional vectors are graft versus host (GvH) and host versus graft (HvG), depending on missing shared alleles [12, 22, 37–40]. In the GvH HLA mismatch (e.g., HLA DRB1 disparity) scenario, the T cells from the donor graft mount an immune response against the recipient because the donor T cells recognize the mismatched recipient's antigens as foreign [12, 22]. Conversely, when residual recipient T and NK cells recognize the incoming donor HSCs as "foreign," they mount an immune response that leads to graft rejection, i.e., HvG response [12, 22].

Treatment-related morbidity and mortality (TRM) are more likely to occur with a mismatched related donor, MUD and UCB as compared to MSD [13–15, 35]. It has been reported that the 3-year post-HSCT TRM is 10% with MSD (95% CI 8–12%), 24% with MUD (95% CI 19–30%), and 27% with mismatched related donor (mMRD) (95% CI 20–34%) [13–15, 35]. Treatment-related mortality is often higher in UCBT as compared to MUD and haploidentical HSCT due to slow initial engraftment associated with UCBT [12–15, 22, 35]. The relapse rates after MUD and UCB HSCT are comparable once engraftment has occurred, though TRM is often higher in UCBT due to delayed engraftment as mentioned above [19, 26].

In an international multicenter trial, overall survival (OS) and disease-free survival (DFS) were shown to be comparable between MSD HSCT and MUD HSCT [13–15, 28, 35]. Four-year disease-free survival was 67% versus 71% in MUD and MSD, respectively [13–15, 28, 35]. There was no statistical difference in outcome between MUD HSCT with 9/10 or 10/10 HLA matches [15]. The overall survival in patients with leukemia who undergo HSCT has been found to be inferior with UCB as compared to MUD and MSD HSCT and is felt to be secondary to increased TRM from delayed

engraftment which increases infection risk [19, 21, 22, 26, 27]. Other factors such as delayed immune reconstitution and possibly a less robust GVL effect are associated with decreased overall survival in UCB recipients. In some studies, however, once engrafted, the disease-free survival of UCBT recipients is comparable to those who underwent MUD or MSD HSCT [19, 21, 22, 26, 27].

HSC Source Options and Selection

The actual procurement and processing of HSCs from bone marrow, peripheral blood, and umbilical vein cord blood are described in detail in Chap. 8.

Summary

In summary, outcomes following allogeneic HSCT for both malignant and nonmalignant disorders have improved tremendously over the past several decades. While a 10/10 MSD bone marrow HSCT is the first choice of donor and stem cell source for pediatric patients, there are alternative donor transplant options available today that were not available in the past. These include UCB, especially for recipients of various ethnic and racial backgrounds that are underrepresented in the bone marrow and UCB registries. The option of using haploidentical donors is being used more frequently nowadays due to better supportive care and GvHD prophylaxis and treatment available today. Lastly, more precise HLA typing at the molecular level as a result of improved technology (i.e., high-resolution sequencing) has directly contributed to improved outcomes post-HSCT over the past 20 years. However, despite all of these advancements, there continues to be significant morbidity and mortality after HSCT. As a result, further work is needed to find the appropriate balance between the risks and benefits of each donor for both malignant and nonmalignant diseases in an effort to minimize morbidity and mortality while maximizing outcomes.

Key Points

- Selection of the most suitable donor is a critical but complex process.
- Human leukocyte antigens (HLA) genes are located on the short arm of chromosome 6 and are inherited together in a cluster.
- The most important HLA determinants are HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, and HLA-DPB1.
- Increased HLA disparity increases posttransplant morbidity and mortality.
- Advancements in HLA typing, particularly the ability to perform high-resolution typing, have resulted in improved overall survival of patients.
- Hematopoietic stem cell sources include bone marrow (most common in pediatric HSCT patients), peripheral blood after cytokine mobilization, and umbilical cord blood. Each HSC source has its advantages and disadvantages. Currently, the HSC source and ideal donor is bone marrow from a matched sibling. Umbilical cord blood is often used as an alternative (even as a mismatch) when no matched sibling or unrelated donor is available.

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8 Donor Evaluation, Selection and Hematopoietic Stem Cell Mobilization, Procurement, and Manipulation

William Ferguson and Aleksandar Babic

Abstract

Donor selection for HSCT is determined by eligibility, suitability, and availability. Donor *eligibility* is mandated by the regulatory requirements designed to minimize the risk of transmitting infectious diseases from the donor blood product to the HSCT recipient. In contrast, donor suitability is a clinical concept: It involves the incorporation of various factors unique to hematopoietic stem cell transplantation (HSCT), with histocompatibility between the donor and recipient the most important. While many suitable donors may be technically "ineligible," HSCT may proceed with proper documentation. The most common scenario is for autologous donors, for whom disease transmission into a disease-naïve donor is not an issue. Selection of a preferred donor must incorporate the availability of potential donors because HSCTs are often time sensitive. Hematopoietic stem cells (HSCs) can be harvested successfully from bone marrow, from umbilical cord blood (which is a rich source of HSCs), and from peripheral blood following cytokine stimulation +/− plerixafor. Each source has its unique set of advantages and drawbacks. Successful engraftment is dependent upon HSC dose which varies by HSC source. A minimum of 2–2.5 × 10^6 CD34+ cells/kg recipient weight is typically required for successful engraftment following bone marrow or peripheral stem cell transplantation. Minimal cell doses for umbilical cord blood are approximately tenfold lower (~2–2.5 × 10^7 total nucleated cells/kg recipient weight), which would typically contain ~2 x 10^5 CD34+ cells/kg recipient weight). Special processing may be required for some donor units; ABO incompatibility between the donor and recipient is one of the most common reasons for special processing. Cryopreservation can allow storage of HSC products for years or even decades. There are many reactions that can occur as a result of HSC infusions, and so careful observation and prompt intervention to treat any reactions are essential.

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Donor Selection and Evaluation

Eligibility, Suitability, and Safety of the Donor

A central component of stem cell donation involves ensuring the *eligibility*, *suitability*, and *safety* of the donor from the beginning through the completion of the collection process. Minimal requirements in these areas are set by various regulatory agencies such as the Food and Drug Administration (FDA) [1] and the Foundation for Accreditation of Cellular Therapy (FACT) [2], although details of implementation can vary among institutions.

The criteria for donor *eligibility* are derived directly from the guidelines used by the FDA to minimize the risk of transmitting infectious diseases from donors of blood or other cellular tissues to the recipients of those products. Eligibility is determined by a combination of medical history and direct testing for the presence of infectious agents (also defined by FDA as *Relevant Communicable Agents and Diseases*). For the vast majority of donated cellular products (e.g., red blood cells and platelets), the donor pool is large and the eventual recipient is unknown, so the regulations are very conservative in order to minimize the risk of having a product that is potentially infectious, even when the magnitude of risk is actually quite small (an example is the exclusion of potential donors who have lived in Europe during outbreaks of mad cow disease).

Donor *suitability* is a broader concept, encompassing the issue of whether HSCT is going to provide an adequate and safe replacement for the recipient's own hematopoietic system and will provide adequate treatment of the underlying disorder. This universally includes the ability of the graft to maintain adequate hematopoiesis (production of erythrocytes, platelets, and granulocytes) and also to reconstitute immune function, while minimizing the potential that the new immune system will react against the recipient and lead to graft-versus-host disease (GvHD). However, in specific circumstances, additional issues may become relevant: For instance, when HSCT is intended to correct an inborn error of metabolism, it would be important to know that the donor cells will provide an adequate level of the missing enzyme [3].

There are also additional infectious disease considerations when evaluating a potential stem cell donor above those involved in determining eligibility. An example that is frequently encountered involves cytomegalovirus (CMV). An individual who becomes infected with CMV will permanently carry a reservoir of CMV-infected leukocytes that could potentially infect an immunocompromised recipient. However, prior CMV infection does not make a donor ineligible for standard blood product donation, especially since the risk of CMV infection from cellular products such as red blood cell or platelets can be minimized by filtration to remove the infected leukocytes [4]. Because leukocyte reduction would also remove the HSCs, it is clearly not applicable to products intended for HSCT, and so CMV-positive products cannot be made "CMV-safe." Thus, careful consideration of both donor and recipient CMV status becomes very important in determining the risk of viral transmission.

As discussed in Chap. 7, the prime determinant of donor suitability is HLA compatibility. For most transplants, a fully matched related donor (usually sibling, occasionally parent or child) is ideal. Because the genes in the HLA cluster are relatively close together, recombination is a rare event and haplotypes are almost always transmitted intact from parent to child. As a result, the probability that two full siblings will be complete HLA matches is ~25%, and the odds of finding a complete match will obviously increase when there are more full siblings. However, with the decrease in average family size over the past several decades and an increasing number of "blended" families, only a small minority of transplant candidates will have a fully matched sibling [5]. For treatment of inherited diseases such as sickle cell disease or thalassemia, the potential donor would also need to be free of the disease, which further decreases the odds of finding a suitable family donor.

Enormous antigenic diversity is one of the hallmarks of the HLA system; thus, the odds are very low that two randomly selected individuals will be complete or near-complete matches. With the development of large donor registries that have millions of potential donors (such as the National Marrow Donor Program's "Be The Match" registry and similar ones worldwide), the overall probability of finding a suitably matched unrelated donor

has increased [6]. However, because there are differences in the distribution of HLA antigens among different ethnic and racial groups, the probability of finding a complete HLA match also depends upon the ethnicity and race of the recipient, and, for certain groups (such as African-Americans), the number of complete HLA matches within the registries may be quite limited [7, 8]. Even for ethnicities and races with greater representation in the donor registries, other donor characteristics (e.g., CMV status as described above) may serve to reduce the number of suitable donors to just a few.

Because the number of suitable donors may be extremely small, one can have a situation where the most suitable donor (or, in extreme cases, the *only* suitable donor) is not considered *eligible* by the usual donor criteria. This can arise either where the actual infectious risk is small (e.g., recent receipt of a tattoo or the mad cow disease example described above) or where the recipient is already infected with the disease that makes the donor ineligible (e.g., hepatitis B). Because the majority of HSCTs occur in situations where the recipient's disorder carries an imminent risk of mortality or severe morbidity, the potential benefit of proceeding with HSCT from an ineligible donor may well outweigh the (presumably small) risk of introducing a new infection into an immunocompromised host. From an operational perspective, proceeding with such an HSCT requires the treating physician to document that the recipient's medical condition represents a medical emergency that requires use of an ineligible product. In addition, FACT requires that both the donor and recipient be informed of the nature of the condition leading to the ineligible status and that both donor and recipient provide written consent that they are willing to assume the risk of transmitting (for the donor) and receiving (for the recipient) the specified condition and agree to proceed with the donation and transplant [2].

A final aspect of donor suitability overlaps with the issue of donor safety; namely, the potential for being able to donate a sufficient number of hematopoietic stem cells to insure timely and adequate engraftment in the recipient. In most situations, this requires administration of a minimum of $2–2.5 \times 10^6$ CD-34+ mononuclear cells per kilogram of recipient body weight. As discussed in more detail below, a large weight discrepancy between a small donor and large recipient may affect the feasibility of obtaining a sufficient number of HSCs, especially when bone marrow is the stem cell source.

Acquisition of HSCs involves a medical procedure (either bone marrow harvest under anesthesia or collection of peripheral stem cell by apheresis) with some degree of associated risk and discomfort. Because the allogeneic donor derives no direct benefit from the procedure, it is vital to make sure that the risks are both minimized and acceptable. Evaluation of donor safety starts with a medical history with a special focus on conditions that might make anesthesia or apheresis unusually risky for the donor. Obviously, there will be overlap between a history focused on donor safety during the collection process and one focused on identifying potential risks to the recipient, and so it is often most efficient to cover all aspects concurrently (see Fig. 8.1 for an example) [9–11]. Note that recommendations for donor screening can change quickly, as illustrated by the recognition of Zika virus as a potential blood-borne pathogen in early 2016 [12]. Similarly, a physical exam of the donor is required to help identify medical conditions that might affect the assessment of the donor's suitability to donate and to preclude conditions that might increase the risk involved with donation. Both ethics and regulations dictate that both the donor exam and the subsequent determination that it is safe for the donor to undergo the HSC collection procedure be performed by a physician who is not part of the recipient's HSCT team [2].

Laboratory evaluation of the donor includes both infectious disease markers (IDM) that are included as part of the eligibility determination mandated by the FDA [13], as well as an assessment of relevant organ function (e.g., liver and kidney function) and information on additional infectious diseases that might be relevant for the HST recipient (e.g., EBV status) (see Table 8.1). Additional donor assessment, such as chest X-ray or EKG, may be indicated especially in older patients undergoing anesthesia as part of the collection.

By FDA regulations, IDM testing for determination of eligibility must be performed within 30 days of HSC collection and the results available prior to collection [2]. Results must also be available prior to the start of the recipient's conditioning regimen because an unexpected positive

marker might indicate that the intended donor is no longer suitable, potentially resulting in a situation in which a recipient's own hematopoietic system has been ablated, and yet there are no stem cells available for hematopoietic reconstitution [2]. With the advent of some longer reduced-intensity conditioning regimens that extend over several weeks, repeat testing of the donor may be required in order to meet both requirements.

Final medical clearance is required just prior to any HSC collection procedure and should include both interval history, physical exam, and a pre-col-

Stem Cell Donor – Medical History Form

Date: ____________

Name: ____ **Birthdate:** ______________

Address: ____

Phone Number: ________________________________

The following questions are used to obtain any information that may help us determine if your blood contains any infections that may be passed to the person receiving your stem cells. The Stem Cell Transplant Coordinator will review your answers with you. All information is confidential.

If you have never participated in sex with another person (for instance, if the donor is a child), answers to questions related to sex activity will be "no".

Please answer yes or no for every question. If the answer is yes to any question, please circle that question and write the date or year it happened or provide other information as requested.

	Yes	No
1. Are you currently taking an antibiotic?	☐	☐
2. Are you currently taking any other medication for an infection?	☐	☐
3. Have you ever taken any of the follow medications? If so, please indicate when the last dose was taken: ☐ Growth Hormone from Human Pituitary Glands—used usually for children with delayed or impaired growth ______ ☐ Hepatitis B Immune Globulin –given following an exposure to hepatitis B (Note: This is different from the hepatitis B Vaccine which is given to prevent future infection ______ ☐ Insulin from Cows (Bovine, or Beef, Insulin)—used to treat diabetes ______ ☐ Any experimental or unlicensed medication or vaccine ______	☐	☐
4. Female donors: Have you been pregnant or are you pregnant now? (Males: check here: ☐)	☐	☐
At any time in the past **12 weeks, have you:**		
5. Had any vaccinations or other shots?	☐	☐
6. Had contact with someone who had a smallpox vaccination?	☐	☐
In the past **6 months have you:**		
7. Had a Zika virus infection?	☐	☐
8. Lived in or traveled to an area with active Zika virus transmission? (Review the list of Zika virus areas of transmission)	☐	☐
9. Had sexual contact with a man, who in the **6 months prior to sexual contact**, has had a Zika virus infection or lived in or traveled to an area with active Zika virus transmission?	☐	☐
In the past **12 months have you:**		
10. Had a major illness or surgery?	☐	☐
11. Been told by a healthcare professional that you have West Nile virus infection or any positive test for West Nile Virus?		
12. Had a blood transfusion?	☐	☐
13. Come into contact with someone else's blood?	☐	☐

Fig. 8.1 An example of a questionnaire designed to cover both donor eligibility and medical conditions that might relate to donor safety. Items that are taken either directly from the current version of the HPC, Apheresis and HPC, Marrow Donor History Questionnaire, version 1.5 or its supporting documents [10] are in *black*. Supplemental items that are solely directed at donor suitability for donation are in *red*

Stem Cell Donor – Medical History Form

Name: Birthdate:	Yes	No
In the past **12 months have you:**		
14. Had acupuncture or an accidental needle-stick?	☐	☐
15. Had a transplant or graft from someone other than yourself, such as an organ, bone marrow, stem cell, cornea, sclera, bone, skin or other tissue?	☐	☐
16. Had sexual contact with anyone who has HIV/AIDS or has had a positive test for the HIV/AIDS virus?	☐	☐
17. Had sexual contact with a prostitute or anyone else who takes money or drugs or other payment for sex?	☐	☐
18. Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything not prescribed by their doctor?	☐	☐
19. Had sexual contact with anyone who has hemophilia or has used clotting factor concentrates?	☐	☐
20. Female donors: Had sexual contact with a male who has ever had sexual contact with another male? (Males: check here: ☐)	☐	☐
21. Had sexual contact with a person who has hepatitis?	☐	☐
22. Lived with a person who has hepatitis?	☐	☐
23. Had a tattoo?	☐	☐
24. Had ear or body piercing?	☐	☐
25. Had or been treated for syphilis, gonorrhea or other sexually transmitted infection?	☐	☐
26. Been in juvenile detention, lockup, jail, or prison for more than 72 hours?	☐	☐
In the past **three years have you:**		
27. Been outside the United States or Canada?	☐	☐
In the past **five years have you:**		
28. Received money, drugs, or other payment for sex?	☐	☐
29. Male donors: had sexual contact with another male, even once? (Females: check here: ☐)	☐	☐
30. Used needles to take drugs, steroids, or anything not prescribed by your doctor?	☐	☐
31. Used clotting factor concentrates?	☐	☐
From **1980 through 1996:**	☐	☐
32. Did you spend time that adds up to three (3) months or more in the United Kingdom (England, Scotland, Northern Ireland, Wales, the Isle of Man, Channel Islands, Gibraltar, Falkland Islands)?	☐	☐
33. Were you a member of the U.S. military, a civilian military employee, or a dependent of a member of the U.S. military? If so, please answer questions 34 and 35.	☐	☐
34. From 1980 through 1990, did you spend a total time of 6 months or more associated with a military base in any of the following countries: Belgium, the Netherlands, or Germany?	☐	☐
35. From 1980 through 1996, did you spend a total time of 6 months or more associated with a military base in any of the following countries: Spain, Portugal, Turkey, Italy, or Greece?	☐	☐
From **1980 to the present, did you**		
36. Spend time that adds up to five (5) years or more in Europe? (Albania, Austria, Azerbaijan, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Republic of Ireland, Italy, Liechtenstein, Luxembourg, Macedonia, Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, the United Kingdom, Federal Republic of Yugoslavia (Serbia and Montenegro))	☐	☐
37. Receive a blood transfusion in the United Kingdom or France?	☐	☐

Fig. 8.1 (continued)

Stem Cell Donor – Medical History Form

Name: Birthdate:	Yes	No
Have you **EVER:**		
38. Had a positive test for the HIV/AIDS virus?	☐	☐
39. Been refused as a blood donor or told not to donate?	☐	☐
40. Had hepatitis or a positive test for hepatitis?	☐	☐
41. Had malaria?	☐	☐
42. Had any other parasitic disease or positive test, including Chagas Disease (*T cruzi)* or babesiosis? (If so, please describe:____)	☐	☐
43. Tested positive for HTLV, had adult T-cell leukemia, or had unexplained paraparesis (partial paralysis affecting the lower limbs)?	☐	☐
44. Received a dura mater (or brain covering) graft?	☐	☐
45. Had sexual contact with anyone who has born or lived in Africa?	☐	☐
46. Been in Africa?	☐	☐
47. Been diagnosed with any neurological disease?	☐	☐
48. Had a transplant or other medical procedure that involved being exposed to live cells, tissues, or organs from an animal?	☐	☐
49. Has your sexual partner or a member of your household ever had a transplant or other medical procedure that involved being exposed to live cells, issues, or organs from an animal?	☐	☐
50. Had any type of cancer, including leukemia?	☐	☐
51. Had any problem with your heart or lungs?	☐	☐
52. Had a bleeding condition or a blood disease?	☐	☐
53. Had a severe allergic reaction?	☐	☐
54. Had asthma?	☐	☐
55. Required treatment for elevated blood pressure?	☐	☐
56. Been diagnosed with diabetes?	☐	☐
57. Been diagnosed with an autoimmune disorders? (Includes any of the following: Rheumatoid arthritis (RA), Systemic lupus erythematosisus (SLE), Fibromyalgia, Multiple sclerosis, Psoriasis, Vitiligo, Guillan-Barre syndrome, Idiopathic thrombocytopenic purpura (ITP), Antiphospholipid syndrome (APS), Sjogen's syndrome, Iritis, Episcleritis, Crohn's disease, Ulcerative colitis, Raynaud's phenomenon, Ankylosing spondylitis)	☐	☐
58. Been diagnosed with Hashimoto's thyroiditis or Grave's disease? (If so, has this been successfully treated and are you currently medically stable? __________________)	☐	☐
59. Have any of your relatives had Creutzfeldt-Jakob disease?	☐	☐
Do you have any of the following:		
60. Unexplained weight loss (10 pounds or more in the last 2 months)?	☐	☐
61. Night sweats?	☐	☐
62. Blue or purple spots on or under the skin or in your mouth?	☐	☐
63. Long-lasting white spots or unusual sores in the mouth?	☐	☐
64. Lumps in the neck, armpits, or groin lasting over a month?	☐	☐
65. Diarrhea lasting over a month?	☐	☐
66. Persistent cough or shortness of breath?	☐	☐
67. Fevers higher than 100.5° F. for more than 10 days?	☐	☐

Donor has received statement of risk of transmission of infectious diseases. **Yes ___** **No____**
Donor's arms have been inspected for parenteral drug use **Yes ___** **No____**
Indicate any positive findings ____
Medical exemption needed **Yes ___** **No____**
NMDP Donor Educational material given **Yes ___** **No____**

Signature: ____
Stem Cell Transplant Coordinator

Fig. 8.1 (continued)

Addendum 1: Pre-donation questionnaire

Date: ______________

Name: ____ **Birthdate:** ________________

	Yes	No
1. Have any of the answers given on the medical history form changed?	☐	☐
2. In the **past 48 hours**, have you taken aspirin or anything that has aspirin in it?	☐	☐
3. In the **past 48 hours**, have you taken any other anti-inflammatory medications, such as Ibuprofen or Naprosyn?	☐	☐
4. In the **past 14 days**, have you taken Plavix© (Clopidogrel) or Ticlid© (Ticlopidine)?	☐	☐
5. In the **past month**, have you taken any other medications? (If so, please list below) ____________________________	☐	☐
6. Have you donated any blood or undergone apheresis for the purpose of donating blood products (such as platelets) in the **last 16 weeks**?	☐	☐
7. Are you feeling healthy and well today?	☐	☐

Donor physical exam meets standards for donation: **Yes ___** **No____**

Female donors only: date of last pregnancy test: ___________________________

Donor meets all criteria for donation: **Yes ___** **No____**
(If no, the medical exemption has been granted and documented: **Yes _____** **No_______** **NA _____)**

Signature: ____
Stem Cell Transplant Physician

Fig. 8.1 (continued)

Addendum 2: STEM CELL DONOR SCREENING QUESTIONNAIRE

Part I: SARS: **Please seek medical attention and call the Cost as Center if you should develop any of these symptoms in the month following donation.**

DEFINITION: **Suspected SARS (Severe Acute Respiratory Syndrome)**
Respiratory illness of unknown etiology with onset since February 1, 2003, which meets the following criteria:

- Measured temperature > 100.4°F (>38°C), **AND**
- One or more clinical findings of respiratory illness (e.g. cough, shortness of breath, difficulty breathing, hypoxia, or radiographic findings of either pneumonia or acute respiratory distress syndrome), **AND**
- Travel within 10 days of onset of symptoms to an area with documented cases or suspected community transmission of SARS (affected areas), **OR** close contact within 10 days of onset of symptoms with either a person with a respiratory illness who traveled to a SARS-affected area or a person known to be a suspect SARS case.
  - Travel includes transit in an airport in an area with documented or suspected community transmission of SARS.
  - Close contact is defined as having cared for, having lived with, or having had direct contact with respiratory secretions and/or body fluids of a patient known to be a suspect SARS case.
  - Probable SARS affected areas include, but are not limited to: China (mainland); Hong Kong; Hanoi,Vietnam; Singapore; Toronto, Canada; Taiwan.

❖ SARS may be transmitted through blood and tissue donation. SARS cannot be transmitted to you as a result of the donation process.

Part II: WNV: **Please seek medical attention and call the hospital if you should develop any of these symptoms in the month following donation.**

DEFINITION: **Suspected WNV (West Nile Virus)**
Viral infection transmitted to humans by a mosquito bite, which meets the following criteria:

- Measured temperature > 100.5°F (>38 °C), **AND**
- Headache, **and possibly**
- One or more clinical findings (flu-like symptoms, eye pain, body aches, generalized weakness, new skin rash, or swollen lymph nodes)
- Convulsions, coma or paralysis

❖ The above may be symptoms of West Nile Virus infection which is known to be transmitted through blood and tissue donation. West Nile Virus cannot be transmitted to you as a result of the donation process.

I understand the information given on this form and will comply with the request to report symptoms to this institution after seeking medical attention should any of the above symptoms occur within four weeks of donation.

Participant Name(print) ______________________ Participant Signature ___________________ Date_______

Fig. 8.1 (continued)

Table 8.1 Donor laboratory evaluations

Tests to be obtained on all potential donors (tests that are *required* by FDA and/or FACT are in **bold**)	Suggested tests for donor safety screening
• **CMV IgG and IgM**	• CBC with differential and reticulocyte count
• **HIV I and II Ab (± p24 Ag)**	• BUN/creatinine
• **HTLV I and II Ab**	• Liver function
• **RPR**	• Urinalysis with micro
• **Hepatitis B panel (sAb, sAg, core Ab IgM)**	• Serum or urine β-hCG (post-menarchal female) • Required prior to donation
• **Hepatitis C Ab (IgG)**	• EKG (adult)
• **Trypanosoma cruzi IgG and IgM**	(–)
• **Hemoglobin electrophoresis**	(–)
• **Type and Screen** (+ identification if Ab screen positive) • Two separate samples required for confirmation of blood type	(–)
• **HCV, HIV, and West Nile Virus (WNV) NAT** – WNV not required for international donors	(–)
• HSV I/II IgG and IgM	(–)
• Varicella zoster IgG/IgM	(–)
• EBV panel (VCA IgG, VCA IgM, Nuclear Ag, Early Ag)	(–)
• Toxoplasma gondii IgG	(–)

lection CBC. Females of childbearing potential should have a current (within 7 days) pregnancy test performed prior to the start of collection (or mobilization if performed); for allogeneic collections, a pregnancy test is also mandated within 7 days prior to the start of the recipient's preparative regimen [2].

The Special Case of the Autologous Donor

While the above description applies to allogeneic HSCT, in some circumstances, the donor is also the recipient. This occurs in situations in which the HSCs are being used to provide hematopoietic recovery following high-dose chemotherapy, and where the likelihood of tumor contamination of the stem cell product is low and there is no need for any alloreactivity (i.e., "graft-vs-leukemia" effect). Autologous donation obviously carries no risk of transmitting a new infection, and so formal determination of donor eligibility—including IDM testing—is not required by the FDA. Furthermore, because the donor will indeed derive personal benefit from the stem cell collection, there may be more tolerance for potential health risks associated with the collection procedure than would be the case for an allogeneic donor.

Consent and the Special Case of the Donor Who Is a Minor

While informed consent is an integral part of any medical procedure, all HSC collection procedures are invasive and so are associated with a substantially greater than minimal risk of complications. As such, written consent following a complete and comprehensive description of the procedure and the risks involved is absolutely required prior to initiation of the procedure and, for the reasons outlined above in the discussion of IDM testing, should be obtained prior to the start of the recipient's conditioning regimen. However, given the time-sensitive nature of most HSCTs, it is prudent to have these discussions early in the donor evaluation process since early identification that a potential donor is unwilling to go through the actual donation process avoids both wasted time and expense, and allows the transplant team to identify an acceptable alternative donor.

The issue of consent becomes more complicated when the optimal donor is a minor. (Take note: this is restricted to situations in which the donors and recipients are related; minors are not considered as potential donors for an unrelated recipient.) Since matched sibling donors are usually felt to be superior to unrelated donors, it is not uncommon for the potential related donor to be a child or young adult. However, in this situation, it

is the parents of the potential donor who must provide consent for the collection, which in turn involves a potential conflict of interest insofar as they are also the parents who are responsible for the health of the sibling recipient. Indeed, some have questioned the ethics of using minor donors in any but exceptional circumstances [14]. However, it is now commonly accepted that many minors—particularly teenagers, but even younger children—can well understand the implications of a proposed procedure and give informed *assent* to participation. Furthermore, they may have a strong desire to help their sick relative and even feel distress if they are arbitrarily excluded merely on the basis of age [15–17]. The American Academy of Pediatrics Committee on Bioethics has issued the following five criteria that should be fulfilled when considering donation of HSCs from a minor [18]:

1. There is no other medically equivalent histocompatible adult relative who is willing and able to donate.
2. There is a strong personal and emotionally positive relationship between the donor and recipient.
3. There is a reasonable likelihood that the recipient will benefit.
4. The clinical, emotional, and psychosocial risks to the donor are minimized and are reasonable in relation to the benefits expected to accrue to the donor and to the recipient.
5. Parental permission and, when appropriate, child assent are obtained.

Additional factors to be considered when choosing among multiple suitable sibling donors include:

1. Donors closer to the age of majority are preferred over younger donors.
2. Heavier donors are generally preferred over lighter ones, especially with larger body weight recipients.

Finally, given the potential for conflict of interest or even coercion on the part of family and medical staff, it is important to engage a patient advocate who is neither a relative nor a member of the medical team treating the recipient. The patient advocate should have appropriate training and experience, so they can help educate the potential donor, determine that the donor's assent is freely given (for older donors), and also provide an independent assessment that the proposed donation is ethically appropriate (which is especially important for very young donors who may be unable to fully comprehend the implications of donation and so are unable to provide informed assent) [15, 16]. Often the role of donor advocate is well fulfilled by a social worker or another nonmedical professional who may have a broad awareness of the overall family situation and dynamics. In many institutions, there is a multidisciplinary team whose focus involves the entire family of a patient with a life-threatening disease, and often members of this team (typically including a member of the pastoral care team) will have a preexisting relationship with the potential donor that allows them to effectively fulfill the role of advocate.

Hematopoietic Stem Cell Source Selection, Mobilization, and Collection

Selection of Stem Cell Source

Current sources of HSCs include bone marrow, peripheral blood, and umbilical cord blood. Bone marrow normally contains a small but clinically relevant number of hematopoietic stem cells and was the first cell source utilized in HSCT. Under basal conditions, there are very few HSCs in the peripheral blood circulation, but that number transiently rises during the period of marrow recovery from chemotherapy to a level that can allow successful collection by apheresis of enough HSCs to provide successful engraftment. This approach was originally used in the 1980s for autologous collections in patients with solid tumors, especially those who had been heavily pretreated with chemotherapy and/or radiation (especially to the pelvis) that compromised the ability to collect sufficient numbers of bone marrow cells [19, 20]. Marrow stimulation by hematopoietic growth factors (G-CSF, GM-CSF) enhances this effect [21], and indeed in normal individu-

als, the administration of G-CSF and/or GM-CSF is often sufficient to mobilize adequate numbers of HSCs without any antecedent chemotherapy, which has allowed adoption of this technique for allogeneic donors [22–24]. Because neutrophil engraftment is generally faster with peripheral blood stem cells compared to bone marrow (in part because higher doses of CD34+ cells can usually be obtained but also probably because of the inclusion of more mature myeloid precursor cells), peripheral blood stem cells rapidly became the predominant stem cell source, especially for adult recipients [25]. In comparison to these HSC sources, umbilical cord blood (UCB) contains a relatively high concentration of HSCs, so much so that successful engraftment can occur at cell doses of approximately 10% of those required for bone marrow or peripheral blood collections [26] (although the volume of cord blood that can typically be collected does limit the total number of cells available per unit).

There are advantages and disadvantages to each of these cell sources, and selection often depends upon multiple factors, including the expertise and experience available at an individual transplant program (see Table 8.2). As a general rule of thumb for pediatric HSCT, peripheral blood stem cell collection is preferred for autologous transplants because an adequate number of cells can often be collected to provide for multiple infusions with fewer risks and less discomfort than are associated with bone marrow harvest. For allogeneic

Table 8.2 Advantages and disadvantages of stem cell sources

Cell source	Advantages	Disadvantages
Bone marrow	• Reliable source of HSCs in most donors • Less potential for graft-vs-host disease (compared to PBSC) • Potential for repeat donation (HSCs or lymphocytes) in case of graft failure • No need for mobilization	• Surgical procedure under general anesthesia • Harvest volume limits, especially in smaller donors • Post-procedure discomfort • Significant red cell contamination may be an issue if donor and recipient are not ABO/Rh compatible • Final product may contain significant amounts of heparin • Identification and screening of donor and scheduling of procedure may be time-consuming
Peripheral blood HSCs	• Potential for greater CD34+ HSC yields • Faster engraftment • Less RBC contamination • Potential for repeat donation (HSCs or lymphocytes) in case of graft failure	• Usually requires placement of special pheresis catheter(s) • Mobilization required (chemotherapy, cytokines, plerixafor) • Timing of mobilization may be difficult to predict • Greater risk for graft-vs-host disease compared to allogeneic bone marrow • Identification and screening of donor and scheduling of procedure may be time-consuming
Umbilical cord blood	• Requires less stringent HLA matching (of particular value for ethnic groups underrepresented in donor registries) • Generally least risk of graft-vs-host disease • Comparable antitumor effect • Units are prescreened for infectious disease markers • Rapid availability • Small volume	• Slower engraftment • Greater risk of graft failure • Slower immune reconstitution • Limited cell dose (may be overcome with multiple unit infusions) • No potential for repeat donation or donor lymphocyte infusions • Expensive (especially for multiple unit infusions)

HSCT, bone marrow has again become the preferred HSC source in many centers due to a lower risk of developing GvHD as compared to peripheral blood stem cells [27–33]; however, collection of bone marrow is a more involved (and potentially unacceptable) process for the donor, and volume considerations may make it unfeasible when the recipient weighs much more than the donor. In these situations, peripheral blood stem cells may be preferable. The use of UCB rather than bone marrow or peripheral blood is influenced by the underlying diagnosis, recipient size (individual UCB units may not have sufficient cell doses to ensure engraftment in larger recipients), and program preferences.

Bone Marrow Harvest: Theoretical and Practical Considerations

The hematopoietic stem cells are contained within the population of mononuclear cells and are identified by expression of the cell surface marker CD34. Doses of 2–2.5 × 10^6 CD34+ cells are considered the minimum to allow effective engraftment in a reasonable time frame, and doses of 3–5 × 10^6 are often preferred to reduce the risk of graft failure or delayed engraftment (these numbers are for bone marrow and peripheral blood; comparable doses for UCB are about 10% of these). A certain number of T cells are also required for successful engraftment and immune reconstitution, although this is usually only a practical concern when the cell product undergoes specific post-collection depletion of T cells.

Bone marrow cells are collected by aspiration through a needle inserted into the marrow space, typically the posterior superior iliac crests although the anterior crests are also at times utilized. The material aspirated from the marrow space is an admixture of marrow spicules and blood; as the volume of an individual aspirate increases over approximately 5 mL, it becomes disproportionately diluted with blood, so typically aliquots of 5–10 mL are obtained. The total nucleated cell count in such an aspirate is usually about 20,000 /μL, of which about 1% will be CD34+. Thus, the concentration of CD34+ cells will be about 2 × 10^5/mL which means that a successful harvest typically requires a minimum of 10 mL/kg of recipient weight, but typically 15 mL/kg of recipient weight is collected. For a 50 kg recipient, that translates into 500 mL, and, with a volume per aspirate of about 5 mL, approximately 100 aspirations will be required.

Even with exceptional local anesthesia of the periosteum and overlying skin and soft tissue, the insertion of the bone marrow needle is, at best, uncomfortable, and the aspiration itself is unavoidably accompanied by transient but significant discomfort or pain. Multiple needle insertions through skin into bone also introduce the potential for introducing infection. Thus, it is only practical to perform a bone marrow harvest in the operating room under general anesthesia and with meticulous attention to appropriate skin preparation, draping, and maintaining strict sterile technique throughout the procedure to avoid either infecting the donor or introducing microbial contamination into the collected product (which would, in turn, infect the immunocompromised recipient). Repeated aspirations are performed at multiple adjacent sites from both posterior superior iliac crests, varying the angle of the needle slightly each time. Over time the aspirates will become increasingly diluted with blood, so sometimes other sites (such as the anterior iliac crests) will also be used [34].

Bone marrow tends to clot very rapidly, so the syringes are rinsed with heparin prior to each aspiration, and the collected marrow is promptly transferred to a container containing enough heparinized saline to maintain adequate anticoagulation. The original collection containers were open to the air (originally metal beakers, later plastic bags suspended from a special frame with an opening on top). While easy to use, these had the potential for introducing airborne contamination, not to mention the catastrophic event of tipping over the container and spilling the product. More recently, closed systems have been developed that significantly reduce the risk of accidental spillage and exposure to air particles. Using either type of

system, the final collection product is passed through a series of filters designed to remove large particles (e.g., bone spicules) and then into a blood collection bag for transfer to the processing lab. The total amount of heparin introduced into the product frequently represents a significant anticoagulant dose for the recipient, a consideration when planning for pre-infusion processing.

Bone marrow aspirates contain essentially the same concentration of red cells as peripheral blood, thus the final marrow product includes a significant volume of red cells. This volume of blood cells can pose a risk of a severe hemolytic reaction if the donor and recipient are not ABO compatible, and so the bone marrow product may require additional processing to deplete the product of red cells (or occasionally plasma) prior to infusion [35, 36]. It may also represent a significant loss of red cells for the donor, which sets a limit on the volume that can be obtained, especially from very small donors. 20 mL/kg donor weight is considered an absolute limit for the total volume harvested. When the anticipated volume exceeds 10–15 mL/kg donor weight, consideration should be given to either autologous red cell collection beforehand (not a standard procedure in smaller children but one that can be specially arranged through blood collection centers) or transfusion of a parental blood unit (if compatible). This can limit the utility of bone marrow harvests when the recipient is much larger than the donor and the maximum harvest volume would provide an insufficient cell dose. While it is not intuitively obvious that administration of G-CSF would increase the number of CD34+ cells in the bone marrow, in practice, there does appear to be some increase which may be sufficient in marginal situations [37–39]. If the disparity in weight is too great, then peripheral stem cell collection may be the only practical option.

Peripheral Blood Stem Cell Collection: Theory

The principle underlying therapeutic apheresis involves subjecting whole blood to centrifugation, which separates blood components on the basis of density. Erythrocytes, being the denser cellular component, will sediment to the gravitational "bottom," plasma will remain on "top," and the white cells and platelets will form an interface between the two, often referred to as the "buffy coat" (see Fig. 8.2 for an example from an umbilical cord blood unit). Modern apheresis machines continuously remove blood from the patient and introduce it into the centrifugation chamber, from which the desired component may be removed and the remaining elements returned to the patient. Because the removal and replacement of blood is a continuous process, it is possible over several hours to process volumes of blood that far exceed the patient's total blood volume. The technology is similar whether being used for therapeutic exchanges of red cells or plasma (in which case replacement of red cells or plasma is also given to the patient) or for removal of platelets or leukocytes. For the purpose of a peripheral blood hematopoietic stem cell harvest, it is the leukocyte layer containing the CD34+ stem cells that is removed and collected.

Fig. 8.2 Umbilical cord blood unit following erythrocyte sedimentation. The light-colored buffy coat (marked by the *blue arrow*) is clearly visible at the interface between erythrocytes (*below*) and plasma (*above*)

For this process to be successful, there must be a sufficient number of CD34+ cells in the peripheral blood circulation to allow the harvesting of an adequate cell dose in some practical time frame. A commonly used minimal threshold for initiating peripheral blood stem cell harvest is 10 CD34+ cells/μL [40], which is equivalent to 1×10^4 /mL. To achieve a minimal target dose of $2–2.5 \times 10^6$ CD34+ cells/kg recipient weight (which, for autologous donors, will obviously be the same as donor weight) would require removing the number of HSCs contained within 200–250 mL/kg whole blood—an amount approximately three times the average blood volume of 70–75 mL/kg. While at first this may seem impractical, the HSC population found in the peripheral blood after chemotherapy or cytokine stimulation is in dynamic equilibrium with the bone marrow, and so over the course of a few hours, there is continuous replenishment of the peripheral stem cell population from the larger marrow population. Given typical conditions, it is quite feasible to process 2.5–3 times the total blood volume over a period of approximately 6 h, and so if the collection process were 100% efficient, it would theoretically be possible to collect enough CD34+ cells in 1 day from an individual with 10 CD34+ cells/microliter to provide for a single HSC infusion. In practice, the efficiency of collection is not 100% (40–50% efficiency is more typical) [41], and many current autologous HSCT regimens involve dual or even triple infusions. Thus, the prudent transplant physician prefers to have an “extra” dose of cells in case of nonengraftment (rare with autologous peripheral stem cell infusions, but not unheard of) or a mishap leading to loss of a stored product (such as rupture of the storage bag for frozen products—again, a rare but potentially catastrophic occurrence without a backup unit). This may require 2–3 days of collection when the CD34+ count is close to the 10 /μL threshold but may be accomplished in a single session when the peripheral CD34+ count is higher.

Peripheral Blood Stem Cell Collection: Practical Considerations

The practical issues involved with peripheral blood stem cell collection in children include *stem cell mobilization*, *vascular access*, *fluid/blood volume* issues, and *procedural risks* [42–44].

As stated above, while peripheral blood stem cell counts may be sufficiently increased during recovery from chemotherapy to allow for successful harvest, counts are generally much increased with the post-chemotherapy use of hematopoietic cytokines. For allogeneic donors who are not receiving chemotherapy, cytokine administration alone is the primary method for inducing mobilization of HSCs into the peripheral blood.

For the autologous donor who is receiving chemotherapy as part of their overall oncology care, successful mobilization is dependent upon both the chemotherapy regimen used and the adequacy of bone marrow reserve. Successful mobilization can be achieved after many different chemotherapy combinations, although some drugs are considered to have a relatively high potential for depleting stem cells and are commonly avoided. Marrow reserve is largely a function of the total exposure to chemotherapy and radiation, which in turn may prompt consideration of relatively early preemptive harvest of patients who may have a relatively high risk of becoming candidates for autologous transplant (e.g., very high-risk lymphoma or germ cell tumors) or for whom autologous transplant is part of frontline therapy (e.g., high-risk neuroblastoma) [45]. For the typical autologous donation, our practice has been to initiate G-CSF at a conventional dose of 5 μg/kg daily (either subcutaneous or intravenous) for 5 days, then escalate to 10 μg/kg. With this regimen, a rise in peripheral CD34+ cells is typically seen around day 10, concurrent with an increase in the total peripheral WBC. For allogeneic donors or autologous donors who are not receiving myelosuppressive chemotherapy, short courses of high-dose G-CSF (up to 10–15 μg/kg/day as either single or divided doses) will often result in an adequate rise in 4–5 days [22–24]. Cytokine stimulation is

sometimes accompanied by bone pain, presumably due to marrow expansion, for which administration of NSAIDs, narcotics, and the antihistamine loratadine can be effective [46].

Mobilization of HSCs into the peripheral blood circulation is a transient phenomenon, and so the window for collection can be relatively narrow (e.g., 1–3 days). Because multiple hospital services are involved in the collection process, ideally, one would plan for the anticipated surge in peripheral blood stem cells to occur early in the week, which would allow for multiple days of collection while avoiding the staffing issues that can arise on weekends. This, in turn, requires coordination between the primary oncology team and the collection team in terms of scheduling the antecedent course of chemotherapy.

Not all donors will mobilize a sufficient number of CD34+ cells into the periphery to allow successful collection. This situation is particularly likely among patients who have been heavily treated with chemotherapy and/or radiation, but it may also happen in normal allogeneic donors who are not receiving chemotherapy as part of their mobilization protocol. Plerixafor, a drug that acts as an antagonist of the CXCR4 molecule which is responsible for the binding of HSCs to the bone marrow stroma, will cause a transient and often dramatic increase in the release of CD-34+ cells into the peripheral blood and, in combination with G-CSF, can be used to facilitate harvest in patients who cannot be mobilized with cytokines alone [47, 48]. Plerixafor is generally administered 11–12 h prior to the planned start of collection and can be repeated for multiple days if needed. Because it is relatively expensive, insurance coverage is sometimes dependent upon demonstration that an adequate collection cannot be obtained with cytokine stimulation alone.

The flow of blood (both removal and replacement) required for efficient peripheral blood stem cell collection in modern devices is generally 10–100 mL/min. While flow rates in this range can usually be achieved in adult donors using large bore peripheral IV catheters, this is impractical in most pediatric donors. While many autologous donors will already have a semipermanent central line in place, the central catheters most commonly used in pediatric patients (Broviac, Hickman, Port-a-Cath, etc.) are insufficiently rigid and will collapse when attempting to remove blood at the required rate. Thus, for allogeneic donors and even the majority of autologous donors, a dedicated double-lumen apheresis catheter will be required. These can generally be placed under conscious sedation without general anesthesia. For a 1–2 day collection, placement of a femoral catheter may be most practical although it does severely limit mobility of the donor. When longer collections are contemplated, placement of a jugular or subclavian catheter may be more comfortable but is associated with different risks (e.g., pneumothorax) [33]. Given the uncertainties outlined above concerning the timing of collection, careful coordination with the personnel responsible for placement of the catheter is essential to avoid unacceptable delays in initiating the collection procedure. Also, for collections that are likely to require more than a single session, placement of a temporary apheresis catheter normally requires the donor to remain inpatient for the duration of the procedures.

An alternative approach for patients for whom autologous donation and HSCT are clearly indicated at the time of initial diagnosis (e.g., high-risk neuroblastoma) is placement of a double-lumen dialysis catheter at the time of diagnosis. Although somewhat larger and cumbersome than a typical transcutaneous CVL and requiring more day-to-day maintenance than a subcutaneous catheter, this type of catheter provides adequate access not only for the stem cell collection (thus bypassing any time delays required for specific apheresis catheter placement) but also for the delivery of routine chemotherapy, supportive care, and the transplant procedure and so may avoid the need for repetitive line placements and removals.

The volume contained within the tubing and circuit of most apheresis machines is 200–250 mL, and for routine collection from adults, the circuit is typically primed with normal saline. For larger donors, this volume of saline is modest compared to the overall blood volume and the flow through the circuit rapid enough to allow timely formation of the erythrocyte-plasma interface from which HSCs will be collected. For

smaller donors, however, infusing this volume of saline while simultaneously removing the same volume of whole blood would cause a rapid and significant dilution of their intravascular red cell mass. Also, because the flow through the machine is slower, it can take much longer for a stable interface to form which, in turn, can markedly prolong the collection procedure. Thus, for smaller donors (i.e., <20 kg), it is standard practice to prime the circuit with red cells diluted to a hematocrit close to that of the donor [49]. While this approach does introduce an additional exposure to blood product(s) for the donor, it avoids dramatic shifts in the donor's hemoglobin concentration and can significantly shorten the "ramp-up" interval between initiating the procedure and actually being able to collect HSCs.

In most cases, the actual collection process is relatively free of side effects. In some cases, there can be alterations in blood pressure or symptoms like nausea or vomiting that result from fluid shifts, but these typically respond to a brief cessation of the procedure or reduction in flow rates. Anticoagulation within the circuit is achieved with citrate which is largely returned to the donor, so there is the potential for symptomatic decreases in ionized calcium levels [44]. In adults, the resultant hypocalcemia is sometimes managed with oral administration of calcium (e.g., Tums) when symptoms arise, but children seem to be more prone to this side effect and may be less capable of recognizing and responding to the early symptoms of hypocalcemia (e.g., facial tingling). Therefore, preemptive infusion of calcium chloride into the blood being returned to the donor, titrated by symptoms and/or ionized calcium levels, is often preferred for the pediatric donor.

Post-collection Care of the Donor

Bone marrow donation and peripheral blood stem cell collections are considered relatively safe procedures with a low incidence of serious complications [33, 50, 51]. Donors should have a CBC performed at the completion of their procedure to determine whether there has been a significant change in blood counts that might warrant transfusion support. Donors undergoing peripheral stem cell collections can generally be discharged home following completion of their collection and removal of their pheresis catheter and commonly experience no more than minor discomfort at the catheter site. Donors undergoing bone marrow harvest are much more likely to have significant procedure site pain even with administration of local anesthetic agents to the skin and periosteum and often require both NSAID and narcotic medications to achieve adequate pain control. We routinely book an overnight bed for pediatric donors undergoing marrow harvest in anticipation that they will require parenteral pain medications following the procedure, although some donors (especially older teenagers) will be adequately controlled with oral pain medications and be discharged home the same day. Regardless, it is also important to follow up with the donor following the procedure to make sure there are no signs or symptoms of complications, including prolonged pain or evidence for procedure site infection. In some cases—especially for large-volume marrow donations that would include harvesting large numbers of erythrocytes—empiric oral iron supplementation to facilitate red cell production may be desirable.

Post-collection Processing: ABO Incompatibility

Introduction

In contrast to solid organ transplantation, in which ABO matching is critical for long-term graft integrity and ABO-mismatched transplants are rarely performed, crossing the ABO barrier is feasible in HSCT because ABO antigens are not expressed on early hematopoietic progenitors and stem cells. Strategies exist to minimize the potential acute issues arising from ABO incompatibility between donor and recipient. Currently, ABO-incompatible HSCTs are performed using all types of grafts including peripheral blood hematopoietic progenitor cells (HPC, apheresis), bone marrow (HPC, marrow), and umbilical cord blood (HPC, cord blood) [52, 53].

ABO compatibility involves A and/or B antigens expressed on either donor or recipient cells together with anti-A and/or anti-B antibodies in

either donor or recipient plasma. Thus, there can be three types of ABO incompatibility. *Major ABO incompatibility* occurs when the recipient's plasma contains antibodies against A and/or B antigens on the donor's cells (e.g., when the recipient is blood type O and the donor is blood type A, B, or AB). *Minor ABO incompatibility* is present when the donor graft contains antibodies against A and/or B antigens on recipient cells (e.g., donor is blood type O and recipient is blood type A, B, or AB). *Bidirectional incompatibility* is the least common type of ABO incompatibility (present in up to 5% of transplants) and occurs when either the donor is blood group B and recipient is blood group A or vice versa. Fig. 8.3a details the donor-recipient combinations that might require post-collection processing.

Management of Major ABO Incompatibility

The presence of erythrocytes in the stem cell graft that can interact with recipient isohemagglutinins may result in immediate hemolysis. Additional complications can occur in the form of delayed RBC donor engraftment and pure red cell aplasia due to continued production of recipient type isohemagglutinins by persistent recipient B lymphocytes [54].

To avoid occurrence of a significant hemolytic reaction in the setting of a major ABO-mismatched HSCT, the volume of infused donor erythrocytes should be minimized. An important parameter that should guide the decision-making process is the maximum volume of ABO-mismatched erythrocytes that can be safely infused. Although this volume is not well defined in the literature, most published studies suggest that a volume between 10 and 40 mL of mismatched erythrocytes can be tolerated by adult recipients without a major reaction. In practice, most institutions limit the volume of infused mismatched erythrocytes to 20–30 mL (in adults) or 0.2–0.4 mL/kg recipient weight (in children or smaller adolescents/adults). The algorithm in Fig. 8.3b describes recommendations for red cell depletion depending upon stem cell source, recipient isohemagglutinin titer, and estimated volume of donor red cells.

The HSC source that is most likely to require red cell depletion is bone marrow because erythrocytes may constitute 25–35% of the final product volume. For example, a typical bone marrow product volume of 10 mL/kg would result in a red cell dose of approximately 3 mL/kg or about ten times the maximally tolerated dose. Peripheral blood stem cell products obtained by apheresis are considerably less likely to require red blood cell depletion because apheresis methodology generally results in significantly less erythrocyte contamination than is typical for bone marrow collections. However, the apheresis collection facility should be instructed to attempt to minimize red blood cell contamination if major ABO incompatibility is a concern.

Major ABO incompatibility is usually not a very significant concern in umbilical cord blood transplantation (UCBT) because most cord blood units undergo a process of red cell depletion prior to cryopreservation [55, 56]. However, if the umbilical cord blood unit had not been red cell depleted, then a significant dose of incompatible red cells might be infused, and indeed there have been multiple reports of adverse events in recipients receiving non-red-cell-depleted incompatible umbilical cord units. Thus, in the rare case in which a cord blood unit that has not been red blood cell (RBC) depleted is to be infused into a small pediatric patient, it is at least necessary to wash the unit after thawing, which usually results in a significant decrease in the post-thaw hematocrit.

Methods for RBC reduction in the processing laboratory include manual centrifugation, hydroxyethyl starch-facilitated sedimentation using manual or automated methodology, density gradient separation, and automated or semiautomated apheresis instrument cell separation [55–58].

Manual centrifugation is the simplest method and involves centrifugation of the product bag at 400–4000 ×*g*. This results in concentration of erythrocytes (RBCs) at the bottom of the bag from which they can be slowly drained or, alternatively, the buffy coat (which contains the CD34+ HSCs) can be removed from the top of RBC layer. In either case, the resulting product is red cell depleted, but, because the separation of RBCs and nucleated cells is incomplete, there is also some depletion of stem cell-containing mononuclear cells.

		Donor Blood Type			
		A	B	AB	O
Recipient Blood Type	A	-	RBC, Plasma	RBC	Plasma
	B	RBC, Plasma	-	RBC	Plasma
	AB	Plasma	Plasma	-	Plasma
	O	RBC	RBC	RBC	-

Guidelines for Red Cell Depletion

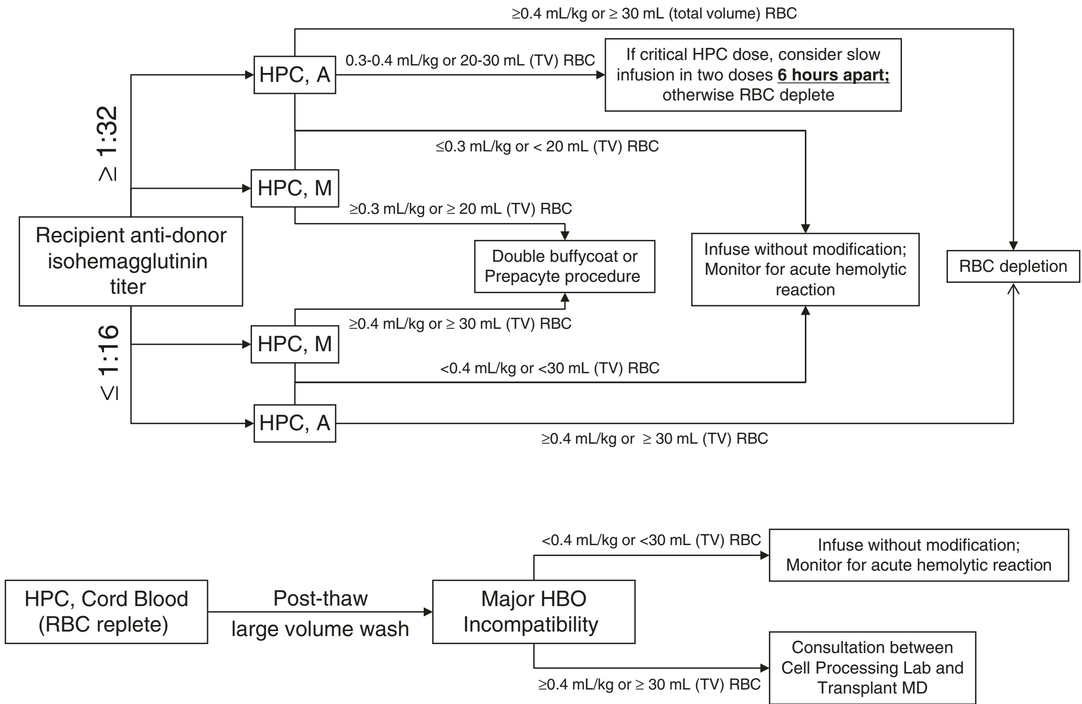

Fig. 8.3 (**a**) Donor-recipient ABO mismatches for which either RBC or plasma depletion should be considered. See figure (**b**) for details regarding RBC depletion. (**b**) Algorithm for RBC depletion depending upon recipient isohemagglutinin titer and stem cell source. *HPC-A* hematopoietic progenitor cell, apheresis (i.e., peripheral stem cell collection), *HPC-M* hematopoietic progenitor cell, marrow (i.e., bone marrow), *TV* total volume

Hydroxyethyl starch (HES) causes clumping (rouleaux) of RBCs and allows for their differential sedimentation to occur without centrifugation. The process involves mixing the HSC product with HES and removal of the sedimented erythrocytes after 30–90 min. Similarly to manual centrifugation, the RBC fraction does contain some contaminating nucleated cells. If the final HSC dose is critical, repeated HES sedimentation can be performed on the removed RBCs in an attempt to recover additional nucleated cells. Using this method, RBCs can be effectively removed while mostly preserving the final nucleated cell content of the product [59].

Density gradient media such as Ficoll-Hypaque can also be used to isolate mononuclear cells. Ficoll-Hypaque has a density intermediate between erythrocytes (and granulocytes) and mononuclear cells (and platelets). The HSC product is initially layered over the Ficoll-Hypaque, and after centrifugation erythrocytes and granulocytes will be under the layer with mononuclear cells and platelets concentrated at the interface between Ficoll-Hypaque and plasma. Mononuclear cells recovered using this method will be of the highest purity in regard to RBC contamination compared with other methods listed here, although the overall recovery of hematopoietic stem and progenitor cells is similar.

Automated or semiautomated instruments based on apheresis technology can also be used for red cell depletion. The method involves centrifugation and separation of mononuclear cells based on their differential density and results in good RBC reduction with expected percent recoveries of CD34+ cells from the low 60s to high 80s [60–62].

Management of Minor ABO Incompatibility

In cases of minor ABO incompatibility, reduction of plasma volume in the donor product can be performed to limit the amount of donor isohemagglutinins that can cause acute hemolysis in the recipient. This is important in pediatric patients in which the relative volume of infused donor plasma might be larger, thus increasing the risk of hemolysis. The method for plasma removal involves centrifugation of the product in a bag followed by the removal of plasma after phase separation. In this process, as much plasma as possible should be removed taking care to avoid disturbance in the cell layer. This can be achieved using a plasma extractor.

Minor ABO-incompatible HSCT can also cause delayed hemolysis due to the presence of passenger donor lymphocytes in the donor HSC product. These B lymphocytes can produce donor-type isohemagglutinins in the recipient which, in turn, can bind to and hemolyze residual recipient erythrocytes. This type of reaction typically develops from 5 to 15 days following stem cell infusion, although less than 15% of patients in this situation will actually experience significant hemolysis. This reaction is best managed by the transfusion of RBCs that are *not* of the recipient's blood group in the posttransplant period.

Management of Bidirectional ABO Incompatibility

The recipient undergoing bidirectional ABO-incompatible HSCT is at risk of developing the complications associated with both minor and major ABO-mismatched transplantation. The best option to address this type of incompatibility is to perform red cell depletion in a fashion that will also result in plasma depletion. However, either delayed hemolysis due to isohemagglutinin production by donor lymphocytes and/or delayed RBC engraftment due to the persistence of recipient lymphocytes is still possible.

Graft Engineering

Whereas depletion of red cells and/or plasma is utilized to avoid hemolysis in the immediate post-infusion period, manipulations of the mononuclear cell population are intended to alter the longer-term function of the graft. This most commonly focuses on minimizing the posttransplant complication of GvHD. While all allogeneic HSCTs carry some risk for GvHD, the incidence and severity increase with HLA mismatching between donor and recipient. Attempts at using related haplo-identical donors were initially plagued with very high rates of GvHD [63, 64]. GvHD is mediated through alloreactive cytotoxic T lymphocytes, and so reducing the number of donor T cells infused into the donor would be expected to ameliorate the risk of developing GvHD. However, bulk depletion of donor T cells is associated with an increase of graft failure or rejection as well as an increased risk of relapse (presumably by decreased graft-vs-malignancy effect, also mediated by cytotoxic T cells) and overall transplant-related mortality [64–66]. While the risk of graft failure can be overcome by infusing higher doses of CD34+ cells (approximately 5–10 times the normal dose; obviously a method that, for the technical reasons discussed earlier, mandates utilization of peripheral blood as the stem cell source) and is associated a with relatively low risk of GvHD, this strategy does not necessarily overcome the tendency for T-cell-depleted grafts to exhibit much slower immune recovery with a resulting increased risk of posttransplant infections [67, 68].

Methods for graft cell manipulation have been available for several decades. These methods involve either selective depletion or enrichment of particular cell populations. Although graft cell depletion can be performed in vivo or ex vivo, cell enrichment can currently only be performed ex vivo.

Ex vivo cell manipulation is most often performed using immunomagnetic separation. Currently, there are modern devices (e.g., CliniMACS, manufactured by Miltenyi Biotec)

that can provide either positive selection of CD34+ cells or negative selection of T cells (CD3+ or T-cell receptor αβ+ cells) and/or B cells (CD19+) in a fashion appropriate for clinical use. Either case involves mixing the stem cell product with paramagnetic beads coated with antibodies reacting with the cell surface molecule(s) of interest. Cells possessing the targeted surface antigen will bind to the paramagnetic beads, which in turn can be immobilized by a magnetic field applied to the periphery of the container. With negative selection, the beads will bind the cell subpopulation(s) to be depleted, and the remaining cells can be washed through and used for infusion into the recipient. With positive selection, the beads will bind the subpopulation targeted for enrichment; after eluting the remainder of the population, the desired cells can be "knocked off" the paramagnetic beads and recovered for subsequent infusion. Positive selection tends to result in a purer cell population (e.g., approximately 90–95% CD34+ cells with an approximately 4.5 log depletion of CD3+ cells).

Ex vivo cell depletion for pediatric patients has been most studied in the setting of haplo-identical transplantation [69]. For example, a study of 41 children with a mixture of malignant and nonmalignant diseases who were transplanted with CD3+/CD19+-depleted grafts following reduced intensity conditioning demonstrated a high rate of engraftment and immune recovery along with low transplant-related mortality (which would include post-transplant infections) [70]. However, both methods will deplete the graft of some CD34− populations, including less mature γδ+ T cells, NK cells, T regulatory cells, dendritic cells, and potentially other immune cell types, that are unlikely to contribute to the development of GvHD but are likely quite important in broader posttransplant immune reconstitution. To minimize loss of beneficial immune cell types, alternative T-cell depletion strategies have been developed to selectively deplete αβ+ T cells, or more specifically alloreactive donor T lymphocytes, but these have not yet been evaluated in large numbers of patients and are still under investigation.

In vivo T-cell depletion was originally provided by anti-thymocyte globulin (ATG). ATG is a polyclonal product obtained from either horses or rabbits that has a broad range of lymphocyte reactivity (predominately, but not exclusively, targeted at T lymphocytes) which has long been utilized as part of pretransplant conditioning regimens in order to remove recipient lymphocytes that might mediate graft rejection. However, some ATG persists through the time of HSC infusion and can also react with infused donor T lymphocytes. Several studies involving all common HSC sources have shown decreased rates of GvHD in patients receiving ATG in comparison to those receiving similar non-ATG conditioning regimens [71–73]. Similar amelioration of GvHD has been shown with conditioning regimens containing more specific T-cell antibodies, such as the anti-CD52 mAb alemtuzumab (Campath) which has become a common component of non-myeloablative conditioning regimens [74, 75]. However, these approaches still result in a relatively nonspecific removal of T-cell populations.

An interesting approach to in vivo T-cell depletion involves posttransplant administration of cyclophosphamide [76–79]. Originally applied to haplo-identical HSCTs with a high risk of GvHD, this approach of in vivo T-cell depletion involves the administration of a moderately high dose of cyclophosphamide to the recipient 2–3 days following HSC infusion. The underlying rationale is that donor T cells that recognize alloantigens in the recipient will be actively proliferating at this point and so be killed by a cell cycle-specific alkylating agent, whereas the remaining T cells (including those reactive to a wide array of viral antigens) will not be proliferating and so be relatively resistant to cyclophosphamide. This approach has led to a low rate of GvHD not only in patients receiving haplo-identical HSCTs but also has been used successfully in more traditional transplant settings [80, 81]. However, because this approach is still relatively new, the long-

term effects on tumor control and immune reconstitution remain to be fully defined.

Cryopreservation of Stem Cell Products

Freshly obtained HSC products (either bone marrow or peripheral HSCs obtained by apheresis) maintain adequate levels of viability for about 48 h, after which time both cell viability and ability to engraft start to decline. This is typically enough time to allow for characterization and minimal processing of products even if transported from a distant collection center, although when utilizing international donors—which is increasingly common—the timeline for long-distance transport can be tight and so might require some flexibility on the part of the transplant center to make sure that cells are infused in a timely fashion. More extensive manipulation of cellular products obviously is more time-consuming and so, for all practical purposes, requires that products be collected either at the center that will be doing the manipulation or one that is geographically located so as to minimize time spent in transport.

Alternatively, products can be frozen when it is impractical to infuse within 1–2 days after collection—for instance, autologous donations when the anticipated time to infusion may be weeks or months in the future, or umbilical cord blood donations. Successful cryopreservation requires the addition of an agent that inhibits intracellular formation of ice crystals, which would otherwise destroy the cells. The most commonly used agent is dimethyl sulfoxide (DMSO), usually added to a final concentration of 10%. Because DMSO is itself somewhat toxic to nucleated cells, the freezing process should be initiated as quickly as possible once it is added to the product. Specially designed controlled-rate freezers are usually used for freezing down the HSC collection because the rate at which cells are frozen is important in preserving their viability. Once frozen, cell products are stored in liquid nitrogen, which maintains them at a temperature of <-150 °C. Under these conditions, cell products can be successfully preserved for years [82, 83].

While frozen HSC products can be maintained for extended periods of time, the actual process of freezing and subsequent thawing does result in some cell damage and death. Typical cell losses range from 10% to as much as 50% even under ideal processing and freezing conditions, although there is evidence that much of the cell loss involves granulocytes, with relative sparing of CD34+ cells [84]. When feasible, this potential for nontrivial cell loss should be taken into account when calculating the number of cells to be collected; thus, one might want to have a prethaw dose of 3–4 × 10^6 CD34+ peripheral blood stem cells collected by apheresis in order to ensure subsequent infusion of ≥2 × 10^6 viable CD34+ cells after freezing and thawing.

One issue that sometimes arises is whether allogeneic stem cell products should be collected and cryopreserved prior to the recipient starting the conditioning regimen. The argument in favor of this would be to avoid the situation in which a recipient has received a myeloablative conditioning regimen, and then something happens to the donor (e.g., illness, accident) that precludes safe collection. However, while prior collection and cryopreservation would avoid that scenario, the potential for cell loss during the freezing and thawing process would mean that the donor would be subjected to a more prolonged collection process than might otherwise not be required. While this might represent a modest increase in risk for apheresis collection, it might represent a significant risk for bone marrow donations and, depending upon donor and recipient size, may not be feasible at all. For this reason, many if not most centers rely on the strategy of planning to collect from the selected allogeneic donor just prior to the planned infusion while taking precautions to avoid the donor's exposure to infections or injury and with some contingency plan that could be quickly implemented if the primary donor becomes unavailable (e.g., alternative related or unrelated donor, umbilical cord blood).

Thawing Cryopreserved Stem Cells

Prior to infusion, cryopreserved HSCs are thawed relatively quickly. Once thawed, HSCs are subject to additional cell damage primarily due to the cellular toxicity of DMSO; in addition, the mixtures of DMSO used for cryopreservation lead to increased intracellular osmolarity, such that exposure to a normal osmotic environment can lead to rapid influx of water and further cell lysis. For this reason, freshly thawed cells are typically diluted with a hyperosmolar solution of albumin and dextran, which serves to minimize osmotic stress and also results in a lower DMSO concentration to which the cells are exposed. Because of the potential for additional cell loss, cryopreserved cells are usually infused within a relatively short period of time (a few hours) after thawing.

Following thawing and dilution, additional manipulations can be performed on HSC units (such as removal of red cells and removal of plasma), but these processes increase the time from thawing to infusion, and the procedures themselves can introduce additional cell loss. For this reason, it is preferable to perform manipulations such as red cell or plasma depletion prior to freezing if feasible. Currently, this is standard practice during preparation of umbilical cord blood units for banking, and so those products will typically have only modest volumes of red cells or plasma. However, older units or those collected outside the United States may not have undergone red cell or plasma depletion, and so it may be necessary to perform post-thaw depletion in these situations even at the risk of some stem cell loss.

An additional instance in which post-thaw washing may be necessary is when a relatively large-volume product is being infused into a small recipient, who might then be exposed to an excessive amount of DMSO. Many of the immediate reactions to infusions as described below are felt to be caused or exacerbated by DMSO. In addition, there have been occasional reports of neurotoxicity attributed to infusion of DMSO. For these reasons, the amount of DMSO infused is typically limited to no more than 1 g/kg recipient body weight. A similar situation might arise with cryopreserved bone marrow, since heparin is typically used to prevent coagulation of bone marrow products; however, alternative interventions (such as concurrent administration of protamine) are available to neutralize the potential anticoagulant effect of heparin.

Hematopoietic Stem Cell Infusion

HSC infusions, regardless of source, have the potential of causing infusion reactions [85, 86]. These can range from mild to severe or even life-threatening. Common reactions include nausea, vomiting, fever, changes in heart rate (either tachycardia or bradycardia), and changes in blood pressure (either hypotension or hypertension). Allergic-type reactions can also occur but are rarely severe. Cryopreserved units are more likely to have such side effects, due to histamine release by DMSO, but any cell source can have complications. Additional factors contributing to side effects include fluid overload, hemolysis due to ABO incompatibility, and reactions to cellular debris (e.g., from lysed granulocytes). Especially in smaller recipients, careful attention to the volumes in the stem cell unit (total volume, red cell and plasma volumes, DMSO) is especially important in anticipating and preventing severe reactions.

Typical preparation for a stem cell infusion might include a short period of fluid restriction (to obviate fluid overload during the infusion) and premedication with antiemetics, acetaminophen, antihistamines (typically diphenhydramine), and steroids (methylprednisolone or hydrocortisone). Often a diuretic (furosemide or mannitol) is administered around the time of HSC infusion in order to both prevent fluid overload and maintain urine flow in situations where there might be red cell lysis and intravascular release of hemoglobin.

As outlined above, there is often a relatively limited time within which HSCs need to be infused in order to maximize viability. However,

it is still important to observe all of the typical safeguards in properly identifying the unit and ensuring that the correct HSC unit is being infused, since administration of an incorrect unit would have catastrophic consequences. Although regular blood products are routinely irradiated and leukoreduced by filtration, both of these *must be avoided* since they would destroy or remove the HSCs and render the product useless.

Because of the potential for severe side effects, an appropriately trained physician or advanced practice professional should be present for the entire duration of the procedure. Infusions should be started slowly, with the rate being adjusted as tolerated. Mild side effects may respond to slowing the infusion rate, but additional medications may be required for allergic-type reactions or hypertension, especially with relatively large-volume infusions. Cells are typically infused by gravity without an infusion pump. Regular filtration sets (*not* leukodepletion filters) may be used, especially if there is concern for cellular aggregates or particles in the infusate (more common with marrow infusions). Especially for smaller patients with small-bore central catheters, there might be significant physical resistance that slows the rate at which the product can be infused. While this is more likely to be an issue with a product that contains a significant number of red cells, it can also occur even with relatively erythrocyte-poor apheresis products. Thus, at times, manual infusion using a syringe might be necessary in order to ensure administration in a timely fashion.

Recipients should be carefully monitored for several hours following the infusion for potential side effects. Of note, DMSO and its metabolites are partially excreted into the urine, but a significant amount enters the lungs and is exhaled into the environment. The distinctive smell of DMSO is variously described as "garlic" or "creamed corn." While the recipient usually becomes quickly acclimated to the smell, families should be advised that it can linger for hours or even days afterward.

Key Points

- Donor *eligibility* is determined by the regulatory requirements established for donors of blood products that are designed to minimize the risk of transmitting infectious diseases.
- Donor *suitability* is a clinical concept that incorporates various factors unique to hematopoietic stem cell transplantation (HSCT), specifically including histocompatibility typing but also potentially including other biologic characteristics of the donor and recipient.
- The most suitable donor may be technically "ineligible," but with appropriate documentation, such transplants may proceed. This is especially true for autologous donors, for whom disease transmission into a disease-naïve donor is not an issue.
- Because HSCTs are often time-sensitive procedures, selection of a preferred donor must incorporate the availability of potential donors.
- Hematopoietic stem cells (HSCs) can be successfully harvested from bone marrow, from umbilical cord blood (which is a rich source of HSCs), and also from peripheral blood following cytokine stimulation +/− plerixafor. Each source has its unique set of advantages and drawbacks.
- Successful engraftment following bone marrow or peripheral stem cell transplantation typically requires a minimum of $2–2.5 \times 10^6$ CD34+ cells/kg recipient weight (which for bone marrow translates into $\sim 2–2.5 \times 10^8$ total nucleated cells/kg recipient weight). Minimal cell doses for umbilical cord blood are approximately tenfold lower ($\sim 2–2.5 \times 10^7$ total nucleated cells/kg recipient weight).
- Donor safety—especially for allogeneic donors, for whom donation provides no direct medical benefit—is a paramount concern in both donor selection and stem cell collection procedures.
- Matched sibling donors (MSD) are usually the ideal donors; in the United States, donation of bone marrow or peripheral stem cells by minor related donors is considered ethical but does require special safeguards to protect the donor.

- Special processing may be required for some donor units; ABO incompatibility between the donor and recipient is one of the most common such situations.
- Cryopreservation can allow storage of stem cell products for years or even decades.
- There are many reactions that can result from stem cell infusions, especially with cryopreserved units; post-thawing processing may decrease the risk of some reactions, but careful observation and prompt intervention to treat any reactions are essential.

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9 Preparing the Patient for HSCT: Conditioning Regimens and Their Scientific Rationale

Carrie-Lynn Kitko, Katie Gatwood, and James Connelly

Abstract

Hematopoietic stem cell transplantation (HSCT) was first developed to cure radiation poisoning and then evolved as a rescue treatment for high-dose cancer therapy. As our understanding of cancer and the donor immune system grew, new conditioning therapies were developed that were safer and emphasized the role of graft-versus-malignancy (GVM) effect (also referred to as graft-versus-tumor (GVT) or graft-versus-leukemia (GVL) effect). Conditioning therapy has three important roles, including anticancer therapy, immunosuppression, and creation of open HSC niches in the bone marrow, but only 0, 1, 2, or 3 of these requirements may be needed depending on the patient and disease. Conditioning regimens range from myeloablative in which the host's hematopoietic stem cells are destroyed by high doses of chemotherapy with or without total body irradiation (TBI) to non-myeloablative (NMA) which relies almost completely on significant immunosuppression to permit the host's immune system to allow the donor HSCs to reside in HSC bone marrow niches and repopulate the host's immune system with the donor's. This chapter discusses the rationale for the need of conditioning; the types of conditioning regimens, including individual agents; and how each is utilized to fulfill these purposes of conditioning therapies. In addition, examples of six common pediatric conditions and how conditioning therapies have been optimized to provide the best chance of cure while minimizing toxicity from HSCT are detailed in this chapter.

Conditioning Therapy: Historical Beginnings

Successful hematopoietic stem cell transplantation (HSCT) requires administration of radiation and/or chemotherapy prior to infusion of hematopoietic stem cells (HSCs). The purpose of this conditioning or preparative regimen is to overcome the immunologic barrier of the host to facilitate allo-

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geneic HSC engraftment and to provide anticancer therapy for malignant diseases. The foundations of HSCT began in the 1940s and 1950s when research was heavily focused on potential protection and treatment of sublethal to lethal doses of radiation that may occur with nuclear reactor accidents or atomic bombs leading to aplastic anemia [1, 2]. Jacobson et al. provided the first hope of an antidote using a mouse model of radiation exposure and curing irradiation-induced aplasia through splenic transplantation [3]. Lorenz et al. subsequently provided the same cure by injecting homologous bone marrow in mice and guinea pigs postirradiation [4]. Additional work proved that the protective effect of spleen and bone marrow transplant was cellular replacement of the bone marrow by donor cells and that successful engraftment of allogeneic cells was only accomplished if a certain threshold dose of radiation was delivered to the animals [2].

The use of HSCT quickly advanced from animal models to human trials secondary to accidental radiation exposures as well as an experimental approach to cure certain cancers. The use of HSCT in humans was first attempted in France by a team of physicians who were caring for five patients who had accidental exposure to high-dose radiation. Bone marrow infusions from donors were used, and four survived, although all were later found to have recovery of autologous hematopoietic cells [5]. Around the same time, Thomas et al. attempted to infuse bone marrow cells in five patients with cancer that had received radiation and/or chemotherapy in attempts to treat subsequent deficiencies in hematopoiesis [6]. There was a temporary "take" of donor cells, later termed engraftment, in two irradiated patients, but no sustained donor hematopoiesis. As in animals, Thomas et al. postulated that more prolonged or permanent engraftment might occur after sufficient dosing of radiation was used to produce marrow aplasia. They also theorized that unlike radiation, chemotherapy agents with extreme marrow toxicity may not be sufficient to engraft donor cells if they lack immunosuppressant properties that are part of irradiation. The importance of sufficient immunosuppression was made more apparent in 1968 when the first successful human engraftment of allogeneic HSCs occurred under the direction of Dr. Robert Good in a patient with severe combined immunodeficiency (SCID) [7].

Conditioning Therapy: Why It Is Necessary

Born out of the atomic era, conditioning therapy with whole-body irradiation followed by infusion of HSCs was discovered and led the way for further advancements in conditioning agents for the treatment of malignancy. Today, there is a wide variety of agents and dosing used for conditioning therapy, and the choice of conditioning regimen is based on multiple factors including underlying disease, status of disease at the time of HSCT, age of the patient, HLA matching, immunocompetency of the recipient, comorbidities of the patient, and physician/treatment center preference. For patients with malignancy, the conditioning therapy must provide a component of anticancer treatment. In the case of autologous HSCT, conditioning therapy allows for administration of otherwise lethal doses of chemotherapy in cancers that exhibit a steep dose-response curve to conditioning agents. The first requirement in allogeneic HSCT is that the anticancer therapy must provide extended cancer control until sufficient immunologic recovery of the donor immune system has occurred to produce a graft-versus-malignancy (GVM) effect (also referred to as graft-versus-tumor (GVT) or graft-versus-leukemia (GVL) effect).

The second requirement for allogeneic HSCT is adequate immunosuppression to overcome the robust rejection barriers of the recipient's innate and adaptive immune systems. Unlike high-dose whole-body irradiation (also referred to as total body irradiation (TBI)) that is both myelo- and immunosuppressive, some agents have only one of these properties, and, therefore, combination therapies have been developed. If the appropriate immunosuppression is not provided to the recipient, the infused cells will be efficiently rejected, resulting in primary graft failure (discussed further in Chap. 11).

The third requirement for successful HSCT revolves around the concept of making "space" within the recipient bone marrow for engraftment of donor HSCs. This "space" within the bone mar-

row is the hematopoietic stem cell niche, including supporting elements such as endothelial cells, adipocytes, specialized osteoblasts, and macrophages [8]. The role of the niche is to keep HSCs protected in a quiescent state, to control HSC self-renewal, and to influence differentiation of HSCs and their progenitors during times of replication, typically following damage to the bone marrow stroma [8]. Current theories assume that the number of niches controls the number of HSCs that can be supported in the bone marrow. As the number HSC niches is small, the total number of HSCs is limited and in order for donor cells to engraft in the recipient, either new niches need to be created (the "augmentation model") or recipient HSCs need to be replaced by incoming donor HSCs (the "replacement model") [9]. There also exists the possibility that leukemia stem cells can occupy the hematopoietic stem cell niche and act as a further barrier to donor HSC engraftment [10].

Conditioning therapy, as part of an allogeneic HSCT, can be used to help create space in the bone marrow by "opening" HSC niches. According to the replacement model, conditioning therapy can deplete recipient HSCs (and presumably leukemia cells) from the HSC niche and provide a more permissible environment for donor HSC engraftment. As HSCs are typically quiescent, the conditioning agents must have activity against non-replicating cells, but not so toxic as to eliminate stromal elements necessary to support HSC engraftment [8]. Busulfan and irradiation are common conditioning agents, and they are very damaging to HSCs. In contrast, cyclophosphamide has no activity against HSCs because of their robust expression of aldehyde dehydrogenase, which converts the active prodrug of cyclophosphamide to its inactivated form [11].

The dose intensity of conditioning agents will influence the number of unoccupied niches and, therefore, the degree of donor engraftment and post-HSCT chimerism, assuming that adequate immunosuppression is also provided. Higher-intensity conditioning regimens result in higher engraftment and donor chimerism. However, the requirement for conditioning therapy to open HSC niches is not absolutely necessary, particularly in situations where a low percentage of donor chimerism is curative for the disease. This has been demonstrated in murine syngeneic and congenic HSCT models (i.e., in experiments where little to no immunologic barrier exists for transplantation) in which a small number of donor HSCs engraft in the absence of conditioning therapy [12]. Similarly, in experimental HSCT of minor histocompatibility mismatches, engraftment of a small number of donor HSCs can also be achieved with T-cell suppression alone [12]. It is uncertain in these experiments if donor cells can actually displace recipient HSCs from the niche or if they engraft unoccupied niches that are created by physiologic migration of stem cells between the bone marrow and peripheral blood. Nonetheless, these experiments prove that donor HSC engraftment can occur without conditioning in the absence of an immunologic barrier. Human HSC engraftment without conditioning therapy has also been established in patients with SCID, the only category of diseases that does not always require conditioning therapy secondary to a lack of T and, in some cases, NK cells to reject donor HSCs.

Rationale and Classification of Conditioning Therapy

Rationale and Graft-Versus-Malignancy (GVM) Effect

In the early days of transplant, it became clear that high-dose chemotherapy alone could not eliminate malignant cells in many patients. There was also growing evidence that the therapeutic benefit of HSCT was not solely due to high-dose conditioning therapy but augmented by a GVM effect [13]. The first suggestion of GVM was introduced in 1956 when irradiated mice receiving an allogeneic HSCT were able to clear their leukemia burden, but recipients of syngeneic transplant could not [14, 15]. It is now believed that both T and NK cells are the principal immune cells to mediate GVM through secretion of cytokines resulting in tumor lysis and by direct cellular interaction through the Fas and perforin pathways [16]. The clinical evidence in humans for GVM by T cells includes (1) higher relapse rates in allogeneic HSCT recipients with T-cell-depleted donor grafts, (2) higher relapse rates in syngeneic HSCT

recipients, (3) lower rates of relapse in HSCT patients with graft-versus-host disease (GvHD), and (4) recognition that donor lymphocyte infusions (DLI) can cure some patients that relapse after HSCT [17–19]. Evidence for a role of NK cells has come from both murine and human studies. These observations include that (1) haploidentical allogeneic HSCT for acute myeloid leukemia (AML) demonstrated higher survival rates if the HLA mismatch was in favor of NK-cell alloreactivity [20] and (2) NK cell incompatibility with donor umbilical cord units was associated with reduced leukemia relapse [21]. Randomized trials and retrospective studies comparing autologous versus allogeneic HSCT for AML [22, 23] and acute lymphoblastic leukemia (ALL) [24, 25] demonstrated lower relapse rates following allogeneic HSCT suggesting a GVM effect. The best evidence to date for an immunologic GVM effect is the re-induction of remission following DLI to treat post-HSCT relapse. Chronic myelogenous leukemia (CML) has been shown to be most responsive to DLI, AML having intermediate sensitivity, and ALL having little to no response to DLI historically [26–28]. Modern strategies currently being developed to exploit GVM include ex vivo expansion and genetic modifications of T and NK cells to specifically target cancer cells [16].

Classification of Conditioning Regimens

Recognition of the GVM effect led investigators to readdress the role and intensity of conditioning therapy. Traditional myeloablative regimens are very toxic. Therefore, a more desirable regimen would be one that provides sufficient immunosuppression to prevent rejection and rely on donor immunoreactivity for HSC engraftment and anticancer therapy. Replacement of more toxic agents was made possible with the introduction of fludarabine to a combination of conditioning regimens, which provides profound T-cell suppression [29, 30]. Initial trials using less toxic conditioning regimens were performed in patients considered ineligible for high-dose therapy secondary to older age or comorbidities [31]. These regimens were quickly extended to pediatric patients with poor performance status from prior intensive chemotherapy and patients with nonmalignant diseases that do not require intensive cytoreductive conditioning treatment. Reduced-intensity regimens in children are particularly attractive given the long-term effects associated with high dose, total body irradiation (TBI), and alkylator chemotherapy including infertility, cognitive deficits, growth retardation, and secondary malignancies.

With a rapid increase in the number and types of conditioning therapies, a universal classification system was needed to categorize the intensity of therapy. In the late 1990s, Dr. Richard Champlin proposed a set of criteria, now known as the "Champlin criteria," during the First International Workshop of Non-myeloablative Stem Cell Transplantation to define the varying intensities of conditioning therapy [32]. From this conference, three classifications of conditioning therapy were defined:

1. Myeloablative conditioning (MAC): Conditioning regimen expected to eradicate the bone marrow and induce profound pancytopenia within 1–3 weeks from administration. The pancytopenia is long lasting, usually irreversible, and in most cases fatal if not rescued by HSC infusion.
2. Non-myeloablative (NMA): Conditioning regimen that will cause minimal cytopenia and does not require HSC infusion for support.
3. Reduced-intensity conditioning (RIC): Conditioning regimen that is not classified as MAC or NMA. RIC regimens result in reversible myelosuppression (recovery within 28 days) when given without HSC infusion, produce mixed chimerism in some patients at time of first assessment (~30 days post-HSCT), and have a low rate of non-hematologic toxicity.

Most RIC regimens combine fludarabine with an alkylating agent or TBI in which the dose of chemotherapy or radiation is reduced by at least 30% [30]. RIC regimens in pediatric patients are not commonly used for malignancy unless the patient has significant comorbidities or an underlying genetic condition predisposing to increased toxicity from a MAC regimen. Head-to-head comparisons

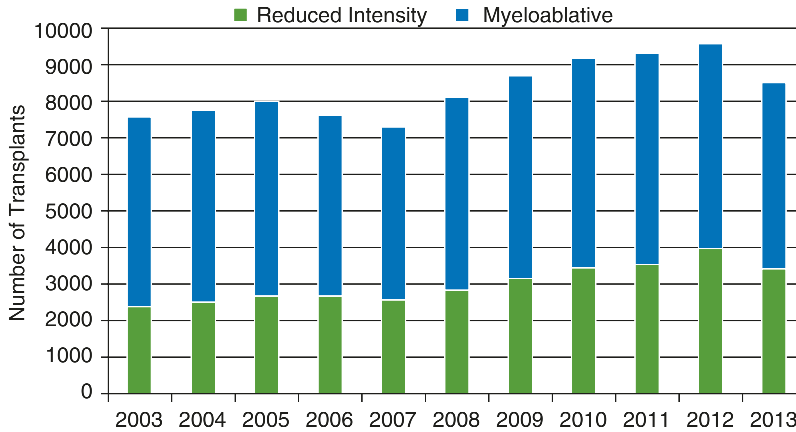

Fig. 9.1 Data from the Center for International Blood and Marrow Transplant Research (CIBMTR) demonstrating rates of both myeloablative and reduced-intensity transplants from 2003 to 2013. The ratio of reduced-intensity to myeloablative transplants has steadily increased with a peak of 42% of all allogeneic adult and pediatric transplants in 2012. (Data from Pasquini MC, Zhu X. Current uses and outcomes of hematopoietic stem cell transplantation: 2015 CIBMTR Summary Slides)

in prospective trials between MAC and RIC for cancer are lacking, but retrospective analyses demonstrate similar rates of relapse and survival in pediatric AML [33, 34] and ALL [35]. For nonmalignant populations, however, RIC has demonstrated similar outcomes compared to MAC regimens for some diseases (sickle cell anemia, thalassemia, primary immunodeficiency disorders, chronic granulomatous disease, Hurler's syndrome) [36–39] or improved outcomes for other diseases (hemophagocytic lymphohistiocytosis, X-linked lymphoproliferative disease) [40, 41]. All of these analyses are retrospective, and prospectively randomized trials comparing these two strategies are lacking. However, because of the perceived safety profile with RIC regimens, the number of both adult and pediatric patients transplanted with this strategy continues to increase (Fig. 9.1) despite the lack of rigorous data to support a RIC approach.

NMA regimens typically consist of very low-dose irradiation (100–300 cGy TBI or TLI), fludarabine, cyclophosphamide, and/or serotherapy, which would include use of various forms of anti-thymocyte globulin (ATG) or alemtuzumab. Excluding patients with bone marrow failure, these regimens are rarely applied to the pediatric population. A final classification, although not originally part of the Champlin classification system, is the designation of "reduced-toxicity conditioning" (RTC), which has been applied to regimens that contain a combination of nucleoside analogs rather than traditional alkylating agents or TBI [42–44]. These regimens typically contain a combination of fludarabine and clofarabine with busulfan or treosulfan and are still considered under the classification of myeloablative but with fewer off-target side effects.

Following this conceptual classification of conditioning therapies, the National Marrow Donor Program (NMDP) and the Center for International Blood and Marrow Transplant Research (CIBMTR) had an expert panel propose operational definitions of RIC regimens to aid in research [32]. These two consortia defined RIC as any regimen that includes (1) TBI of ≤500 cGy as a single fraction or ≤800 cGy if fractionated, (2) <9 mg/kg of oral busulfan (or intravenous equivalent), (3) <140 mg/m^2 of melphalan, (4) <10 mg/kg thiotepa, and (5) BEAM (BCNU, etoposide, cytarabine, melphalan). The acceptability of these definitions were tested in 2006 by a survey to participants at the BMT Tandem Meetings, with more than 60% of the respondents agreeing or strongly agreeing with the first four operational definitions, but only 32% agreed or strongly agreed that BEAM should be downgraded to a RIC regimen [32].

A summary of MAC, RTC, RIC, and NMA allogeneic conditioning regimens used in pediatric patients is provided in Table 9.1. It is important to

Table 9.1 Short- and long-term toxicities associated with myeloablative total body irradiation

Timing	Side effect	Comments
During	• Nausea/vomiting	• Treat with scheduled antiemetics
	• Parotitis	• Typically resolves within 1–2 days
	• Skin irritation/erythema	• Avoid use of lotions during treatment
Early onset	• Mucositis	• Supplementation with glutamine
	• Alopecia	• Rarely permanent
	• Diarrhea	• Treat with antimotility agents if necessary
	• Interstitial pneumonitis	• Typically occurs 1–4 months post-HSCT
	• Veno-occlusive disease	• 5–22% [215, 216]
	• Marrow suppression	• Ablation with fractionated doses >800 cGy
Late onset	• Hypothyroidism	• 27–57% [217, 218] • Annual TSH/free T4 screening
	• Cataracts	• 30–62% [219] • Annual screening • Ophthalmology visit every 1–3 years [220]
	• Puberty and fertility	• Delayed puberty (47–68% of females, 38–77% of males [221]) • Infertility (pregnancy in 2% of female and 6% of male (partner) adult TBI recipients [222]) • Consider pre-HSCT sperm banking/oocyte preservation
	• Short stature	• 20–84% [223, 224] • Monitor growth velocity • Early referral to endocrine
	• Dental complications	• Monitor for dental caries • Monitor for salivary dysfunction • Monitor for damage to developing teeth
	• Chronic renal impairment	• 17% [225] • Annual screen with serum creatinine, urinalysis, and blood pressure [220]
	• Cardiovascular complications	• Cerebrovascular disease • Coronary artery disease • Congestive heart failure • Conduction abnormalities • Valve disease • Metabolic syndrome • Annual exams • Fasting glucose and lipid panel every 2 years • 2D echocardiography every 1–5 years [220]
	• Pulmonary complications	• Restrictive phenotype and diffusion impairment [226] • Screen with pulmonary function testing [220] • Avoid tobacco use
	• Osteonecrosis and low bone mineral density	• Measurement of bone mineral density • MRI of painful joints • Supplement Vitamin D
	• Neurodevelopmental deficits	• Deficits in executive functioning, attention, memory, intelligence quotient, and math and reading [220, 227] Screen with neuropsychiatric testing and early academic intervention
	• Secondary malignancies	• Therapy-related AML/MDS, melanoma, oral, brain, liver, uterine, cervix, breast, bone, and connective tissue cancers [228] Age-appropriate screening (skin and oral checks, Pap smear, colonoscopy, mammogram) and counseling of lifestyle modifications

Common pediatric allogeneic conditioning regimens plotted according to their relative immunosuppressive and myelo-suppressive properties

ATG anti-thymocyte globulin, *Bu* busulfan, *Clo* clofarabine, *Cy* cyclophosphamide, *Flu* fludarabine, *Mel* melphalan, *Treo* treosulfan, *TBI* total body irradiation

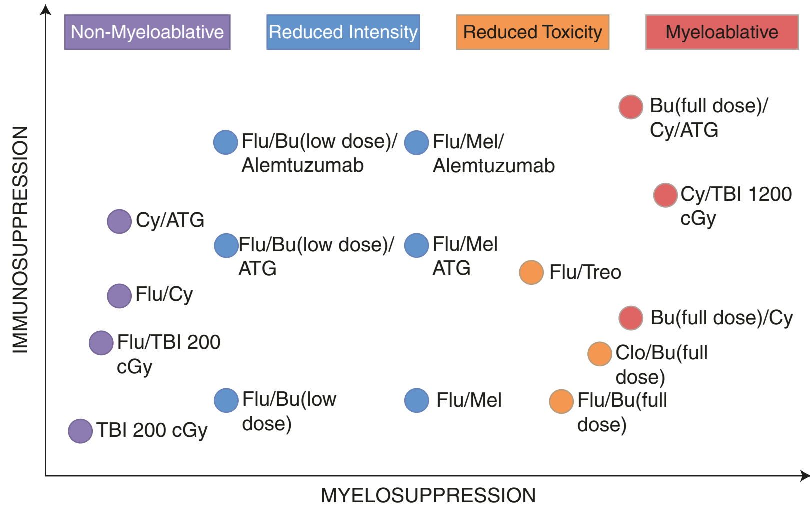

Fig. 9.2 Common pediatric allogeneic conditioning regimens plotted according to their relative immunosuppressive and myelosuppressive properties. *ATG* anti-thymocyte globulin, *Bu* busulfan, *Clo* clofarabine, *Cy* cyclophosphamide, *Flu* fludarabine, *Mel* melphalan, *Treo* treosulfan, *TBI* total body irradiation

realize that each of these conditioning regimens represents a continuum of myelo- and immunosuppression as opposed to discrete categories of toxicity. This alternative way of looking at conditioning regimens is depicted in Fig. 9.2 that plots individual regimens based on myelo- and immunosuppressive properties.

Conditioning Therapy Agents

Total Body Irradiation (TBI)

Despite its toxicity, total body irradiation (TBI) remains a frequently used conditioning agent secondary to its ideal conditioning properties including extreme immuno- and myelosuppression, effectiveness against leukemia and lymphoma cells, and the ability to penetrate sanctuary sites including the testes and CNS [45]. The mechanism of action of irradiation is the induction of both single- and double-stranded DNA breaks and cellular membrane damage [46]. This injury leads to cell death through active apoptosis and passive necrosis as the damaged cell attempts to undergo mitosis with unrepairable chromosomal damage [46]. The initial dose of radiation given to humans was based on experiments in animals and was given as a single fraction of 1000 cGy [30]. Administration of this large, single dose of irradiation was associated with high rates of interstitial pneumonitis and treatment-related mortality [47]. By fractionating the dose of TBI delivered (and thus reducing the rate of administration), the toxicity profile was improved [47, 48]. Most modern-day regimens fractionate or hyperfractionate a total dose of 1200–1600 cGy to decrease organ toxicity yet maintain antileukemic activity. Higher-dose radiation is associated with decreased rates of relapse in adults but not increased survival secondary to increased treatment-related mortality [49]. The most commonly employed regimen in pediatric myeloablative conditioning is 1200–1325 cGy fractionated twice a day over 3–4 days in combination with chemotherapy. Cyclophosphamide was the first chemotherapeutic agent added to TBI as initial trials with TBI alone resulted in fatal tumor lysis, and "pretreatment" with cyclophosphamide reduced this risk [44]. Other agents included in TBI-based conditioning regimens include cytarabine, etoposide, melphalan, and busulfan.

Morbidity from TBI is extensive as irradiation can induce tissue injury throughout the body. The list of toxicities, both acute and long-term, is extensive and summarized in Table 9.1. The extent and severity of late effects are dependent on the method and dose of radiation as well as the age of the patient. Fractionation and dose reduction decrease the risk of interstitial pneumonitis and cataract formation, while most acute toxicities correlate with overall TBI dose and dose rate [47, 50]. Radiation at a younger age (particularly under the age of 2–3 years) has been associated with higher rates of neurocognitive defects and secondary malignancies [51, 52]. While TBI is a very effective component of conditioning regimens

Table 9.2 Commonly used conditioning regimens for pediatric allogeneic transplantation

Conditioning regimen	Standard dosing	Disease indications
Myeloablative regimens		
Cyclophosphamide/total body irradiation	Cyclophosphamide (120 mg/kg over 2 days) TBI (1200–1320 cGy fractionated over 3 days)	ALL (standard in the USA)
Busulfan/cyclophosphamide +/− anti-thymocyte globulin (ATG)	Busulfan[a] (12.8 mg/kg IV over 4 days) Cyclophosphamide (120–200 mg/kg over 2–4 days) Thymoglobulin (ATG) (6 mg/kg over 3 days)	AML/MDS Sickle cell anemia Thalassemia
Reduced-toxicity regimens		
Fludarabine/busulfan +/− ATG/ alemtuzumab	Fludarabine (160 mg/m^2 over 4 days) Busulfan[a] (12.8 mg/kg over 4 days) Thymoglobulin (ATG) (6 mg/kg over 3 days) Alemtuzumab (variable)	AML/MDS (standard in the USA) Nonmalignant disorders
Fludarabine/treosulfan	Fludarabine (150 mg/m^2 over 5 days) Treosulfan (30–42 g/m^2 over 3 days)	AML/MDS ALL Nonmalignant disorders
Clofarabine/busulfan[a]	Clofarabine (150 mg/m^2 over 5 days) Busulfan[a] (12.8 mg/kg IV over 4 days)	AML/MDS
Reduced-intensity regimens		
Fludarabine/reduced busulfan[a] +/− ATG/alemtuzumab	Fludarabine (160 mg/m^2 over 4 days) Busulfan[a] (6.4 mg/kg over 2–4 days) Thymoglobulin (ATG) (6 mg/kg over 3 days) Alemtuzumab (variable)	AML Nonmalignant disorders
Alemtuzumab/fludarabine/ melphalan	Fludarabine (150 mg/m^2 over 5 days) Melphalan (140 mg/m^2) Alemtuzumab (variable)	Nonmalignant disorders
Non-myeloablative regimens		
Cyclophosphamide/ATG +/− total body irradiation (TBI)/ total lymphoid irradiation (TLI)	Cyclophosphamide (200 mg/m^2 over 4 days) Thymoglobulin (ATG) (7.5 mg/kg over 3 days) TBI (200 cGy) TLI (400 cGy)	Aplastic anemia (CyATG standard in the USA for matched sibling donor)

[a]Sarting dose of busulfan based on age and weight; subsequent doses adjusted based on PK studies

for successful engraftment and elimination of cancer cells (particularly leukemia), it is associated with a number of significant short- and long-term toxicities such that alternative conditioning regimens are designed to exclude TBI from the conditioning regimen.

In principle, tissues outside of the lymph tissue, the bone marrow, and, for leukemia, the CNS and testes should not require irradiation for successful engraftment and anti-malignancy therapy. Therefore, tissue blocks are used commonly nowadays to decrease radiation exposure to the lungs and prevent the development of interstitial pneumonitis [53]. Irradiation of lymphoid tissue only (known as total lymphoid irradiation (TLI)) can have significant immunosuppressive effects without myelosuppression and reduced tissue toxicity. Also, low-dose irradiation (TBI or TLI) has been employed in reduced-intensity and non-myeloablative regimens and exerts a primarily immunosuppressive effect on the recipient. Doses as low as 200 cGy along with additional agents such as fludarabine and cyclophosphamide are commonly employed with successful engraftment in both pediatric and adult patients (Table 9.2).

High-Dose Chemotherapy

Development of alternative, solely chemotherapy-based regimens was necessary in the early days of HSCT secondary to the lack of access to TBI at some centers [44]. Today, alternative conditioning regimens are purposely chosen to avoid short- and long-term toxicities of TBI. There are particular therapeutic properties that guide selection of combination chemotherapy, including (1) steep dose-response curve with myelotoxicity being the usual dose-limiting toxicity, (2) tolerability at higher doses, (3) cell cycle nonspecific-

ity, (4) wide biologic activity (active against multiple tumor types), and (5) synergistic effects when combined with other agents without overlapping toxicity.

Alkylator Chemotherapy

The most widely used class of drugs that encompasses many of the above properties is alkylator chemotherapy. Alkylating agents are antitumor drugs that act through the production of reactive intermediates that covalently bind to a variety of cellular molecules, with DNA molecules as the primary target. The binding of an alkylating agent to DNA molecules results in the formation of both inter- and intra-strand DNA cross-links, preventing further DNA synthesis and ultimately resulting in cytotoxicity [54]. Alkylating agents can bind to DNA independent of its replication, thus making this class of drugs cell cycle-nonspecific. This property, combined with the fact that these agents exhibit a linear dose-response relationship, has made them one of the primary drug classes used in HSCT for a variety of diseases.

The most commonly used alkylating agents used today are busulfan, cyclophosphamide, melphalan, thiotepa, and treosulfan. As described below, these agents differ in their toxicity against bone marrow precursors, cancer cells, and immune cells, as well as their side effect profiles, all of which impact the decision of which and how much of each agent to use in conditioning. Fludarabine is typically paired with an alkylator as it inhibits DNA repair following DNA alkylation and therefore provides synergistic activity against cancer and lymphoid cells [45].

Busulfan (Bu): Busulfan (Busulfex®, Myleran®) [55] is a bifunctional alkylating agent that is widely used in HSCT conditioning regimens for both malignant and nonmalignant diseases. Cells of the myeloid lineage are highly susceptible to the cytotoxic effects of busulfan, and this drug is particularly toxic to hematopoietic stem cells [56]. However, the effects of busulfan on mature lymphocytes are limited, and, therefore, it has minimal immunosuppressive properties. The total daily dose for HSCT is typically 3.2 mg/kg IV given either once daily or divided every 6 h, depending on age and weight. It is routinely administered over 2–4 consecutive days, with longer therapy durations achieving myeloablation. Because of its relatively narrow therapeutic index, busulfan dosing is adjusted using pharmacokinetic monitoring to achieve a target steady-state plasma concentration (Cpss) or area under the curve (AUC) level [57]. An AUC is calculated following several measurements of drug plasma concentrations typically following the first administered dose. A target Cpss of 600–900 ng/mL or AUC of 900–1200 μM min is commonly used during high-dose busulfan therapy [58]. Dosing for subsequent doses may be adjusted based on the calculated Cpss or AUC. Alternatively, utilization of a small test dose of busulfan (0.8 mg/kg) has been shown to be an effective method to calculate the dose required to reach the target Cpss or AUC [59].

Busulfan is available in oral and intravenous (IV) formulations. Oral bioavailability of busulfan is highly variable, with up to a threefold difference in busulfan AUC levels after oral dosing observed within individual patients [60]. In adults, the relationship between dose and AUC within the same patient has been shown to be much more predictable over multiple days of IV busulfan administration [60]. However, due to the many challenges associated with oral busulfan use, such as limited pill size, need for re-dosing after vomiting, and increased liver toxicity, IV is the preferred route of administration for pediatric HSCT patients.

Busulfan undergoes extensive metabolism in the liver, primarily through conjugation with glutathione. Several studies have demonstrated that busulfan clearance declines with increasing age and body weight, which leads to the potential for underdosing in pediatric patients [61]. Thus, younger children require drug administration every 6 h compared to once daily in adolescents and adults. Also, secondary to a larger volume of distribution, larger doses (1.1 mg/kg/dose) must be used in children younger than age 4 or ≤12 kg in order to achieve the same cytotoxic effects [62]. Regardless of age, all dosing of busulfan should be delivered based upon the results of pharmacokinetic monitoring.

Busulfan is highly lipophilic and exhibits a low level of protein binding, allowing it to readily cross the blood-brain barrier to penetrate both the brain and cerebrospinal fluid [63]. This property not only enhances the activity of

busulfan against leukemia and lymphoma cells in the CNS but also results in the propensity to cause seizures, particularly in the setting of high-dose therapy. Therefore, all patients receiving busulfan require seizure prophylaxis with an anticonvulsant medication, starting 12–24 h before busulfan therapy and continuing through 24–48 h after therapy completion. Levetiracetam (Keppra®) is the preferred anticonvulsant prophylaxis agent as it has few drug interactions, is not metabolized in the liver, and will rapidly achieve therapeutic concentrations following an initial loading dose.

Common toxicities that occur with high-dose busulfan therapy include nausea and vomiting, diarrhea, and mucositis, which occur in more than 80% of the patients [64]. Busulfan may also cause hyperpigmentation of the skin, especially in skin folds and nail beds, and may be accompanied by rash and pruritus. Skin discoloration typically resolves 7–10 days following drug administration, but, in rare cases, it can be permanent. Seizures are also a common adverse effect, as mentioned above. More rare but serious toxicities associated with high-dose busulfan are pulmonary toxicity and sinusoidal obstructive syndrome (SOS), also known as veno-occlusive disease (VOD) of the liver. SOS has been reported as occurring in up to 20% of the patients receiving high-dose busulfan [65]. However, it is important to note that much of the data regarding the incidence of busulfan-induced SOS dates from an era when the drug was most commonly combined with cyclophosphamide, which also carries a high risk of VOD [60]. Therefore, the true incidence of VOD from high-dose busulfan alone is likely lower with the use of newer, reduced-toxicity regimens. Busulfan-associated pulmonary toxicity manifests as bronchopulmonary dysplasia and interstitial pulmonary fibrosis, often referred to as “busulfan lung” [66]. It is characterized by progressive, restrictive lung disease defined by a decrease in forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO). Busulfan pulmonary toxicity occurs in up to 5% of the patients, and onset may often be delayed, occurring 4 months to 10 years following therapy [66]. Late effects of high-dose busulfan therapy include gonadal atrophy and infertility and secondary malignancies.

Busulfan has several clinically important drug interactions. The administration of acetaminophen (Tylenol®) use should be avoided within 72 h of busulfan administration as it can increase busulfan concentrations and lead to an increased risk of toxicity [67]. Acetaminophen depletes glutathione stores needed for the conjugation and elimination of busulfan, resulting in decreased busulfan clearance and higher levels of drug exposure [67]. The use of the azole class of antifungals (e.g., fluconazole, voriconazole, posaconazole) should also be avoided within 24 h of busulfan administration because they can decrease the clearance of busulfan by approximately 20% through suspected inhibition of hepatic CYP3A4-mediated metabolism [68]. Metronidazole (Flagyl®) also decreases busulfan clearance, and concurrent use of these agents has been shown to cause a dramatic increase in serum busulfan concentrations by up to 79–87%. Therefore, the use of metronidazole should be avoided within 24 h of busulfan administration [69].

Cyclophosphamide (Cy): Cyclophosphamide (Cytoxan®) is another commonly used alkylator and is most commonly used in HSCT in preparative regimens for malignancies and as prophylaxis for graft-versus-host disease (GvHD) [70]. Cyclophosphamide is unique among the alkylating agents due to its significant immunosuppressive properties. It affects both T- and B-cell functions in a dose-dependent manner. At low doses, cyclophosphamide selectively inhibits the function of regulatory T cells and can also induce B-cell tolerance [71]. At high doses, cyclophosphamide leads to profound lymphocyte depletion while having little effect on HSCs and platelets [72]. The total daily dose of cyclophosphamide used in HSCT is 50–60 mg/kg/day and is typically administered for 2–4 consecutive days.

Cyclophosphamide requires metabolism by the CYP P450 enzymes in order to form two metabolites, 4-hydroxycyclophosphamide and aldophosphamide. Aldophosphamide is further converted to the active metabolites, phosphoramide, and acrolein [73]. Aldophosphamide can also be inactivated to a nontoxic metabolite via oxidation by aldehyde dehydrogenase (ALDH) [74]. Hematopoietic progenitor cells have high levels of ALDH activity and thus are relatively unaffected by the cytotoxic

effects of cyclophosphamide [11]. This explains why high doses of cyclophosphamide can be administered without resulting in myeloablation. It also plays an important role in the efficacy of post-HSCT cyclophosphamide therapy for GvHD prophylaxis by permitting selective cytotoxicity to rapidly proliferating lymphocytes without concomitant damage to the newly infused HSCs.

The most common toxicities that occur with high-dose cyclophosphamide are nausea, vomiting, and mucositis [75]. Another important toxicity is hemorrhagic cystitis, which is due to an interaction with one of the active metabolites, acrolein, with the bladder wall resulting in irritation and epithelial damage [76]. It typically occurs shortly after administration of cyclophosphamide, and there are very few treatment options once it occurs. Therefore, aggressive preventative strategies are employed, including hyperhydration, the administration of MESNA (2-*m*ercapto*e*thane *s*ulfonate *Na*) at 60–100% of the cyclophosphamide dose, and monitoring urine hemoglobin and specific gravity during 24 hours following cyclophosphamide administration (see Chaps. 16 and 22 for further discussion of hemorrhagic cystitis) [77]. More rarely occurring toxicities associated with high-dose cyclophosphamide use are cardiotoxicity, SOS, and syndrome of inappropriate antidiuretic hormone (SIADH) secretion [75]. The dose-limiting toxicity of cyclophosphamide is cardiotoxicity, which manifests in its most severe form as a hemorrhagic myocarditis that presents as acute heart failure leading to cardiogenic shock and death [78]. Less severe forms of cardiac toxicities include tachyarrhythmias, hypotension, and myocarditis. These toxicities are associated with the magnitude of the dose in a single treatment course, most commonly occurring with doses >150 mg/kg. It is typically acute, occurring within 48 h to 10 days of administration; it occurs in <7% of HSCT patients (see Chap. 23 for further discussion of cyclophosphamide-induced cardiotoxicity). Although rare, cyclophosphamide therapy is also associated with an increased risk of developing a secondary leukemia or myelodysplasia [75].

Melphalan (Mel): Melphalan (Alkeran®, Evomela®) [79] is another alkylating agent and, like busulfan, is particularly toxic to hematopoietic stem cells [54]. Melphalan is routinely used in autologous HSCT conditioning regimens for lymphoma and neuroblastoma. It is typically given as a single dose of 140–200 mg/m^2, and the dose must be reduced for renal dysfunction. A minimum dose of 100 mg/m^2 is required for myelosuppression and doses ≥140 mg/m^2 are myeloablative. The primary adverse effect associated with melphalan therapy is gastrointestinal (GI) toxicity, including nausea/vomiting, diarrhea, and mucositis, throughout the entire GI tract [80]. Using cryotherapy during administration can reduce the severity of mucositis. Cryotherapy causes vasoconstriction of the blood vessels in the oral mucosa and, thus, reduces drug exposure to the area by keeping the mouth cold (using ice or other cold liquids/foods), starting 10 min prior to and continuing until 30 min after melphalan infusion [81]. Diarrhea caused by melphalan therapy is typically delayed, occurring 5–7 days following its administration, and is a direct result of sloughing of the GI mucosa. Development of a secondary leukemia or myelodysplastic syndrome is a rare delayed toxicity associated with melphalan use [80].

Thiotepa (Thio): Thiotepa (Tepadina®) [82] is an alkylating agent metabolized by CYP P450 to its primary metabolite, triethylenephosphoramide (TEPA), along with several other metabolites, all of which have cytotoxic activity [54]. Thiotepa has potent antitumor properties but is myeloablative at high doses. Therefore, it has traditionally been used in autologous HSCT preparation regimens for CNS tumors and neuroblastoma. However, it is also felt to have some immunosuppressive effects and has been used increasingly in conditioning regimens for allogeneic HSCT, particularly in haploidentical HSCT regimens, due to improved engraftment rates with its use [83, 84]. The usual dose of thiotepa is 5–15 mg/kg/day for 1–3 days, with doses ≥10 mg/kg/day achieving full myeloablation. Thiotepa is highly lipid-soluble and readily penetrates the blood-brain barrier, thus explaining its utility in CNS malignancies [85].

Toxicities most commonly associated with thiotepa use are nausea/vomiting and mucositis [86]. Thiotepa is also excreted into sweat and can cause skin rash and discoloration, flaking,

peeling, and chemical burns if left on the skin [87]. Therefore, a strict skin care regimen must be utilized during high-dose thiotepa therapy. Beginning with the first dose and continuing until 24 h after completion of the last dose of thiotepa, it is recommended for the patient to bathe with a mild soap and change clothes, dressings, and bedding every 6 h. Patients should wear loose clothing and should not apply any skin lotions, ointments, or other occlusive substances or dressings during this time. It is also important that any caregivers avoid direct skin-to-skin contact with the patient or their clothing/bedding and they need to wear gloves when providing care to avoid accidental drug exposure. Severe, but less commonly occurring, toxicities associated with thiotepa use are CNS toxicity; hepatic toxicity, including SOS; and development of secondary leukemia [86]. These side effects are described in more detail in Chaps. 15 and 24–26.

Treosulfan (Treo): Treosulfan (Ovastat®) is a busulfan analog first approved for use in Europe for the treatment of advanced ovarian cancer and is now used as an investigational agent in HSCT conditioning regimens. Treosulfan has potent effects on both committed and primitive stem cells, as compared to busulfan which primarily effects primitive stem cells [88]. Treosulfan also has immunosuppressive properties with activity against T, B, and NK cells [89, 90]. It is typically dosed at 14 g/m^2/day IV for 3 consecutive days. In contrast to busulfan, treosulfan exhibits a linear correlation between AUC levels and dose with little interpatient variability and, therefore, does not require pharmacokinetic monitoring and dose adjustment [88]. Treosulfan also does not undergo hepatic metabolism, another major difference from busulfan.

One of the primary advantages of treosulfan is its markedly decreased toxicity profile in comparison to busulfan. Since treosulfan does not undergo hepatic metabolism and does not penetrate extramedullary sites with great affinity, it is associated with much lower rates of hepatic toxicity and VOD than busulfan. Penetration of treosulfan into the CNS is low and therefore does not require seizure prophylaxis. However, increased brain exposure has been demonstrated in young compared to old rats, and treosulfan administration in children less than 6 months has been associated with irritability, hypertonicity, and rarely seizures, indicating a potential higher CNS exposure for very young patients [91, 92]. GI side effects such as mucositis and diarrhea are the most common adverse effects seen with treosulfan therapy and are typically less severe than with what is observed with busulfan, with the incidence of severe grade III–IV toxicity reported as low as 5–20.7% [88].

Carmustine (BCNU): Carmustine [93] belongs to the nitrosourea group of alkylating agents and is most commonly used in preparation for autologous HSCT for lymphoma. It is usually administered as a single dose of 300 mg/m^2. Carmustine is highly lipid-soluble resulting in CSF concentrations >50% of blood plasma levels and, thus, has potent activity in the CNS [54]. The most common toxicities associated with carmustine use are phlebitis, CNS toxicity, and acute nausea and vomiting, typically only lasting 4–6 h following drug administration [94]. The phlebitis and CNS toxicity caused by carmustine are due to the IV formulation, which is solubilized in ethanol. Therefore, the CNS effects are similar to those of alcohol intoxication (dizziness, blurred vision, etc.), and pediatric patients tend to experience these effects to a greater degree. Carmustine also causes pulmonary toxicity, and doses >450 mg/m^2 should be avoided. When doses of 450 mg/m^2 have been used as part of the conditioning regimen, pneumonitis developed in about 20% of the patients [95]. The incidence has been reduced by decreasing the dose of carmustine to 300 mg/m^2. Patients should be monitored for new pulmonary symptoms such as dyspnea, particularly in the first 2 months post-HSCT, with prompt steroid treatment if BCNU pneumonitis is suspected.

Antimetabolite Chemotherapy

Cytarabine (ara-C): Cytarabine (Cytosar®) [96] is an antimetabolite cytotoxic agent. It is an analog of cytidine and is cell cycle-specific for S phase. Upon entry into the cell, cytarabine is phosphorylated to its active form, arabinosylcytosine triphosphate (ara-CTP), which is then responsible for the subsequent inhibition of DNA polymerase and chain elongation. The antimetabolites exert their antineoplastic effects by mimicking the structure of the naturally occurring nucleic acids to incorporate into DNA to terminate DNA replication [54]. It is most commonly

used in autologous HSCT conditioning regimens for lymphoma and is typically dosed at 400 mg/m^2/day for 4 consecutive days. Cytarabine is highly water-soluble, distributes rapidly into total body water, and yet also has the ability to penetrate the CNS [97].

The toxicity of cytarabine therapy is dependent on the dose and duration of therapy. Cytarabine is one of the few agents used in HSCT conditioning regimens that is dosed similar to or lower than doses used for other indications. Therefore, toxicity associated with cytarabine at HSCT doses is generally mild, with the most common being nausea and vomiting [98]. These GI effects are highly associated with dose administration and usually subside quickly following completion of the infusion. Cerebellar and ophthalmic toxicities typically seen with high-dose cytarabine therapy are quite rare when used in HSCT.

Fludarabine (Flu): Fludarabine (Fludara®) is a member of the purine nucleoside analog group of antimetabolites, specifically an analog of adenosine [99]. It exerts its cytotoxic effects by incorporation into DNA as a false nucleotide and subsequently inhibits key DNA replication enzymes that ends in DNA chain termination [54]. Fludarabine also impairs DNA repair mechanisms, owing to its ability to have both cell cycle-specific and cell cycle-nonspecific activity, and, when paired with an alkylating agent, this effect is synergistic [100]. It also has pronounced immunosuppressive properties through its potent effects on lymphocytes and can cause a decrease in the CD4+/CD8+ ratio for up to 24 months following treatment [101]. Fludarabine is used in a wide variety of HSCT conditioning regimens for both malignant and nonmalignant diseases. It is typically dosed at 30–40 mg/m^2/day for 4–5 consecutive days.

Fludarabine is one of the most well-tolerated agents used in HSCT conditioning regimens. It has a low toxicity profile, and this, along with its potent immunosuppressive effects, is why it has become widely used in both reduced-intensity and reduced-toxicity HSCT regimens [45]. The primary adverse effects associated with fludarabine use are mild myelosuppression and increased infection risk, which is expected with any agent used in preparation for HSCT. Outside of these, the most common toxicities are mild nausea and vomiting, occurring in approximately 30% of the patients [102]. Neurotoxicity is a rare side effect that is associated with high doses of fludarabine (>50 mg/m^2/dose); however, doses this high are rarely used in HSCT [101].

Clofarabine (Clo): Clofarabine [103] is a second-generation purine antimetabolite. In comparison to fludarabine, it is incorporated into DNA more readily, and its structure makes it resistant to intracellular enzymatic inactivation [54]. It also causes mitochondrial damage to induce apoptosis, which is an effect not observed with fludarabine. These key pharmacokinetic properties of clofarabine render it to have enhanced antileukemic activity as compared to the first-generation purine analogs and make it an ideal agent for use in reduced-intensity or reduced-toxicity regimens for leukemia in an effort to reduce post-HSCT relapse rates. It is currently utilized in reduced-toxicity HSCT conditioning regimens for leukemia, particularly in patients with active disease at the time of HSCT. Clofarabine is typically dosed at 30–40 mg/m^2/day for 4–5 consecutive days and used in combination with an alkylating agent.

Similarly to fludarabine, clofarabine is a potent immunosuppressive agent, and thus one of its primary toxicities is risk for infection [104]. Other commonly occurring toxicities are mild nausea and vomiting, palmar-plantar erythrodysesthesia, and hepatotoxicity. Hepatotoxicity occurs in up to 25–40% of the patients and manifests as transient elevation of transaminases and serum bilirubin that typically resolves within 15 days following clofarabine administration [105]. Less common but serious toxicities associated with clofarabine use include capillary leak syndrome (CLS) and severe dermatologic reactions. CLS is rare, occurring in approximately 4% of the patients, but is generally severe. Premedicate with corticosteroids prior to each dose is recommended. In the event that CLS develops, subsequent doses should be dose-reduced and infused over a longer period of time. Clofarabine can cause a variety of dermatologic side effects, most commonly manifesting as

pruritic rash and hand-foot syndrome; however, in rare cases these reactions can progress to Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Clofarabine therapy should be discontinued if any sign of an exfoliative rash occurs [105].

Other Chemotherapeutic Agents

Carboplatin (Carbo): Carboplatin is a member of the platinum analog group of antineoplastic agents that exert their cytotoxic effects by binding to purine bases in DNA creating DNA cross-links that ultimately result in double-strand breaks. When compared to the other platinum compounds, carboplatin produces greater myelosuppression [54]. Carboplatin is most commonly used in autologous HSCT conditioning regimens for CNS tumors, neuroblastoma, and germ cell tumors. It is usually dosed per kilogram of body weight in pediatric patients weighing ≤12 kg and by body surface area or AUC-based dosing in older patients. Typical dosing for HSCT ranges from 10 to 17 mg/kg/day for 2–4 days; 400 to 700 mg/m^2 for 3–4 days; or a target AUC of 7 mg/mL min per dose for 1 day. Carboplatin dosing must be adjusted for renal dysfunction, and the dosing method utilized can vary depending on the presence of preexisting renal insufficiency [106].

The most common toxicities associated with carboplatin use are nausea and vomiting, renal toxicity, ototoxicity, and neuropathy [106]. Upward of 80% of the patients will experience nausea and vomiting with carboplatin, and it is often delayed up to 5 days after receipt of the drug. Utilization of aggressive antiemetic regimens for prevention is essential. Renal toxicity occurs in up to 25% of the patients and is due to interaction between the drug and the distal renal tubules. Often, renal function can decline without an associated increase in serum creatinine. The renal damage also results in wasting of cations, specifically magnesium, potassium, and calcium; as a result, many patients require supplementation during and immediately following carboplatin therapy [107]. Therefore, IV hydration during carboplatin therapy is essential to minimize renal toxicity. It is also important to note that carboplatin can cause hypersensitivity reactions that typically occur within minutes of drug administration [106]. Appropriate anaphylaxis precautions should be in place, and patients need to be closely monitored for signs and symptoms of reaction.

Etoposide (VP-16): Etoposide (Toposar®) [108] is a epipodophyllotoxin-type topoisomerase II inhibitor that acts by creating cleavage complexes on DNA that interact with topoisomerase II and lead to double-strand DNA breaks that halt cell cycle progression, typically in early G2 or S phase [54]. It is one of the few cell cycle-specific agents used in HSCT conditioning regimens. Etoposide is most commonly used in autologous conditioning regimens for lymphoma, neuroblastoma, and germ cell tumors. It is also used in some allogeneic HSCT conditioning regimens for acute lymphoblastic leukemia (ALL). Etoposide is most commonly dosed at 200 mg/m^2/day for 3–4 consecutive days but may be dosed based on kilograms of body weight in smaller pediatric patients. Etoposide is highly protein-bound (94–98%) and, as a result, has very little penetration into the CNS. Etoposide is metabolized in the liver, primarily by CYP P450. Etoposide requires dose adjustments for renal dysfunction and elevated serum bilirubin levels [109]. It is important to note that the drug is highly unstable and can quickly precipitate at concentrations <0.4 mg/mL. This often results in large fluid volumes for adequate dilution of the dose and may require total daily or hourly fluid restriction requirements in small or fluid-sensitive patients.

The most common toxicities associated with high-dose etoposide use are nausea and vomiting, mucositis, hypotension, and palmar-plantar erythrodysesthesia (hand-foot syndrome) [109]. Hypotension with etoposide therapy occurs during the infusion and is due to the IV diluents polysorbate 80 and polyethylene glycol. It can typically be managed by slowing of the infusion rate. A rare but important delayed toxicity of etoposide therapy is secondary leukemia; fortunately, the risk is much less when used in HSCT regimens compared to other treatment regimens where patients receive a total of ≥6 g/m^2 of etoposide [110].

Preparative Regimens for Common Pediatric Conditions

Acute Lymphoblastic Leukemia (ALL)

ALL is the most common pediatric cancer. Approximately 3000 children and teenagers are diagnosed with ALL annually in the USA [111]. Well-designed clinical trials have improved survival rates from 10% to 20% observed in the 1950s [112] to greater than 90% survival reported in the most recent clinical trials [113, 114]. Despite these excellent survival rates, the outcome for patients who relapse is very poor, though timing of relapse is critical. Patients that have late relapses have survival rates of about 50%, compared to only 20–30% if they relapse while still receiving therapy [115]. Which patients should receive transplant and when to perform the transplant remain controversial. While these topics are addressed in Chaps. 4 and 5, a table of common indications is provided in Table 9.3.

As outlined earlier, ideal agents used in the preparative regimen for ALL should provide sufficient immunosuppression to support donor engraftment, provide antileukemic treatment, and penetrate the CNS and testes, which are the second and third most common locations of relapse, respectively, after the bone marrow. Early reports of successful sibling donor allogeneic bone marrow transplant for acute leukemia started appearing in the late 1970s and early 1980s [116, 117]. At that time, studies included both AML and ALL, as well as both pediatric and adult patients altogether. The regimens used in these early studies involved a single fraction of 1000 cGy TBI plus 2 days of cyclophosphamide (60 mg/kg IV X two doses). The disease status in these early reports ranged from second complete remission (CR2) to active disease, and patients in CR2 had improved outcomes as compared to patients with higher stage disease. The next evolution of conditioning regimens for ALL involved changing TBI from a single dose to hyperfractionated regimens. The goal with hyperfractionation was to further improve the reduction in leukemic burden by providing a higher total dose of radiation while decreasing toxicity to normal tissues, particularly

Table 9.3 Acute lymphoblastic leukemia: possible indications for transplant

CR1 (currently being studied)
Primary induction failure
Persistent minimal residual disease (MRD) after consolidation
Extreme hypodiploidy (<44 chromosomes)
Infants with MLL rearrangements <6 months with high-risk characteristics
Emerging CR1 HR features (unknown if improved with HSCT)
Early T-cell precursor ALL
T-Cell ALL lacking biallelic TCR gamma locus deletions
IKZF1 deletions
CR2 (currently being studied)
High risk
• Isolated marrow relapse on treatment or within 6 mo of completion of therapy (36 months from diagnosis)
• Combined marrow and extramedullary relapse within 18 months of diagnosis
Intermediate risk
• Isolated extramedullary relapse within 18 months of diagnosis
• Marrow relapse (isolated or combined) more than 6 months after completion of therapy or 36 months from diagnosis (moves to HR if persistent MRD after re-induction)
CR3+ (currently being studied)
Any second or greater relapse, whether bone marrow, isolated extramedullary or combined relapse

Transplant in the first remission is still considered somewhat controversial. Indications for HSCT for relapsed disease would be considered more standard. Table is adapted from Pulsipher et al. [229]

pulmonary toxicity. An early report from Memorial Sloan Kettering Cancer Center in 1981 included both pediatric and adult patients with acute leukemia (both ALL and AML). This report included 47 patients that received cyclophosphamide plus 1320 cGy TBI divided in 11 total fractions and compared the outcomes to their previous transplant protocol that included 12 patients that received the same dose of cyclophosphamide followed by a single dose of 1000 cGy TBI. Patients receiving hyperfractionated TBI had improved disease-free survival (DFS) as well as decreased incidence of interstitial pneumonitis (33% vs 70%) and resulted in

improved overall survival [48]. A follow-up study expanded the pediatric cohort, again including both ALL (n = 59) and AML (n = 38) patients using the same regimen. A low rate of interstitial pneumonitis was again observed with improved DFS. Among the 59 patients with ALL, those transplanted in CR2 had a DFS of 64%, as compared to only 42% for those in CR3 and only 23% in those in CR4/relapse [118].

Cyclophosphamide with TBI (Cy/TBI) is generally considered the standard of care conditioning regimen for pediatric ALL. However, relapse remains a significant problem, and, therefore, some centers have tried other chemotherapy agents with TBI or with Cy/TBI with the goal of further decreasing relapse rates. Other agents partnered with TBI, including etoposide (VP-16) [119, 120], cytarabine (ara-C) [121], thiotepa [122], and melphalan [123, 124], have produced variable results. A retrospective study performed by the Center for International Blood and Marrow Transplant Research (CIBMTR) compared various HSCT conditioning regimens in 765 children transplanted in CR2 or greater with either a related or unrelated donor from 1998 to 2007. This study compared four groups categorized into high- or low-dose TBI (≤1200 cGy vs ≥1300 cGy) with each group further divided into cyclophosphamide as a single chemotherapeutic agent versus cyclophosphamide plus etoposide. This large retrospective study found no decrease in relapse rate from the higher dose of TBI or the addition of etoposide. Furthermore, the treatment-related mortality was significantly increased in patients receiving high-dose TBI and the addition of etoposide [125].

Because of the significant long-term toxicities associated with TBI, many transplant centers have explored chemotherapy-only approaches to conditioning regimens for ALL. The most commonly studied chemotherapy regimen is cyclophosphamide with busulfan. There are two combinations of these agents that have been used, Bu/Cy2 (busulfan 16 mg/kg given over 4 days plus cyclophosphamide 120 mg/kg given over 2 days) and Bu/Cy4 (busulfan 16 mg/kg given over 4 days plus cyclophosphamide 200 mg/kg given over 4 days). A retrospective study from the CIBMTR compared outcomes for patients that received Cy/TBI (n = 451) to those that received Bu/Cy regimens (n = 176) between 1988 and 1995. The authors found that recipients of Cy/TBI had an improved 3-year overall survival (55% vs 40%, p = 0.003) and that recipients of Bu/Cy regimens were more likely to experience death and treatment failure [126]. It should be noted that this was studied in an era before IV busulfan was routinely available and monitoring for busulfan pharmacokinetics was not consistently performed.

These studies have led to the practice of using Cy/TBI as the standard conditioning regimen, particularly in the USA. International centers, however, have favored different combinations. A large retrospective study from the Japan Society for Hematopoietic Cell Transplantation studied 767 children transplanted for ALL with one of four chemotherapy combinations with TBI, and they found a 5-year event-free survival (EFS) of 71% for those children that received TBI plus melphalan which was superior to the other three comparator arms, including Cy/TBI [124]. There is also emerging data for newer chemotherapeutic agents with improved toxicity profiles, such as treosulfan. In a retrospective study from the European Society for Blood and Marrow Transplantation (EBMT) database, 71 patients with ALL received a chemotherapy-only regimen that included treosulfan and resulted in very low rates of early regimen-related toxicity and a 3-year EFS of 51%. These results have opened the door to challenge the accepted paradigm that Cy/TBI should be considered the standard of care conditioning regimen for ALL. There is a current trial in Europe called FORUM (NCT01949129) that is randomizing patients to receive etoposide/TBI versus two possible chemotherapy-only approaches—fludarabine/thiotepa/treosulfan or fludarabine/thiotepa/busulfan (IV). This study opened in 2013 and has a target enrollment of 1000 patients. The results of this study could have a major impact on how pediatric ALL patients are transplanted in the future.

Acute Myelogenous Leukemia (AML)

AML is the second most common form of leukemia in the pediatric population with more than

700 children and adolescents diagnosed per year in the USA [111]. In the 1970s, patients with AML did quite poorly despite initial responses to chemotherapy with DFS rates ranging from 30% to 50%. This led investigators to try alternative treatments such as allogeneic HSCT if an initial remission could be obtained. With more intensive chemotherapy regimens, survival rates with chemotherapy have approached those provided with allogeneic HSCT in the first remission. Therefore, allogeneic HSCT is typically reserved for those patients with high-risk features at diagnosis (Table 9.4) or those who relapse after initial therapy. This topic is reviewed in more detail in Chap. 4.

As discussed in the ALL section above, much of the proof of concept that allogeneic HSCT could provide long-term disease control was reported in several studies that included both ALL and AML patients and used the Cy/TBI regimen [48, 116, 117]. The first report of an exclusively AML population transplanted in CR1 was published in 1979. Thomas et al. reported the results of 19 patients, including six pediatric patients that received Cy/TBI (single dose) followed by a matched sibling donor bone marrow transplant. The overall survival was about 60%, including five of six pediatric patients. Pulmonary toxicity was common, however, with five deaths from interstitial pneumonitis [127]. This study was followed by several single-institution reports in an exclusively pediatric patient population that confirmed long-term DFS of about 60–65% when transplanted in CR1 [128, 129].

In order to potentially decrease rates of transplant-related mortality (TRM) and late effects, approaches using chemotherapy only were developed. The regimen most commonly used in pediatrics involved myeloablative doses of busulfan with high-dose cyclophosphamide. A randomized trial comparing Cy/TBI to Bu/Cy (cyclophosphamide 120 mg/kg) that included both pediatric and adult patients revealed similar 3-year EFS rates between the regimens (66% vs 67%) when only the pediatric cohort was analyzed. When the entire cohort was analyzed, the EFS was comparable only in those patients with CR1, as compared to those with advanced disease where the EFS was superior in the Cy/TBI group (22% versus 66%; $p = 0.002$) [130]. In the 1990s, there were four randomized controlled trials that compared Cy/TBI to Bu/Cy in leukemia patients. The studies included only a small number of pediatric patients, and only two of the studies included AML patients [130, 131]. A comparison study to better assess the long-term outcomes of patients enrolled on these four studies found that AML patients receiving Bu/Cy had a 10% lower survival, which was not statistically significant, with similar rates of long-term complications [132].

For more than 20 years, the Bu/Cy combination has been the preparative regimen used on successive studies sponsored by the various pediatric cooperative groups, with the only change being the introduction of IV instead of PO busulfan. While this combination had a long track record in sibling donor HSCT of achieving EFS

Table 9.4 AML-risk stratification according to the most recent COG study, AAML1031 (NCT01371981)

	Low risk		High risk[a]		
Risk assignment	LR group 1	LR group 2	HR group 1	HR group 2	HR group 3
FLT3 ITD allelic ratio[b]	Low/neg	Low/neg	**High**	Low/neg	Low/neg
Good-risk molecular markers[c]	**Present**	Absent	Any	Absent	Absent
Poor-risk cytogenetic markers[d]	Any	Absent	Any	**Present**	Absent
Minimal residual disease at the end of induction (>0.1%)	Any	Negative	Any	Any	**Positive**

Bold indicates overriding risk factor in risk group assignment
[a]Patients meeting the high-risk criteria are recommended to proceed to transplant in CR1 from the best available donor
[b]Fms-like tyrosine kinase 3 (FLT3) internal tandem duplication (ITD) allelic ratio is determined by dividing the ITD product peak by the wild-type product peak. High-risk status is defined as an allelic ration >0.4
[c]NPM1, CEBPa, t(8;21), or inv.(16)
[d]Monosomy 7, monosomy 5, or del(5q)

of 60% [133], there is a high rate of toxicity including SOS (VOD) (20–50%) [134, 135] and hemorrhagic cystitis (16–24%) [130, 136], resulting in unacceptably high TRM. In order to reduce toxicity, fludarabine is being used more frequently to replace cyclophosphamide, and, in several adult studies, fludarabine has dramatically reduced the incidence of 1-year TRM to 1–15% [137, 138]. In 2009, Pulsipher et al. reported the results of a prospective clinical trial for pediatric patients with high-risk hematologic malignancy ($n = 47$) that were ineligible for traditional myeloablative regimens, including 15 patients with AML, that received fludarabine with myeloablative busulfan (FluBu4). The entire cohort had a 2-year EFS of 40% and a 2-year TRM of 11% [139]. In a single-institution retrospective report, 20 pediatric patients with a myeloid malignancy were transplanted using the Flu/Bu4 regimen with a 2-year overall survival of 63% and 2-year TRM of 14% [140]. Based on these results and a strong desire to both limit TRM while improving EFS in the pediatric population, the Children's Oncology Group has moved to Flu/Bu4 as the standard conditioning regimen on its most recent clinical trial (AAML1031; NCT0137198).

A challenging scenario faced by pediatric HSCT physicians is what to do with refractory AML patients because standard conditioning regimens result in relapse rates exceeding 50% and overall survival of <20%. Clofarabine is an agent felt to have increased antileukemic effects and has been combined with fludarabine (CloBu4) for refractory leukemia patients. A study from the University of Michigan investigated this high-risk population, including 31 patients (adult and pediatric) with refractory AML, and found 100% of the AML patients achieved a CR by D30 post-HSCT, and the 1-year OS was encouraging at 48% [141].

Neuroblastoma

Neuroblastoma is the most common extracranial solid tumor of childhood and the most common cancer diagnosed in patients under a year of age. Approximately 700 children in the USA are diagnosed with this disease each year [111]. The diagnosis is rarely made after the age of 10 years and is most commonly diagnosed before the age of 2. The clinical severity of the disease is quite variable, with low- and intermediate-risk patients having excellent long-term survival, while those with high-risk disease having <40% EFS with conventional chemotherapy alone. Defining high-risk disease in neuroblastoma requires a complicated algorithm that includes disease staging, age of the patient, and biologic and pathologic characteristics of the tumor. A simplified version of this algorithm is provided in Table 9.5.

Initial chemotherapy regimens for high-risk neuroblastoma patients in the late 1970s and early 1980s resulted in dismal long-term survival rates of only 9%. Subsequent studies from the Children's Cancer Group (CCG) intensified chemotherapy dosing and improved a 4-year survival to 30% [142]. Improvements in cryopreservation in the mid-1980s led to the feasibility of collecting bone marrow HSCs to be stored for future use, opening up the possibility of myeloablative chemotherapy followed by autologous HSC rescue. The agents chosen for the myeloablative

Table 9.5 Characteristics of high-risk neuroblastoma: presence of these high-risk features helps define a population of patients that may benefit from autologous transplantation as consolidation following response to initial induction therapy

Stage[a]	Age	MYCN	Ploidy	INPC[b]
3	Any	**Amplified**	Any	Any
3	≥18 months	Not amplified	Any	**Unfavorable**
4	<365 days	**Amplified**	Any	Any
4	365 days (18 months)	**Amplified**	Any	Any
4	365 days (18 months)	Any	**DI = 1**	Any
4	365 days (18 months)	Any	Any	**Unfavorable**
4	**>18 months**	Any	Any	Any

Bold indicates overriding risk factor in risk group assignment
[a]Stage as defined by the International Neuroblastoma Staging System
[b]INPC, International Neuroblastoma Pathology Classification

conditioning regimen were quite variable but included melphalan +/− TBI [143], cisplatin/etoposide/melphalan + TBI [144], carboplatin/etoposide/melphalan (CEM) + TBI [144], and thiotepa/cyclophosphamide [145]. Initial reports of HSCT included patients with high-risk disease, but they could be in CR or have progressive disease. When limited to those in CR1 following induction chemotherapy, these various regimens provided a 3-year EFS of 32–50%. These encouraging results prompted the CCG to perform a randomized clinical trial (CCG 3891) to help determine if patients with high-risk neuroblastoma did better following consolidation with prolonged chemotherapy ($n = 190$) or with consolidation consisting of myeloablative chemotherapy (CEM + TBI) followed by autologous HSC rescue ($n = 189$). Patients in the autologous transplantation arm had improved 3-year EFS 34% vs 22% ($p = 0.03$), establishing autologous HSCT as part of the standard of care for high-risk neuroblastoma patients [146].

Because inclusion of a myeloablative autologous HSCT improved outcomes, some groups tested whether survival could be further improved by performing tandem autologous HSCT (i.e., two sequential autologous HSCTs). Tandem transplantation would permit further dose intensification as well as introduction of additional non-cross-reactive anti-neuroblastoma agents. The largest study included 156 patients who received a first transplant with CEM followed by a second transplant using melphalan + TBI. The 3-year EFS for these patients approached 55% [147, 148]. Based on these favorable results, the COG performed a phase III clinical trial (ANBL0532; NCT00567567) that randomized patients with high-risk neuroblastoma to receive one myeloablative HSCT (CEM) versus two (thiotepa/Cytoxan followed by CEM). Although TBI was included in many early transplant protocols, radiation was omitted from this study over concern of long-term toxicity in a cohort of patients in which the majority of participants are under 3 years of age. This study has completed accrual, and initial analysis of the data appears to indicate a superior EFS in those patients that received tandem HSCTs, but the final results have not yet been published.

The European Society for Paediatric Oncology (SIOPE) conducted a randomized study comparing CEM to a newer combination of busulfan/melphalan (Bu/Mel) in high-risk neuroblastoma patients. The induction chemotherapy leading up to HSCT is also a different backbone from what is traditionally used in the COG protocols. Approximately one third of the patients enrolled on the clinical trial were eligible for HSCT and underwent randomization ($n = 598$). The study found an improved 3-year EFS for those randomized to Bu/Mel as compared to CEM (49% vs 33%; $p < 0.001$), with a lower incidence of severe toxicity except for a higher incidence of VOD in the Bu/Mel arm (18% vs 4%; $p < 0.001$). Of note, there were no deaths attributable to SOS/VOD on this study [149, 150]. In response to these results, the COG designed the next high-risk neuroblastoma protocol to assess the toxicity profile of Bu/Mel when given as part of the standard COG backbone (induction chemotherapy pretransplant and posttransplant maintenance therapy +/− radiation therapy). This trial, ANBL12P1 (NCT01798004), opened in April 2013 and completed its accrual goals in April of 2015, having enrolled 150 total patients and 99 patients completing the Bu/Mel preparative regimen. The final results of the study have not been published, but the study progress report indicates that there have been six patients with severe SOS/VOD and three patients with severe pulmonary complications. Once more long-term follow-up data is obtained, we may learn if Bu/Mel should be accepted as a new standard of care.

The aforementioned trials included high-risk patients who demonstrated chemotherapy response to induction regimens, but autologous HSCT is not curative for patients with relapsed or progressive disease. One experimental strategy for treating these patients includes giving radiolabeled metaiodobenzylguanidine (mIBG) as therapy. mIBG is a norepinephrine analog that concentrates in the sympathetic nervous tissue and pathologic neuroblastoma tumors. Radiolabeling mIBG with iodine isotope I-131 results in the therapeutic compound ^{131}I-mIBG, in contrast to labeling with iodine-123, which is used for diagnostic imaging. Initial studies of

infused ^{131}I-mIBG as a single agent to help concentrate the radiotherapy within sites of disease resulted in short-term disease responses in 10–55% of the patients [151]. One of the main toxicities of mIBG therapy is bone marrow suppression, and many patients require stem cell rescue. This led researchers from the University of Michigan to perform a pilot study that combined ^{131}I-mIBG treatment on day −21 followed by standard myeloablative CEM on days −7 to −4 with infusion of autologous peripheral blood stem cells on day 0. Twelve patients who failed induction therapy were enrolled; responses were seen in eight patients [152]. In a follow-up multicenter study, 22 patients with refractory or progressive neuroblastoma received ^{131}I-mIBG followed by CEM and autologous peripheral blood HSCT. Of the 22 patients, six had a CR/PR, and an additional 15 had either a mixed response or stable disease [153]. Given the potential improved outcomes with Bu/Mel, a pilot study of eight patients with refractory neuroblastoma first received ^{131}I-mIBG treatment followed by autologous peripheral blood HSC rescue, followed 8 weeks later by Bu/Mel and autologous peripheral blood HSC infusion. Of the eight patients enrolled, there were three CRs and two PRs [154]. This promising approach has been brought forward as the initial treatment of high-risk neuroblastoma patients in a COG pilot study (ANBL09P1; NCT0175356). Patients received five cycles of induction therapy followed by ^{131}I-mIBG treatment with peripheral blood HSC rescue. Patients without progressive disease received Bu/Mel followed by autologous peripheral blood HSC rescue 12 weeks after ^{131}I-mIBG treatment. The trial enrolled 99 patients, and 27 received Bu/Mel myeloablative chemotherapy followed by autologous peripheral blood HSCT. The trial is closed to accrual as of January 2016, and patients are still in active follow-up.

Idiopathic Aplastic Anemia

Aplastic anemia is characterized by pancytopenia and a hypocellular bone marrow in the absence of an infiltrative process. The etiology is variable, including idiopathic, environmental exposure, as well as the presenting feature of a bone marrow failure disorder. In the pediatric patient population, it is essential to rule out genetic causes such as Fanconi's anemia, dyskeratosis congenita, and Shwachman-Diamond syndrome since these disorders can impact both donor selection and conditioning regimen. Approximately 70–80% of the pediatric patients with aplastic anemia will be classified as idiopathic, though children under the age of 5 years have a higher likelihood of having a genetic cause [155]. Current outcomes following a sibling donor allogeneic HSCT are superior to immunosuppression therapy (anti-thymocyte globulin (ATG) plus cyclosporine), and selection of a sibling donor HSCT is the standard treatment for severe or very severe aplastic anemia.

Initial studies of sibling donor HSCT from Seattle in the 1970s used high-dose cyclophosphamide (50 mg/kg/day × 4 days) and demonstrated poor results, with survival in only 12 of 22 patients 1-year post-HSCT. Graft rejection occurred in 5 of 22 patients [156]. Other reports emerged regarding a high rate of graft failure when patients were conditioned with cyclophosphamide alone, and the mechanism of this finding was felt to be inadequate eradication of the recipient immune system. This assumption was supported by the observation that graft rejection rates were higher if recipients had received significant pretransplant transfusions [157, 158]. To increase the level of immunosuppression, many centers added TBI to cyclophosphamide. A retrospective report from the CIBMTR found that patients that did not receive radiation ($n = 290$) had nearly three times the risk of graft failure (RR = 3.2; $p < 0.0001$) as compared to those patients that did receive a radiation-based conditioning regimen ($n = 334$), but this did not translate into improvements in overall survival secondary to the toxicity associated with TBI [159].

The next generation of studies focused on decreasing toxicity from TBI but improving the degree of immunosuppression provided by cyclophosphamide alone with the addition of ATG (Cy/ATG) to the conditioning regimen. In a prospective study of 39 patients that received a matched related donor bone marrow transplant for severe aplastic anemia, only two patients

experienced graft rejection (5%), and the 3-year OS was 92%, comparing favorably to a matched historical control conditioned with cyclophosphamide alone (3-year YR OS of 72%) [160]. These findings were confirmed by several other groups, including a multicenter prospective study of 94 patients that demonstrated a low rate of graft rejection (4%) with outstanding 6-year OS of 88% [161]. This combination is now considered the standard upfront therapy for all children and young adults (< 40 years) with severe aplastic anemia and a suitably matched sibling donor. The dose of anti-thymocyte globulin use can vary depending on the formulation used, but generally horse ATG (ATGAM [Pfizer, USA]) is given at 40 mg/kg/day × 3 days, rabbit ATG (thymoglobulin [Sanofi, France]) is given at 2.5 mg/kg × 3 days, or rabbit ATG [Fresenius, Germany] is given at 10 mg/kg/day × 3 days [162].

The course for patients without a suitable sibling (or related) donor has been more challenging. Due to higher risks of long-term toxicity, alternative donor HSCT has been reserved in general for those aplastic anemia patients that fail initial therapy with immunosuppression. Initial response rates with immunosuppression approach 70%, but there are continued issues with clonal evolution with as many as 20% of the patients developing MDS or AML [163]. Initial attempts to apply the Cy/ATG regimen used in the related donor setting were dismal, with two early deaths and three patients with very poor engraftment [164]. A retrospective evaluation of 141 patients with aplastic anemia that received an unrelated donor HSCT through the assistance of the NMDP demonstrated similar poor results. In this cohort, the majority of patients received a conditioning regimen that included TBI (86%), and the majority were a 6/6 HLA serologic match (74%). Rates of non-engraftment were still high (11%), and high rates of both acute and chronic GVHD led to an overall survival of only 36% [165].

One of the first advances in unrelated donor HSCT was optimization of the dose of TBI required to assist engraftment while limiting HSCT-associated toxicities. In a dose de-escalation study, the addition of a single fraction of 200 cGy to the standard Cy/ATG regimen achieved engraftment in 95% of the patients and resulted in an overall survival in the HLA-matched recipients of 55% [166]. In an attempt to further improve the toxicity profile, many centers transplanted unrelated donor recipients with combinations that included fludarabine (30 mg/m^2 × 4 days) for its attractive immunosuppressive properties while removing the low-dose TBI from the preparative regimen with impressive overall survival rates in the range of 68–73% [167, 168]. Recently, there has been an effort to replace ATG with alemtuzumab, a CD52 monoclonal antibody with extensive immunosuppressive effects. A retrospective study from the British Society for Blood and Marrow Transplantation included 55 unrelated donor recipients that received alemtuzumab in addition to fludarabine and cyclophosphamide. The total median dose of alemtuzumab delivered was approximately 1 mg/kg, and all doses were delivered pre-HSCT, though further information regarding the exact timing was not provided. The 5-year OS for these patients was excellent (88%), approaching outcomes with upfront HSCTs using matched related donors [169]. Improved results in the unrelated setting have brought into question if patients with a suitably matched unrelated donor available should also receive upfront HSCT over a traditional trial of immunosuppression. However, this debate is yet to be solved, and, unlike patients with a matched related donor, upfront unrelated HSCT is not considered the current standard of care.

Sickle Cell Disease

In the USA, there are approximately 100,000 children and adults with sickle cell disease. It is the most common inherited blood disorder in the USA, and it will affect 1 in 400 African-American newborns annually. Despite improvements in supportive care, the natural history of this disease is one that evolves into a disabling chronic condition, with less than 50% of adult patients able to work [170]. Allogeneic HSCT can be curative, but concerns of excessive toxicity and lack of appropriate donors have resulted in less than 1300 total transplants in the USA and Europe combined [171]. The indications for HSCT

Table 9.6 Potential indications for allogeneic HSCT for sickle cell disease

Degree of acceptance	Sickle cell complication
• Commonly accepted	• Stroke/cerebral ischemia
	• Silent cerebral infarction
• Frequently accepted	• Recurrent acute chest syndrome[a]
	• Frequent vaso-occlusive/pain crises[a]
	• Persistent transcranial Doppler velocity > 200 cm/s
	• Red cell alloimmunization
	• Sickle nephropathy
	• Sickle lung disease
	• Tricuspid regurgitant velocity > 2.5 m/s

[a]Patients should have failed an adequate trial of hydroxyurea

remain controversial, but the generally accepted indications have been summarized in Table 9.6.

The first successful HSCT in a patient with sickle cell disease was performed in an 8-year-old child diagnosed with AML in 1982 who received a MSD, myeloablative HSCT conditioned with Cy/TBI as directed by her enrollment on a clinical AML trial. Her donor was her brother who had sickle cell trait. Her AML and sickle cell disease were cured by HSCT, and her level of hemoglobin S fell to 30%, consistent with sickle cell trait [172]. Subsequent HSCT trials for patients with sickle cell disease used a backbone of busulfan and cyclophosphamide (Bu/Cy) with or without ATG. After adjustments to busulfan dosing and then the addition of ATG in an attempt to decrease the risk of graft rejection [173], myeloablative Bu/Cy (busulfan 12.8 mg/kg IV, cyclophosphamide 200 mg/kg) with ATG became the standard of care. Recognition that patients with sickle cell disease are more vulnerable to CNS toxicity post-HSCT (including seizures, intracranial hemorrhage, and recurrent stroke) led to further improvements in supportive care. Survival was improved by optimization of supportive care such as maintaining antiseizure prophylaxis through the duration of calcineurin inhibitor therapy, higher platelet thresholds (maintain platelets above 50,000/μL), prevention of severe anemia and hyperviscosity (Hgb maintained between 9 and 11 g/dL), and strict blood pressure control (within ±20% of the baseline). Employment of these interventions with myeloablative Bu/Cy plus ATG conditioning in a MSD setting has achieved excellent HSCT outcomes with overall survival rates of over 90% and disease-free survival of 85% [173–176].

These early trials of allogeneic HSCT were limited to younger patients (<16 years of age) and often focused on those with more severe manifestations of disease. In order to expand the indications for HSCT to older patients and to those with more severe comorbidities, non-myeloablative conditioning regimens were investigated. Non-myeloablative approaches are attractive even for patients without additional high-risk features to prevent long-term toxicity, including the potential to preserve future fertility. Decreased intensity of the preparative regimen needs to be balanced by the increased risk of graft rejection. Since only 10–18% of sickle cell patients will have an appropriate HLA-matched sibling donor [177], some studies investigating non-myeloablative approaches also included HLA-matched unrelated donors as well. An initial study of non-myeloablative conditioning (fludarabine 180 mg/m^2, busulfan 6.4 mg/kg + ATG) included three patients with sickle cell disease (1 MSD, 2 MUD). The outcome of these three patients was poor with the MSD recipient dying of GVHD and both MUD recipients alive but with engraftment failure [178].

An alternative reduced-intensity approach was studied in 43 MSD recipients and included the use of alemtuzumab starting at day −22 over 4 days (total dose 48 mg), fludarabine starting at day −8 (150 mg/m^2 divided over 5 days), and melphalan on day −3 (140 mg/m^2). One patient who received a related umbilical cord blood transplant (UCBT) without additional bone marrow cells failed to engraft. The OS and ESF were 93% and 91%, respectively. The three deaths in this study were secondary to complications from GvHD. Recipient chimerism was present in 28% of the patients at 1-year post-HSCT, requiring the use of donor lymphocyte infusions in two patients [36]. Given these promising results, this reduced-intensity platform was tested for unrelated donors

in a multicenter study through the BMT CTN (BMT CTN 0601; Sickle Cell Unrelated Donor Transplant Trial; NCT00745420). Initially, the trial allowed both 8/8 HLA MUD and 5–6/6 HLA-matched unrelated cord blood (UCB) donors. There was a very high rate of graft failure in UCBT recipients, with only three of eight patients having sustained donor engraftment. Therefore, the trial was closed to further accrual on the UCBT arm [179]. The data on the MUD arm of the trial was presented at the American Society for Blood and Marrow Transplantation (ASBMT) in February 2016. Twenty-nine patients received a MUD, and three suffered early graft rejection with autologous recovery. The 1-year OS and EFS were 86% and 76%, respectively; however, there was a very high rate of chronic GvHD at 62%. There was also a very high rate of posterior reversible encephalopathy syndrome (PRES; 35%), leading to changes in GvHD prophylaxis and/or treatment which may have contributed to the high rates of chronic GvHD [180].

Other investigators have evaluated a reduced-toxicity regimen with busulfan (3.2–4 mg/kg/day × 4 days), fludarabine (30 mg/m^2 × 6 days), and alemtuzumab (52 mg/m^2 total with escalating doses × 4 days), with very promising results in the MSD setting (n = 18). The OS and ESF were both 100%, including three recipients of a sibling UCB unit. The patients achieved high levels of donor chimerism, with no patient requiring a DLI [181]. Unfortunately, when this same approach was attempted with eight unrelated cord blood recipients, three patients had primary graft failure and subsequently died of infection [182].

Given the high rate of graft failure with cord blood and difficulty finding a matched unrelated donor (only 16–19% of African-Americans currently have an 8/8 HLA match in the NMDP registry) [183], familial haploidentical (FHI) allogeneic HSCT has become a viable option for sickle cell patients without a matched sibling donor. One study of mostly adult patients (median age of 30 years; range, 15–46 years) investigated a reduced-intensity regimen consisting of ATG (days 9–7), cyclophosphamide (14.5 mg/kg/d on days −6 and −5), fludarabine (30 mg/m^2/day on days −6 to −2), and TBI (200 cGy on day −1). There were 14 patients that received FHI allogeneic HSCT, and three patients received MSD. GvHD prophylaxis included post-HSCT cyclophosphamide (50 mg/kg on day +3 and +4), followed by mycophenolate mofetil (MMF) and either tacrolimus or sirolimus. All of the MSD recipients were engrafted, but 43% of the FHI allogenic HSCT experienced graft rejection. Of the ten patients that were engrafted, five became full donor chimeras and were able to discontinue immunosuppression, whereas the other five were mixed chimeras and required ongoing immunosuppression [184]. For patients who can tolerate a myeloablative conditioning approach, there is emerging evidence that a myeloablative regimen followed by a FHI allogeneic HSCT with peripheral blood HSCs that are enriched for stem cells (CD34+ selection) with a T-cell add-back (2.0 × 10^5 CD3 cells/kg) may have promise. In an abstract presented at ASBMT, 14 patients received a myeloablative regimen with hydroxyurea and azathioprine (days −59 to −11), fludarabine (30 mg/m^2/day days −17 to −13), busulfan (3.2 mg/kg/day on days −12 to −9), thiotepa (10 mg/kg on day −6), cyclophosphamide (50 mg/kg/day on days −7 to −4), rabbit ATG (2 mg/kg/day on days −5 to −2), and total lymphocyte irradiation (500 cGy on day −2). The authors report that 100% of patients were engrafted and had a very low rate of acute GvHD (<15%) and a 1-year EFS of 92% with stable donor chimerism [185].

Finally, for those patients with a sibling donor option but with significant concern for chemotherapy- associated toxicity, there are data that non-myeloablative HSCT may be feasible. A single-center prospective study investigated the use of alemtuzumab (1 mg/kg in divided doses on days −7 to −3) and TBI 300 cGy on day −2. The patients received G-CSF-stimulated peripheral blood HSCs from an HLA MSD. Patients were maintained on sirolimus starting on day −1 and continued until at least 1-year post-HSCT with demonstration of stable donor chimerism. Thirty patients were enrolled on this study, with a median age of 29 years (range, 17–65 years). At 1-year post-HSCT, 25 patients had full donor-type

hemoglobin. Four patients had late graft rejection with autologous recovery. The EFS was 87% with no patient developing acute or chronic GVHD [177]. These results were recently confirmed in the second single-center prospective study that enrolled 13 patients, with a DFS of 92% and with no patients developing acute or chronic GvHD [186].

Allogeneic HSCT for patients with sickle cell disease remains a controversial and complex topic. Once the decision has been made to evaluate a patient for HSCT, many factors contribute to the determination of the best conditioning regimen, including donor type (MSD, MUD, FHI) and patient comorbidities. Future studies through the BMT CTN will continue to explore FHI allogeneic transplantation using a reduced-intensity backbone and may help further expand both the donor pool and patient eligibility.

Severe Combined Immunodeficiency (SCID)

Severe combined immunodeficiency (SCID) is a heterogeneous disorder that results in profound deficiency of T-, B-, and, in some cases, natural killer (NK)-cell function. There are over 18 different genetic mutations associated with SCID which can be categorized into defects of cytokine signaling, V(D)J recombination, T-cell receptor signaling, hematopoietic cell precursor survival, and toxic accumulation of metabolites [187–189]. Subtypes of SCID are additionally defined by the presence or absence of NK cells (NK+ or NK−) and B cells (B+ or B−), although humoral immunity is always impaired either directly from the genetic defect or indirectly by the absence of T-cell-dependent maturation.

Without immune reconstitution, the disorder is uniformly fatal by 2 years of life secondary to severe infection and/or autoimmune complications. HSCT may correct part or the entire immune defect for each of these disorders. Other potential options include gene therapy, but this is currently only available for common gamma chain and adenosine deaminase (ADA) deficiency [190]. Enzyme replacement for ADA deficiency is also available but is associated with waning immunity, autoimmunity, and malignancy with long-term administration [191].

HSCT for SCID is generally associated with favorable outcomes for patients, but important allogeneic HSCT decisions, including whether to use conditioning therapy, and, if so, how much, are difficult as different subtypes of SCID can present unique challenges for engraftment and toxicity. For example, radiosensitive SCID, which includes Artemis, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), Cernunnos-XLF, and DNA ligase IV defects, imparts the conflicting challenge of having increased risk of failed engraftment but heightened sensitivity to DNA-damaging agents. In a recent report detailing the outcomes of patients with Artemis mutations with follow-up >5 years, 86% had growth deficiency, and 48% had dental abnormalities if alkylator chemotherapy was administered as part of the HSCT conditioning regimen [192]. Although mixed chimerism is curative of the immune defect in SCID, failure to eliminate recipient hematopoietic stem cells may impart a lifetime cancer risk in certain subtypes including myelodysplastic syndrome in reticular dysgenesis and lymphoma in cartilage hair hypoplasia and hypomorphic forms of radiosensitive SCID [193–196]. Barriers to engraftment include patients with hypomorphic mutations resulting in low numbers of functioning T cells and leaky SCID, a subtype in which an oligoclonal population of T cells is produced that can occasionally lead to Omenn syndrome, an autoimmune condition characterized by erythroderma, lymphadenopathy, and hepatosplenomegaly. The presence of any immune barrier, weak as it may be, increases the risk of graft rejection, especially if no conditioning regimen is provided [197].

The rarity of disease, with an incidence of 1/58,000 newborns, limits our knowledge of the disease, and most literature to date consists of retrospective experience with small patient cohorts [198]. Because the best outcomes occur before the onset of infection, HSCT during the newborn period is also recommended, but knowledge of chemotherapy pharmacokinetics and pharmacodynamics is poor in this age group. Although our knowledge of busulfan is improving,

very little is known about other drugs that may result in increased toxicity [199, 200]. Melphalan metabolism is not well defined and demonstrated extreme pulmonary complications in 17/30 (57%) infants less than 1 year of age with nonmalignant conditions when combined with fludarabine and alemtuzumab [200]. Use of treosulfan in infants less than 6 months of age is associated with transient CNS toxicity including hypertonicity and irritability, likely from a more immature blood-brain barrier [201]. Patients with SCID commonly have multiple long-term complications secondary to the underlying disease, a history of severe infections, and likely from chemotherapy given at a young age. A study of SCID patients greater than 2 years out from HSCT identified mortality in 7% of patients with infection, organ failure, and chronic GVHD as the most common etiologies [202]. SCID patients have lower IQ in comparison to unaffected siblings, but surprisingly use of conditioning in a study of 56 SCID patients did not impact IQ significantly [203].

Despite this variability, the commonality between SCID patients is a weakened immune system that may or may not be significant enough to require a conditioning therapy for donor engraftment to be successful. In general, a successful HSCT for SCID results in the reconstitution of T-cell immunity. Other components of the immune system, such as B- and NK-cell immunity, may also be missing secondary to the genetic defect and may or may not be reconstituted along with T-cell donor engraftment. Patients who do not obtain B-cell function require lifelong intravenous immunoglobulin (IVIG). Long-term complications from failure to reconstitute NK-cell immunity are not well understood, but lack of NK cells is concerning for continued immune compromise, particularly to viral infections such as HPV.

The engraftment of T cells is dependent on many factors including the "permissiveness" of the recipient to accept a donor T-cell graft. Recipient factors that increase the likelihood of successful T-cell engraftment include the absence of NK cells [38], empty "T-cell niches" that are not occupied by recipient pro-T cells [204], presence of maternal T cells [205–207], and absence of autologous T cells that can mediate donor graft rejection [197]. Non-recipient factors important for donor T-cell engraftment include donor-type and conditioning therapy. Bone marrow from a matched sibling donor, which is typically infused without a preceding conditioning regimen, results in higher T-cell counts in comparison to other donor types [38]. The impact of conditioning therapy on T-cell engraftment depends on the SCID subtype and donor source. When only looking at the impact of conditioning regimen across all SCID and donor subtypes, a retrospective study by the Pediatric Immune Deficiency Treatment Consortium (PIDTC) demonstrated that neither the use of nor the intensity of conditioning was significantly associated with graft failure and need for second transplant [38]. However, the study showed that use of a conditioning regimen increased the $CD3^+$ and naïve $CD4^+$ ($CD4^+/CD45RA^+$) populations, but not the overall $CD4^+$ population.

The same factors that influence T-cell engraftment can also influence donor B-cell engraftment. However, engraftment of donor B cells has proven more difficult than T cells. Factors that increase the likelihood of donor B-cell engraftment include a matched sibling donor [38], development of acute GvHD [207], and absence of NK cells [207], although the impact of NK cells is less understood for B-cell reconstitution [208]. It is now becoming clear that conditioning therapy is highly associated with donor B-cell engraftment. In a retrospective review of European centers, patients who received conditioning therapy had a higher percentage of donor B-cell chimerism, B-cell function, and lower percentage of HSCT recipients on IVIG therapy [209]. The retrospective PIDTC study found that B-cell chimerism was more common with conditioning (23/26 vs 7/31) in non-matched sibling donors and conditioning therapy was associated with normal IgA levels and independence from IVIG [38]. A third study demonstrated that all 19 patients with primary immunodeficiencies who received a MAC regimen with busulfan and cyclophosphamide were off IVIG in comparison to 5 out of 33 patients who received RIC and

were still on IVIG [210]. A review of seven published studies demonstrated that use of busulfan resulted in better B-cell function but did not guarantee B-cell function as 35–45% of patients still required IVIG despite receiving conditioning therapy [211].

As we continue to accumulate knowledge of this enigmatic disease, recommendations on use of conditioning therapy will be better delineated. At this time, however, the conditioning approach for SCID is extremely variable among centers. For matched sibling donors, the expert consensus is to use no conditioning unless the patient has leaky SCID, Omenn syndrome, or reticular dysgenesis [212]. However, recent publications indicate that 10–34% of sibling donor HSCT recipients receive a conditioning regimen [38, 210]. When conditioning therapy is omitted prior to matched sibling donor HSCT, outcomes are excellent with survival ranging from 89% to 95% and donor B-cell engraftment in 40–50% [211, 212].

Mismatched related HSCTs are commonly administered after ex vivo T-cell depletion to lower the risk of GvHD and are infused without preceding conditioning therapy. The survival outcomes with this approach are good (OS 79%), but approximately 25% will require "booster" infusions to improve T-cell engraftment, and 58% require long-term IVIG [38, 213, 214]. In North America, currently 66% of mismatched related transplants are given without conditioning [38].

The majority of unrelated HSCTs, including UCBT, have historically been administered with chemotherapy. Recent data demonstrate that only 10% of unrelated bone marrow, peripheral blood, and UCB HSCTs are given without conditioning, 24% with immunosuppression only, and the remaining receiving either RIC (35%) or MAC (31%) [38]. Because of low patient numbers, there is minimal data regarding patients that receive unrelated donor transplants with no conditioning. The largest study to date compared 37 patients who received an unrelated donor bone marrow, peripheral blood, or cord transplant UCB HSCT either without conditioning or with serotherapy only with 66 non-conditioned matched sibling donor bone marrow or cord patients [207]. The need for a second HSCT was similar in both donor sources (19% unrelated and 15% related) but highest in the unrelated cohort who received serotherapy (28%). Despite the need for second transplants, the overall survival in the serotherapy cohort was 100%, similar to the non-conditioned matched related donor cohort (91%) and superior to the non-conditioned, unrelated donor cohort (56%) likely secondary to the protective effect against GvHD.

For patients who do receive conditioning in the unrelated donor setting, the intensity of therapy also impacts outcome. In a historical comparison of MAC and RIC HSCTs for unrelated donors, OS was significantly better in patients who received a RIC regimen (94% vs 53%) [210]. The retrospective PIDTC cohort identified a higher incidence of organ toxicity from MAC, with 64% of deaths secondary to pulmonary toxicity and an additional 21% of the deaths related to other organ toxicity [38]. In contrast, death in the RIC cohort was largely attributed to infection (71%), with only 18% of patients dying from pulmonary or other organ toxicity.

Another important factor in the decision to use chemotherapy is the infection status of the patient. Recent data indicate that use of a conditioning regimen can be detrimental to survival in the setting of active infection. The PIDTC retrospective study specifically evaluated the impact of conditioning on patients with active infection receiving a mismatched related donor HSCT and identified a significant decrease in survival (65% vs 39%) when a conditioning regimen was used [38].

In conclusion, the use of chemotherapy for SCID is variable and dependent on multiple factors including the underlying genetic condition, the donor, the presence of autologous or maternal T cells, the need for B-cell engraftment, and the infection status of the patient. Current consensus is that chemotherapy is not necessary for matched sibling donors or maternal donors when maternal T cells are already detected at birth. Conditioning is likely necessary for patients for the following groups: (1) leaky SCID, particularly when lacking a matched sibling donor; (2) Omenn syndrome; (3) reticular dysgenesis given

a high rate of rejection and risk of MDS; and (4) SCID variants, including hypomorphic mutations, that present with decreased but not absent immunity. Conditioning therapy will improve the chances of a donor B-cell chimerism and naïve T-cell production, but donor B cells are not necessary for B-cell function in certain SCID subtypes with normal B-cell phenotypes such as IL-7Rα, CD3δ and ε chain deficiencies, and ADA deficiency following adequate detoxification by donor cells. Finally, conditioning should be used with extreme caution in patients with radiosensitivity disorders secondary to increased long-term effects and in patients with active infection as current data suggests decreased survival when chemotherapy is administered.

Key Points

- The three key roles of a conditioning regimen in HSCT are to provide immunosuppression, to provide anticancer therapy, and to create openings within the bone marrow's HSC niches.
- Conditioning regimens range from the most intensive (myeloablative) to the least intensive (non-myeloablative).
- Myeloablative conditioning results in myeloablation of the host's bone marrow resulting in full bone marrow aplasia that requires ex vivo HSCs for immune reconstitution. Common myeloablative conditioning regimens include cyclophosphamide/TBI and busulfan/cyclophosphamide/ATG.
- Non-myeloablative conditioning relies predominantly on profound immunosuppression in contrast to myeloablation to permit engraftment of donor HSCs. If given enough time, the patient's own bone marrow will have autologous reconstitution if the immunosuppression is removed and no donor engraftment is present.
- The selection of the appropriate conditioning regimen is multifactorial and depends primarily upon the underlying disease being treated and the physical status and age of the potential HSCT recipient.

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Part III

The Peri- HSCT Period: Pre-Engraftment (Days 0–30) and Early Post- HSCT (Days 30–100) Periods

10 Engraftment and Chimerism

Valerie I. Brown

Abstract

The goal of hematopoietic stem cell transplantation (HSCT) is sustained engraftment. Engraftment is defined as neutrophil and platelet recovery after a period of aplasia. In order to be considered engrafted, the patient post-HSCT needs to have an absolute neutrophil count (ANC) >500 mm^2 for the first 3 consecutive days. Platelet recovery occurs when the patient has sustained a platelet count >20,000 μL^{-1} for 3 consecutive days without a platelet transfusion in the 7 preceding days. Typically, neutrophil engraftment occurs first with platelet engraftment occurring 1–3 weeks later, depending on the donor and hematopoietic stem cell (HSC) source. Other factors that influence engraftment include the use of growth factors during the peri-HSCT period, the bone marrow microenvironment of the recipient, the HSC dose, and donor graft manipulation. Often at the time of engraftment, patients will develop a "flu-like" syndrome with fevers, chills, worsening mucositis, peripheral edema, joint and muscle aches, and/or abdominal pain due to a cytokine storm driven by donor T cells. The degree of donor engraftment is measured by the percentage of donor-derived blood cells in the HSCT recipient, termed donor chimerism. Multiple studies have shown that sustained donor chimerism of >90% to 95% is associated with an improved event-free survival (EFS) and overall survival (OS). More importantly, a decrease in donor chimerism over time can predict the relapse of the underlying disease for which the patient was transplanted. Decrease and/or elimination of immunosuppression and donor lymphocyte infusions (DLI) may be able to reverse decreasing

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chimerism. This chapter reviews the timing of engraftment and the factors which influence it. Graft failure and rejection are addressed in Chap. 11. Methods to measure donor chimerism and the implications of changing donor chimerism percentage are also addressed.

Engraftment

Introduction

Generally, engraftment occurs when hematopoietic stem (or progenitor) cells from the donor have taken up residence in the recipient's (or host's) bone marrow and then give rise to all types of mature blood cells. According to the Foundation for the Accreditation of Cellular Therapy (FACT) and the Center for International Blood and Marrow Transplantation Research (CIBMTR), engraftment is defined as neutrophil and platelet recovery following a period of aplasia. Neutrophil recovery is defined as an absolute neutrophil count (ANC) >500 mm^2 for first 3 consecutive daily measurements, and platelet recovery is defined as a platelet count >20,000 μL^{-1} without a platelet transfusion in the 7 days preceding the first of 3 consecutive days.

Typically, there is a period of pancytopenia (or aplasia) that develops as a result of the conditioning regimen (or may be present without conditioning because of the underlying condition such as severe aplastic anemia or primary immunodeficiency). Pancytopenia is defined as prolonged reduction of red blood cells, platelets, and white blood cells in the circulating blood. Pancytopenia places the HSCT patient at very high risk for infections, bleeding, and potential organ dysfunction. This period of profound neutropenia, anemia, and thrombocytopenia persists until hematopoietic stem cells (HSCs) home to the bone marrow and begin to produce all blood cell components, i.e., HSC engraftment.

Thrombocytopenia in the peri-HSCT setting is due to both decreased production and increased consumption. The decreased production is due to the conditioning regimen, particularly myeloablative regimens, which directly affects the capacity of HSCs to produce platelets. Decreased platelet production may also be due to the underlying disease, such as severe aplastic anemia. In contrast, factors leading to increased platelet consumption include fever, sepsis, mucositis, disseminated intravascular coagulation (DIC), sinusoidal obstructive syndrome/veno-occlusive disease (SOS/VOD) of the liver, and fungal infections. The most common sites of bleeding are mucosal membranes, skin, GI tract, respiratory system, GU tract, and intracranial.

Growth factors, including granulocyte colony-stimulating factor (G-CSF) (also known as filgrastim) and granulocyte macrophage colony-stimulating factor (GM-CSF) (also known as sargramostim), are sometimes used after HSC graft infusion to shorten the duration of profound neutropenia to neutrophil engraftment. These agents are used most often in the context of autologous and umbilical cord blood transplantation (UCBT). Unfortunately, there is no analogous agent available for the use to hasten platelet recovery.

Mechanism of Engraftment

Donor cells home to the bone marrow and adhere to the vascular endothelium in specific areas of the bone marrow microenvironment called niches that set up hospitable environments for HSCs. This initial process is mediated by selectins. The adhesion is firmed up by the interaction between other sets of molecules, which include integrin superfamily molecules expressed on the cell surface of HSCs and endothelial immunoglobulin superfamily receptors and hyaluronan receptors (CD44) on the bone marrow stroma. Following this reinforced adhesion, the HSCs migrate to the hematopoietic niches within the inner endosteal surface of the bone. This migration is regulated by a gradient of extracellular matrix-bound SDF1 (CXCL12) binding to CXC4 receptors located on the cell surface of HSCs. Many other cell types participate in this homing process, including

mesenchymal stromal cells, osteoblasts, bone marrow sinusoidal endothelial cells, certain subpopulations of T cells, adipocytes, and fibroblasts, resulting in successful HSC engraftment (see Chap. 3 for more details). Despite the complexity of this process, HSC graft failure is relatively rare. Damage to the HSC niches and immune-mediated host-versus-graft and graft-versus-host reactions can hinder engraftment (see Chap. 11 for further discussion of graft failure).

Timing of Engraftment

Each blood cell lineage engrafts at different times. In general, neutrophils are the first cell type to engraft, typically within 1–4 weeks after HSC infusion. Neutrophil engraftment is characteristically followed by platelet engraftment occurring 3–8 weeks after HSC infusion and then red blood cell engraftment in 1–3 months after HSC infusion. It may take up to 1–3 months for a normal platelet count to be achieved. The lymphoid lineage is typically the last to engraft and may take up to 1–2 years post-HSCT (see Chap. 26 for an in-depth discussion regarding immune reconstitution).

The timing of engraftment is multifactorial. These factors include the donor source (allogeneic versus autologous), the HSC source (bone marrow versus growth factor-mobilized peripheral blood HSCs (PBSCs) versus umbilical cord blood (UCB)), the presence of concurrent infection(s), the bone marrow microenvironment of the recipient, growth factor administration, donor graft manipulation, the agents used for graft-versus-host disease (GvHD) prophylaxis, and drug toxicity. In terms of neutrophil engraftment by donor source, autologous growth factor-mobilized PBSCs typically engraft the fastest (7–14 days post-HSCT). In contrast, peripheral blood HSCs from an allogeneic donor engrafts in 10–21 days after HSC infusion [1]. In comparison, related allogeneic bone marrow takes approximately 14–21 days to engraft [1]. UCB HSCs generally are the slowest to engraft at 15–40 days [1, 2].

Factors That Influence Engraftment

HSC Source

The source of the HSCs (growth factor-mobilized peripheral blood, bone marrow, and umbilical cord blood) greatly influences the success and timing of engraftment. Table 10.1 summarizes a comparison of median neutrophil and platelet engraftment by donor and HSC source. In general, neutrophil and platelet engraftment occur 2–6 and 5–8 days earlier with growth factor-mobilized peripheral blood HSCs versus bone marrow [1, 2]. In addition, immune function recovers more quickly with PBSCs than bone marrow. This is most likely due to the higher

Table 10.1 Comparison of median time to neutrophil and platelet engraftment by donor and hematopoietic stem cell (HSC) source

Donor source	HSC source	Median time to neutrophil engraftment in days (range)[a]	Median time to platelet engraftment in days (range)[b]
• Autologous	• Growth factor- mobilized peripheral blood	12 (7–14)	21
• Autologous	• Purged bone marrow	28	35
• Allogeneic: related and unrelated	• Peripheral blood	12 (10–21)	20 (7–39)
• Allogeneic: matched, related	• Bone marrow	16 (12–35)	28
• Allogeneic: unrelated	• Bone marrow	18 (10–40)	29 (8–141)
• Allogeneic: unrelated	• Umbilical cord blood	23 (15–133)	56 (16–159)
• Allogeneic: T-cell depleted	• Bone marrow	16 (9–40)	29 (8–165)

[a]Neutrophil engraftment as defined by an ANC >500 mm^2 for the first 3 consecutive days post-HSCT

[b]Platelet engraftment as defined by a platelet count >20,000/uL for the first 3 consecutive days without a platelet transfusion in the preceding 7 days

number of mature T cells in PBSC-derived grafts versus an intermediate number of T cells in bone marrow versus the relatively low number of mature T cells in umbilical cord blood [3, 4]. Generally, the lower the T-cell number in the graft, the longer time to hematopoietic recovery. Alternatively, engraftment after UCBT may be due to HLA mismatch, as a higher number of HLA mismatched UCBTs have been performed as compared to both unrelated bone marrow and PBSC HSCT [5].

HSC Dose

The dose of total nucleated cells (TNCs) and of $CD34^+$ HSCs infused into the recipient may affect the time to engraftment and decrease the risk of rejection. Typically, 2.5×10^6 cells/kg is the minimum number of HSCs from bone marrow of peripheral blood that is required for engraftment. In general, a higher TNC and $CD34^+$ cell dose from bone marrow donors have been found to result in faster neutrophil engraftment, and PBSC $CD34^+$ dose has been found to be associated with faster platelet engraftment but also correlated with increased mortality and relapse of underlying disease [6]. Other studies have shown that a much lower dose of $CD34^+$ HSCs is needed for the high probability of engraftment after UCBT. Wagner et al. [7], reported that UCB HSC grafts that contain at least 0.17×10^6 $CD34^+$ cells/kg resulted in engraftment and a high probability of survival despite up to two HLA loci mismatches. In addition, a dose of at least 2.5×10^7 TNC/kg is needed to have a high probability of engraftment [8].

Because a unit of umbilical cord blood has a fixed number of HSCs and the “donor” of the unrelated UCB unit is not available for obtaining supplemental HSCs, the use of UCB as the donor source had been limited to children and small adults in the past. However, because of its less restrictive parameters on HLA matching, the availability of UCB as an HSC source has permitted a multitude of patients, particularly those of ethnic minorities, to undergo HSCT [8]. Thus, strategies to increase the number of UCB HSCs are being actively explored. One approach that has been successful is the infusion of two UCB units such that the absolute TNC dose is > $2–3 \times 10^8$ TNC/kg. A number of studies have reported similar neutrophil recovery times, and, shortly after engraftment, one of the UCB types dominates although this predominance of one unit over the other is not related to cell dose. In one study in which pediatric patients with leukemia were randomized to receive one versus two UCB units with a minimum of 2.5×10^7 TNC/kg, the incidence of neutrophil recovery was 89% and 88% in one- versus two-unit recipients, respectively [2]. The median time to neutrophil engraftment was similar: 21 days (range, 11–62) versus 23 (range, 11–133) in one versus two UCB units, respectively. In contrast, the timing of platelet recovery differed significantly. Platelet recovery was higher in recipients of one- as compared to two-UCB-unit HSCTs (76% versus 65%, respectively; $p = 0.04$), and the median time to recovery was faster in the one UCB unit at 58 days (range, 28–295) as compared to 84 days (range, 22–716) in recipients of a two-UCB-unit HSCT. However, this study reported that the 1-year overall survival rates were similar and not statistically significant (73% versus 65% for one- versus two-UCB-unit HSCTs, respectively, $p = 0.17$). If full donor chimerism is not achieved by day 90 or decreasing donor chimerism is noted, then donor chimerism should be monitored weekly. If donor chimerism continues to decline, then interventions are warranted (see the “Implications of Donor Chimerism on Event-Free Survival (EFS) and Overall Survival (OS)” and “Interventions to Improve Donor Chimerism” sections and see Chap. 12, Graft Failure).

Graft Manipulation

Donor graft manipulation can influence the engraftment timeframe negatively and positively, theoretically, by T-cell depletion either ex vivo of the donor HSC product or in vivo by the infusion of antithymocyte globulin (ATG) or Campath. However, in one large study of 541 pediatric patients with acute leukemia who received an unmanipulated bone marrow ($N = 262$), T-cell-depleted bone marrow ($N = 180$), or umbilical cord blood ($N = 99$) unre-

lated donor HSCT, there was no statistically significant difference in neutrophil or platelet engraftment between the unmanipulated and the T-cell-depleted bone marrow donor graft groups [5]. In contrast, engraftment was significantly delayed and had the highest frequency of graft failure in those who underwent UCBT.

The process of cryopreservation and rethawing of the HSC product can negatively impact the integrity of the graft (see Chap. 8). In addition, manipulation of the HSC product to eliminate red blood cells in the setting of ABO incompatibility may also affect the HSC product negatively (see Chap. 8).

In contrast, the administration of growth factors (i.e., G-CSF and/or GM-CSF) may be employed to shorten the time to neutrophil engraftment [9]. The use of growth factors post-HSCT is dependent on many factors, including donor source (i.e., autologous versus allogeneic), HSC source, underlying disease being treated by HSCT, and the conditioning regimen used. This approach is most commonly used in the setting of autologous HSCT and often after UCBT [10, 11]. The use of G-CSF post-HSCT is particularly important in HSCT recipients who received a relatively low dose of HSCs [12].

Bone Marrow Microenvironment of Recipient (Host)

The state of the patient's bone marrow microenvironment and the patient's underlying disease influence HSC engraftment. In addition, previously administered chemotherapy and radiation and the conditioning regimen are thought to contribute to creating an inhospitable bone marrow microenvironment that is unable to robustly support hematopoiesis. The damage to the host's HSC niches involves injury to endosteal, perivascular, and vascular endothelial cells that make up the bone marrow stroma. Thus, this impaired bone marrow microenvironment and damage to the HSC niches can hinder hematopoietic recovery post-HSCT [13].

Graft-Versus-Host Disease (GvHD) Prophylaxis

Immunosuppression used for GvHD prophylaxis may affect hematopoietic recovery. It has been well established that methotrexate in a dose-dependent manner can delay engraftment at least by 5 days when used as GvHD prophylaxis [14].

Clinical Manifestations of Engraftment

Very often, patients manifest signs and symptoms of engraftment prior to objective evidence of engraftment such as increasing white blood cell (WBC) count in the peripheral blood. These signs and symptoms are reminiscent of a “flu-like” syndrome with new-onset fevers as the most common manifestation of early engraftment. In addition, patients can develop chills, a rash (typically erythema), malaise, joint and muscle aches, and abdominal pain. Mucositis becomes much worse with increased mucosal friability, thickened and copious oral secretions and perioral edema. Peripheral edema and increased platelet consumption often occurs as well. This constellation of findings are due to increased cytokine secretion from the new donor cells (often referred to as a “cytokine storm”). If this process becomes significantly worse, it is termed hyperengraftment syndrome and may require treatment with a short course of corticosteroids (see Chap. 12). However, one cannot assume that the development of these signs and symptoms is only due to engraftment. Infection(s) will also present in this manner; thus, blood cultures and blood for viral PCR testing should be obtained, and empiric broad-spectrum antibiotics need to be initiated immediately with the onset of new fevers.

Shortly after engraftment becomes evident in the bloodstream as a rising WBC count and a measurable ANC, mucositis begins to improve and rapidly resolves as the ANC rises. Also, the post-HSCT recipient begins to self-diurese the retained fluids without pharmacologic intervention. A patient's need for blood product transfusions also decreases. At this time, patients may develop acute GvHD as early as initial engraftment.

Implications of Engraftment

Persistent or chronic thrombocytopenia has been found to be a poor prognostic factor, especially if

it is in association with chronic graft-versus-host disease (GvHD). It also may be an indicator of disease relapse. In addition, thrombocytopenia may be due to viral or fungal infections, delayed engraftment, or drug toxicity. Thus, the etiology of persistent thrombocytopenia warrants prompt investigation.

Chimerism

Introduction

The term, chimerism, originates from Chimera, an ancient Greek mythological, fire-breathing creature that was first described in Homer's Iliad. She was a creature composed of the parts of other creatures: the body of a lion with the head of a goat arising from her back, a serpent for a tail, and a hind part of a dragon. Donor chimerism is defined as the percentage of donor-derived blood cells and is used to confirm donor engraftment. Donor chimerism is a valuable tool to assess the risk of disease relapse and overall survival of recipients after allogeneic HSCT. In other words, the presence of host-derived cells after HSCT may be the result of inadequate full myeloablation and/or inadequate elimination of frank disease or minimal residual disease (MRD) that can ultimately lead to an overt disease relapse.

Monitoring and Testing Methods of Donor Chimerism

Testing Methods

Strategies to measure donor chimerism rely on the unique pattern of genetic markers that distinguishes donor- from recipient-derived cells. The two most widely used tests are (1) fluorescence in situ hybridization (FISH) using a set of sex chromosome-specific probes and (2) typing of polymorphisms by DNA amplification (PCR). XY-FISH can only be performed if there is a donor-recipient mismatch, i.e., the donor and recipient are of different sexes, and thus, this method has limited utility. Polymorphism testing by DNA amplification is much more versatile. The most commonly used method currently is short tandem repeat (STR) DNA analysis. STRs are genomic polymorphisms of loci that consist of tandemly repeated DNA sequences that have a variable number of base pairs ranging from two to eight. These loci may have alleles that differ among individuals. They are inherited in a codominant Mendelian fashion. The conserved, nonpolymorphic flanking regions of the STR DNA are used to create oligonucleotide primers to the STRs. These primers are used to amplify via PCR the STRs which are separated by gel electrophoresis to determine the origin (donor or recipient) of the post-HSCT patient's cells. A prior sample from the donor is needed in order to interpret the results of the STR analysis appropriately. Analysis on whole blood from the HSCT recipient measures the origin (i.e., donor versus recipient) of both lymphoid and myeloid lineage components together. However, chimerism can be quantitated for individual cell lines [15].

Monitoring Time Frame

Donor chimerism is dynamic with patients spontaneously converting from mixed chimerism to full donor chimerism. Commonly, patient with full donor chimerism may experience transient mixed donor chimerism early on in the post-HSCT period (up to 100 days post-HSCT) without untoward consequences. However, mixed chimerism may ultimately result in graft failure and thus needs to be monitored closely early on post-HSCT in order to identify patients who may benefit from interventions to promote full donor chimerism. Typically, donor chimerism is first checked around day 30 (as long as the patients show signs of engraftment by that time). However, Dubovsky et al. reported in 1999 the results of a prospective study of a heterogeneous pediatric patient population that underwent HSCT [16]. Chimerism was assessed by STR-PCR every 1–3 days post-HSCT. They reported that donor engraftment could be detected approximately 7 days before hematologic engraftment was detected. They went on to report that mixed donor chimerism was

associated with a very high risk of relapse. In comparison, donor chimerism analysis can be discontinued once full (100%) donor chimerism and complete blood count recovery have been achieved in patients who received an unmanipulated donor HSCT after myeloablative conditioning. However, for patient who received a reduced intensity conditioning regimen (RIC), donor chimerism typically needs to be monitored for a longer period of time and specific cell lineages monitored for engraftment because RIC regimens are associated with incomplete eradication of host immune cells. Thus, these patients may have multi-lineage mixed donor chimerism. In order to be considered engrafted in patients who undergo RIC HSCT, patients must have ≥5% donor $CD3^+$ T cells as well as normal blood cell counts. Otherwise, patients are considered to have primary graft failure (see Chap. 11 for further discussion of graft failure).

Full Versus Mixed (or Partial) Donor Chimerism

Full donor chimerism refers to the setting in which all of the hematopoietic cells are of allogeneic donor origin. While full donor chimerism is what is ideally achieved in recipients of an allogeneic HSCT, mixed (or partial) donor chimerism may occur. Mixed donor chimerism refers to the scenario in which a fraction of the recipient-derived hematopoietic cells (or just cells of lymphoid lineage) persists. Mixed donor chimerism may be compartmental in which not all of the cell lines are of both donor and recipient origin. For example, a post-HSCT patient may demonstrate full donor chimerism of the T-cell lineage but not of the myeloid lineage. Certain nonmalignant conditions, such as those of chromosomal instability (e.g., Fanconi anemia), autoimmune proliferative disorders, and Bloom's syndrome, and the vast majority of malignant conditions require full donor chimerism for successful outcomes. In contrast, many nonmalignant syndromes, including primary immunodeficiencies, metabolic disorders, and hemoglobinopathies as well as HLH, do not require full donor chimerism as long as the underlying disorder is adequately corrected. However, mixed donor chimerism is associated with graft rejection in some circumstances. Thus, the ultimate goal is to achieve full donor chimerism for all HSCT recipients.

Implications of Donor Chimerism on Event-Free Survival (EFS) and Overall Survival (OS)

Donor chimerism may fluctuate early on post-HSCT, and mixed donor chimerism may not necessarily lead to graft failure. However, a progressive rise in the percentage of recipient cells often is a harbinger of disease recurrence and/or graft failure. Furthermore, it is the rate of change of donor chimerism that is most prognostic. Multiple studies have shown that donor chimerism greater than 90–95% is associated with an improved event-free survival (EFS) and overall survival (OS). More importantly, a decrease in donor chimerism over time (0–6 months post-HSCT) can predict relapse in patients with acute leukemia. In one study of a heterogeneous patient population ages 2–63 years who underwent allogeneic HSCT for a variety of malignant diseases, Lamba et al. [17] reported that 37% of patients with mixed donor chimerism at day 30 post-HSCT relapsed as compared to only 23% of patients who had achieved full donor chimerism at this same time point. Furthermore, 23% of patients with mixed chimerism versus only 7% at day 90 relapsed by 6 months post-HSCT. Thus, the overall survival was significantly lower in the group of patients with mixed chimerism at day 90.

In a retrospective study of adult patients with myelodysplastic syndrome (MDS) or AML who underwent RIC allogeneic HSCT, Tang et al. [18] found that having a higher percentage of donor-derived cells of myeloid origin only at day 90 post-allogeneic HSCT had a positive impact on EFS and OS. In contrast, a higher percentage of both myeloid and T-cell origin at day 60 had a positive impact on EFS only and not OS. Because donor chimerism is a process that can change

over time, these relative fluctuations are more prognostic than the absolute values in one patient at one time point. In this study, increased donor chimerism over time was associated with a better OS but not EFS. Conversely, a decreasing chimerism over time was associated with a significantly worse EFS and OS. Patients who had the highest OS (at 83%) at one-year post-HSCT were those whose donor chimerism increased from 95% at day 30 to 100% by day 90. In contrast, patients whose donor chimerism that decreased from 95% at day 30 to 90% by day 90 had a predictably worse 1-year OS of only 30%. This effect was even more evident in patients who were transplanted with active disease present, had poor cytogenetics, and had a matched unrelated donor.

In a prospective, multicenter study of 163 pediatric patients who underwent HSCT for acute lymphoblastic leukemia (ALL), serial, quantitative donor chimerism analyses were performed on peripheral blood weekly for the first 100 days and then monthly thereafter [19]. Patients who had complete donor or low-level mixed donor chimerism and increasing donor chimerism had a 3-year EFS of 66%. In contrast, patients whose donor chimerism decreased over time had an EFS of only 23%. In another study of 81 AML pediatric allogeneic HSCT patients, decreasing donor chimerism was found to be a poor prognostic indicator. The 3-year EFS for patients with full or low-level mixed donor chimerism was 59%, whereas those with increasing donor chimerism was 60%. In contrast, the 3-year EFS of patients with decreasing chimerism was only 28% [20]. These two studies demonstrate that decreasing donor chimerism can identify post-HSCT ALL and AML patients who are at highest risk for relapse prior to overt, hematologic relapse is evident.

Interventions to Improve Donor Chimerism

If decreasing donor chimerism is noted in patients who underwent allogeneic HSCT for hematologic malignancy, then interventions should be initiated at the time that the decrease in donor chimerism is identified in order to prevent a frank hematologic relapse. If the patient is on immunosuppression at the time of decreasing donor chimerism, withdrawal of all immunosuppression is warranted. If the patient is not on immunosuppression, then donor lymphocyte infusion (DLI) should be given. In a group of 46 ALL patients who had decreasing donor chimerism post-HSCT, 31 patients received immunotherapy (i.e., DLI) and withdrawal of immunosuppression [19]. Their disease-free survival (DFS) was 37%. In contrast, the DFS of those 15 patients who did not receive immunotherapy was 0%. Patients who responded to cessation of immunosuppression showed evidence of rising donor chimerism in peripheral blood in 1 week, whereas patients who responded to DLI showed an improvement in donor chimerism in 2–3 weeks post-DLI. Furthermore, patients who responded to withdrawal of immunosuppression as the first-line intervention had a higher rate of achieving complete/full donor chimerism than those whose first-line therapy was DLI.

In a study of pediatric AML patients who underwent allogeneic HSCT, patients who had a decreasing donor chimerism and received immunologic intervention early on had an EFS of 36% as compared to 0% for those who received no immunologic intervention [20]. This study supports the notion that early interventions can improve outcomes by predicting hematologic relapse of AML. In another study of childhood AML, donor chimerism from peripheral blood was monitored weekly for the first 300 days post-HSCT and then monthly thereafter [21]. The bone marrow was assessed for donor chimerism at days 30, 60, and 100 and then 6, 9, 12, 15, and 18 months post-HSCT. If a patient had mixed donor chimerism of >1% recipient-derived cells in the peripheral blood or bone marrow, then another sample was obtained within 1 week. If >1% recipient-derived cells were detected in two consecutive samples, then immunosuppression was discontinued, and then DLI was administered. Of the 71 patients, 51 had continuous full donor chimerism. In the other 20 patients with mixed donor chimerism, 13 went on to receive immunotherapy. Of the 13, six achieved full donor chimerism without toxicity, and they remained in long-term remission. All of the

patients with mixed chimerism who received no intervention relapsed. This study confirms that donor chimerism post-HSCT is a prognostic indicator in pediatric AML that warrants immediate intervention.

In a retrospective study of 104 pediatric HSCT patients with acute leukemia who had mixed donor chimerism, Horn et al. [22], reported that 51 of the 104 patients received preemptive immunotherapy. All had withdrawal of immunosuppression, and 30 (59%) went on to receive DLI for persistent mixed donor chimerism. These patients who received preemptive immunotherapy had similar EFS rates whether or not residual disease was present pre-HSCT. Furthermore, chimerism status and minimal residual disease (MRD) status pre-HSCT were no longer predictors of a poor outcome in patients with mixed donor chimerism who received preemptive immunotherapy with a 5-year EFS of 68 to 69%.

Collectively, these studies demonstrate that donor chimerism needs to be monitored closely, and, in those at high risk for relapse, intervention should be instituted if complete donor chimerism is not achieved by day 90 or if decreasing donor chimerism is noted at any time post-HSCT. Firstly, immunosuppression should be withdrawn. If complete/full donor chimerism is not achieved, then DLI should be administered. If the patient is not on immunosuppression at the time that decreasing chimerism is discovered, then DLI should begin immediately.

Key Points

- Sustained engraftment is the goal of HSCT.
- Engraftment is defined as neutrophil and platelet recovery after a period of aplasia.
- Engraftment is dependent upon the donor source, the HSC source, the HSC dose, donor graft manipulation, and the underlying disease.
- Patients typically experience a "flu-like" syndrome at the time of engraftment.
- Chimerism measures the percentage of donor-derived blood cells in the HSCT recipient.
- Donor chimerism (both lymphoid and myeloid lineages) should be monitored in all post- allogeneic HSCT patients at least at days 30, 60, and 90 post-HSCT until full (100%) donor chimerism has been achieved.
- If full donor chimerism is not achieved or is decreasing, then intervention(s) is warranted. These interventions include the discontinuation of all immunosuppression and the administration of donor lymphocyte infusion (DLI) if stopping immunosuppression did not achieve 100% donor chimerism or if the patient is not on immunosuppression at that time.
- For leukemia, a decline or not achieving full donor chimerism is often a harbinger of relapse.

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Graft Failure

11

Valerie I. Brown

Abstract

Graft failure is a very serious, life-threatening complication of hematopoietic stem cell transplantation (HSCT) that is fortunately rare. A patient is considered to have graft failure if the patient lacks hematopoietic cell engraftment after HSCT. Graft failure can occur in both the autologous and allogeneic HSCT settings. Graft failure can be classified as primary or secondary. Both primary and secondary graft failure are defined as an absolute neutrophil count (ANC) <500/mm^2. However, they differ in that primary graft failure has no evidence of engraftment by Day 28 after bone marrow or peripheral blood hematopoietic stem cell transplantation and by Day 42 after umbilical cord blood transplantation, whereas secondary graft failure occurs after hematopoietic stem cell (HSC) engraftment had already been established. Graft failure is multifactorial which can be categorized as quantitative, qualitative, or immunologic. Graft rejection is a cause of graft failure and is immune mediated. Poor graft function may result after HSCT as well. An HSCT recipient is considered to have poor graft function if the recipient does not have adequate, sustained blood counts but has full or nearly full donor chimerism and often responds to a boost of CD34$^+$ donor stem cells via infusion. Because graft failure is associated with significant morbidity and mortality, every effort needs to be made to minimize the risk of graft failure. If graft failure occurs, then donor lymphocyte infusion(s) (DLI) and use of a CD34$^+$ donor stem cell boost followed by G-CSF mobilization are employed. If these measures fail, and the patient

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dose not reconstitute with autologous HSCs, then the patient needs to undergo a second HSCT. This chapter discusses the risk factors, etiologies, and strategies to minimize the risk and to treat graft failure.

Introduction and Definitions

Graft failure is a serious, sometimes deadly, complication of hematopoietic stem cell transplantation (HSCT). Graft failure is defined as the lack of hematopoietic cell engraftment after allogeneic or autologous HSCT and can be classified as primary or secondary. Graft failure is considered primary if there is no evidence of initial engraftment. Specifically, in primary graft failure, the patient's absolute neutrophil count (ANC) is $<500/\text{mm}^2$ by Day 28 after myeloablative conditioning followed by bone marrow or peripheral blood HSCT or by Day 42 after myeloablative conditioning followed by umbilical cord blood transplantation (UCBT). Graft failure following myeloablative conditioning is also considered primary if the ANC is $<500/\text{mm}^2$, platelet count is $<20{,}000/\mu\text{L}$, and hemoglobin is ≤ 8 g/dL, and the patient is still transfusion-dependent. In comparison, a patient who underwent a reduced intensity conditioning (RIC) allogeneic HSCT with an ANC $<500/\text{mm}^2$ by Day 28 post-HSCT and failure to have $>5\%$ donor cells even with the other blood counts being normal is considered to have primary graft failure.

A patient who underwent myeloablative HSCT is considered to have secondary graft failure if the patient's ANC is $<500/\text{mm}^2$ for three consecutive days after already meeting the definition of engraftment (see Chap. 10). The decrease in ANC must not be related to an infection, medication, or disease progression. Patients develop pancytopenia and bone marrow aplasia, and their percent of donor chimerism will significantly decrease or will be 0% (i.e., 100% recipient). In the context of RIC HSCT, secondary graft failure is defined as an ANC $<500/\text{mm}^2$ after initial engraftment and loss of donor hematopoiesis to $<5\%$ despite normal blood counts. Secondary graft failure can occur early or late in the course post-HSCT with late occurring more commonly in the setting of allogeneic versus autologous HSCT. In contrast, graft rejection is due to immune-mediated rejection of the donor graft by residual recipient immune cells. Graft rejection is a type of graft failure.

A patient is considered to have poor graft function if the patient does not achieve adequate blood counts despite having full or nearly full donor chimerism. Poor graft function requires at least two cell lines to be cytopenic.

Incidence

The incidence of graft failure is difficult to measure because it is attributed frequently to the underlying cause and not graft failure itself. A rate of 1–3% has been estimated in autologous HSCT recipients as determined by the number of patients who needed the infusion of backup HSCs [1]. The incidence of graft failure in allogeneic HSCT recipients has been estimated at 2–20%, depending on the presence of known risk factors such as HLA disparities, T-cell depletion of the HSC graft product, or use of umbilical cord blood as the donor HSC source. Generally, the risk of graft failure is highest in donor UCBT recipients at 10–20%, whereas the risk is only 2% in matched sibling donor (MSD) HSCT recipients. The risk of primary graft failure for matched unrelated donor (MUD) HSCT recipients has been reported to be 9–12% [2].

Risk Factors and Etiologies

The most common risk factors for graft failure can be categorized as quantitative, qualitative, or immunologic (see Table 11.1). The most common risk factors for primary and secondary graft failure differ. The most common risk factors for primary graft failure include increased HLA disparity (most likely in the setting of haploidentical HSCT), poor bone marrow microenvironment of the recipient, low HSC dose, HSC product manipulation (e.g., T-cell depletion) and/or

Table 11.1 Risk factors of graft failure

Quantitative	Qualitative	Immunologic
• Donor graft source – Cord blood > bone marrow > peripheral blood	• Older donor	• HLA disparity between donor and recipient
• Low CD34+ stem cell dose	• Underlying disease	• Allosensitization – Exposure to multiple blood products pre-HSCT – Prior pregnancy
• Presence of splenomegaly	• Disease status	• Presence of pre-HSCT donor-specific antibodies (DSAs)
–	• Iron overload	• Graft manipulation
–	• Bone marrow fibrosis	• Type and intensity of the conditioning regimen
–	• Storage techniques affecting HSC integrity	• Post-HSCT immunosuppression regimen
–	• Pre-HSCT treatment with chemotherapy and/or irradiation	• Presence of acute or chronic graft versus host disease
–	–	• Viral infection – HHV-6 – CMV – Parvovirus

integrity, the disease status at the time of HSCT, the type and intensity of the conditioning regimen with UCB associated with the highest risk, less immunosuppression post-HSCT, and allograft-damaging drugs. The patient's underlying disease (i.e., bone marrow failure syndromes and leukemia) as well as pretreatment may play a significant role for increased risk of primary graft failure [3]. Allosensitization which may occur with a history of multiple blood product transfusions can cause wither primary or secondary graft failure.

While many of the risk factors for primary graft failure also increase the risk of secondary graft failure, graft-versus-host disease (GvHD) and certain pharmacologic agents that can cause myelosuppression can increase the risk of secondary graft failure. The etiologies of secondary graft failure include myelosuppression and immunosuppression from any etiology. While infection may cause primary graft failure, infection, particularly HHV-6 and CMV, more commonly cause secondary graft failure by suppressing the newly engrafted donor HSCs. The most common etiology of late secondary graft failure is graft rejection due to the presence of residual recipient cells. In addition, progression of the underlying disease, other infections, drug effects, immunosuppression, and GvHD can cause late secondary graft failure. The risk factors and etiologies of graft failure are detailed below.

Donor HSC Source

In pediatric patients who undergo HSCT after myeloablative conditioning, the overall rate of irreversible graft failure has been found to be approximately 10% [4]. In general, UCB used as the HSC source has been found to have the highest risk of graft failure, reported at 10–20% [2]. In contrast, MSD HSCT has been reported to carry the lowest risk of graft failure at 2%, whereas the graft failure rates for those undergoing MUD HSCT range from 9 to 12.3% [2]. This difference may be related directly to the intrinsic properties of the different HSC product sources. Alternatively, this difference may be due to HLA disparity, as the number of mismatched HSCTs use UCB as the HSC source, as compared to bone marrow and peripheral blood HSCs. Thus, HLA disparity rather than the HSC product itself may account for the different rates of graft failure by HSC source. In a retrospec-

tive study of 309 children who underwent allogeneic bone marrow transplantation (BMT), a total of 11 patients (3.5%) had graft failure with four cases of primary and seven cases of secondary graft failure [3]. In this study, primary graft failure was defined as the absence of donor cell engraftment by Day 42 post-HSCT, whereas secondary graft failure was defined as the loss of donor cell engraftment after initial donor cell engraftment. Furthermore, a diagnosis of nonmalignant disorder, lower total nuclear cell (TNC) dose, conditioning regimen without total body irradiation (TBI) were associated with a higher incidence of graft failure, whereas donor source, CMV status of the donor and recipient, the $CD34^+$ stem cell dose, and alloimmunization were not in this study.

HSC Dose and Graft Manipulation

Data regarding the impact of $CD34^+$ stem cell dose on graft failure after full myeloablative conditioning as inconsistent because as many cells as possible are infused at the time of HSCT to afford the best chance of rapid and robust hematopoietic recovery. Thus, it is difficult to determine the effect of $CD34^+$ stem cell dose on graft failure [5, 6]. However, there are some data showing that the $CD34^+$ stem cell dose may impact the time to engraftment in patients who received mismatched or haploidentical HSCTs. Most centers use a target dose of >10 × 106 cells/kg recipient weight for mismatched or haploidentical HSCTs. In contrast, patients who undergo RIC HSCTs have more rapid T-cell donor engraftment with a lower rate of graft failure [7].

Historically, T-cell depletion, either pan $CD3^+$ or selective $CD8^+$, may reduce the rate of acute GvHD, but it is associated with an increased risk of graft failure [8].

Age

As we age, telomeres shorten and damage accumulates in stem cells, including HSCs. These damaged stem cells have a diminished capacity for hematopoietic reconstitution. Interestingly, shortly after HSCT, the recipient's newly engrafted donor-derived HSCs have significantly shorter telomeres as compared to his/her donor and age-matched controls with the end result of accelerated aging of the donor HSCs [9, 10]. HSCs from older donors which are more likely to have partially damaged and/or have shortened telomeres are more likely to become exhausted and not provide sustained engraftment. Thus, HSCT recipients whose donor is older are more likely to end up with graft failure.

Underlying Disease/Disorder

In general, disorders that require multiple blood product transfusion or possess bone marrow dysfunction or failure due to poor bone marrow stromal milieu are associated with higher rates of graft failure post-HSCT.

Hemoglobinopathies: Thalassemia is associated with a relatively high rate of HSCT graft failure. In pediatric patients <16 years old, risk factors for graft failure include hepatomegaly and the presence of portal fibrosis prior to HSCT. The quality of iron chelation therapy prior to HSCT also plays a role in the risk of developing graft failure [11, 12]. In one study of patients with thalassemia who underwent a MSD HSCT with myeloablative conditioning, none of the three risk factors (hepatomegaly, presence of portal fibrosis, and poor chelation therapy) had a rate of graft failure of 10% as compared to 25% in patients who had all three risk factors present at the time of HSCT [11]. In contrast, another study reported that graft failure was seen in only three of 75 pediatric patients (4%) after MSD BMT following myeloablative conditioning [13]. All were secondary graft failure with varying degrees of risk factors.

In patients with sickle cell disease, similar results regarding graft failure have been reported. In a CIBMTR study that evaluated data from a 13-year period, the rate of graft failure after myeloablative conditioning with busulfan and cyclophosphamide followed by MSD BMT was 15%

(9 of 67 patients) [14]. In other studies, graft failure rates were <10% using RIC regimens of alemtuzumab or ATG plus post-HST cyclophosphamide [15]. However, using this regimen in haploidentical donor HSCT, the graft failure rate was >40% [16].

Myelodysplastic Syndrome (MDS) and Myeloproliferative Disorders (MPD): Patients undergoing HSCT for myelodysplastic syndrome (MDS) or chronic myeloproliferative disorders (MPD), such as chronic myelogenous leukemia (CML), are at increased risk for graft failure, particularly if they had received a RIC regimen [3]. Often, these patients may have a prolonged period of cytopenia (>50 days after HSCT). The increased risk of graft failure may be due to a defective bone marrow microenvironment and/or the presence of residual host (recipient) immune effector T cells. Very often, these patients do not receive intensive chemotherapy pre-HSCT and thus may have intact host immune cells to reject donor HSCs.

Allosensitization: Patients such as those with thalassemia, sickle cell disease, and severe aplastic anemia will become sensitized to histocompatibility antigens over time as they are exposed to multiple blood products. Often, this sensitization to allogeneic minor histocompatibility antigens prior to HSCT can result in graft failure. Thus, HSCT early in the disease course (i.e., before allosensitization has occurred) will reduce the likelihood of graft failure [17].

Prior Exposures and Type and Intensity of the Conditioning Regimen: A prior exposure to intensive chemotherapy and/or radiation may impair the bone marrow microenvironment such that graft function is negatively impacted. Furthermore, increasing the intensity of the conditioning regimen does not necessarily ameliorate the risk for graft failure. In patients who received a RIC regimen, approximately 30% of patients developed graft failure if their Day 30 post-HSCT T-cell donor chimerism was <50% [18]. In another study, the rate was 50% in patients whose post-HSCT Day 30 T-cell donor chimerism was <25% versus 4% if the T-cell donor chimerism was 51–75% [19].

Post-HSCT Immunosuppression: Post-HSCT cyclophosphamide is being used successfully to reduce graft failure in patients receiving RIC haploidentical BMT or EIC UCBT; NK cells are highly sensitive to cyclophosphamide and may contribute to cyclophosphamide's impact on graft failure in HSCT patients. In a study of adult and pediatric patients with leukemia, MDS, and lymphoma who received a RIC HLA-haploidentical BMT and received post-HSCT cyclophosphamide, the graft failure rate was 13% [20].

Graft Rejection

Introduction and Definitions: Graft rejection is a cause of graft failure. It is mainly immune mediated. Because it is immune mediated, graft rejection, by definition, only occurs in the setting of allogeneic (and not autologous) HSCT. Early primary graft rejection is defined by the absence of donor hematopoiesis after 1 month post-allogeneic HSCT, whereas late (or secondary) rejection is defined as a loss of donor cells after initially engrafting.

Risk Factors: The risk factors of graft rejection include the presence of pre-formed antibodies which may initiate graft rejection. This is most commonly seen in the setting in which the haploidentical donor has Class I or Class II anti-HLA antibodies present but is also seen in MUD HSCT [21, 22]. Also, patients who have received multiple blood product transfusions prior to HSCT are at a higher risk for graft rejection.

Donor-Related Immune-Mediated Graft Rejection: Donor T cells are required to achieve successful donor HSC engraftment. Thus, graft manipulation of T-cell depletion places a patient at a higher risk for graft rejection. The role of T cells in successful engraftment is not clear, though. They may be playing a direct role by suppressing or eliminating host-derived effector T cells. Alternatively, donor T cells may secrete key cytokines needed for the donor HSCs to home to the HSC niche in the bone marrow or augment HSC proliferation. $CD8^+$ T

cells are thought to drive this process. In mice, natural killer (NK) cells derived from the host play a role in graft rejection and that regulatory T cells (Tregs) of donor or host origin can regulate NK-mediated graft rejection [23, 24]. Correlative data in humans are lacking at this time, so the role of NK cells in mediating HSC graft rejection is unclear.

Host (Recipient)-Related Immune-Mediated Graft Rejection: Host T cells are thought to play a role in mediating graft rejection. These memory T cells persist despite chemotherapy and irradiation and are thought to arise after a patient is exposed to white blood cells (WBCs) contaminated within blood products. These radio-resistant memory T cells develop against minor histocompatibility antigens on the WBCs. These T cells which recognize these minor allo-antigens contribute to donor graft rejection, accounting for the observation that exposure to multiple blood product transfusions prior to HSCT is a risk factor for graft rejection [17].

Additionally, B cells which produce antibodies are thought to play a role in graft rejection via antibody-dependent, cell-mediated, or complement-mediated cytotoxicity [25]. Typically, pre-formed antibodies are not cleared with conventional conditioning regimens. Studies have shown that the presence of donor-specific antibodies (DSAs) is associated with a high rate of graft rejection [26]. This seems to be particularly important in haploidentical and UCB HSCTs [21, 27].

Diagnostic Studies

To establish the diagnosis of graft failure, donor chimerism analysis, which measures the percentage of hematopoietic-derived cells that are of donor origin in the HSCT recipient, is performed (see Chap. 10, Engraftment and Chimerism). Donor chimerism may be evaluated from peripheral blood or bone marrow. The lack of donor cells, the presence of recipient T cells, an ANC $<500/mm^2$, and the requirement of blood product transfusions are diagnostic of graft failure. The absence of loss of donor chimerism is seen with graft rejection.

Strategies to Decrease the Risk of Graft Failure

Strategies used to help mitigate the risk of graft failure include prescreening for the presence of donor-specific antibodies when considering a mismatched, unrelated, or haploidentical donor graft. If these antibodies are present, then one should consider an alternative donor [28]. If available, a younger, unrelated donor or even an UCB donor (if the cell dose is adequate) should be considered over an old, related donor. If a patient has massive splenomegaly, then it may be advantageous to treat him/her to reduce the spleen size prior to HSCT. Depending on the indication for HSCT, such as severe aplastic anemia, a higher $CD34^+$ cell dose should be considered [29]. Other measures to minimize the risk of graft rejection include the use of irradiated and leukocyte-reduced blood products, performing HSCT before exposure to multiple blood product transfusions has occurred, the use of antithymocyte globulin (ATG) in the conditioning regimen, and the continuation of post-HSCT immunosuppression. The use of G-CSF-mobilized peripheral blood stem cells as the HSC source may be advantageous, as this will increase the $CD34^+$ stem cell count in the HSC product.

Interventions and Outcomes

For autologous HSCT, one approach is to collect and store an unmanipulated portion of cells to be used as a backup infusion if graft failure develops. If autologous cells are not available, then a search for an allogeneic donor should be initiated immediately. Having a backup of autologous stem cells for patients undergoing allogeneic HSCT should be considered in certain circumstances, such as nonmalignant disorders. Growth factors, such as G-CSF and GM-CSF, are often initiated as a temporizing measure while implementing a more definitive treatment strategy, such as donor lymphocyte infusion (DLI), $CD34^+$ stem cell boost or a second HSCT with the same of different donor. Preemptive DLI has been used

successfully to prevent secondary graft failure in patients with declining donor CD3+ T-cell chimerism. However, administration of DLI for the treatment and prevention of graft failure needs to be balanced with the risk of GvHD.

In patients with poor graft function who have full donor or nearly full donor chimerism, the use of a CD34+ donor stem cell boost followed by G-CSF mobilization may result in improved graft function [30].

Consideration of a second HSCT is warranted in patients who do not respond to the above interventions or do not reconstitute with autologous HSCs; RIC over myeloablative conditioning regimens is preferred for second HSCTs, but the use of the same of different donor is not so important, but one should check for the presence of DSAs. If DSAs are present, then one should consider using a different donor.

Outcomes of graft failure are very variable and depend upon many factors including the underlying condition and type and intensity of the conditioning regimen and if infection is involved. The 1-year overall survival has been reported to be only 24% for patients with primary graft failure and 25% for patients who developed secondary graft failure [31].

Key Points

- Graft failure is a rare occurrence, but its development is associated with significant morbidity and mortality.
- Graft failure is defined as the lack of hematopoietic cell engraftment after allogeneic or autologous HSCT and is considered as primary or secondary graft failure.
- Primary graft failure is defined as an ANC <500/mm² by Day 28 following BMT or PBSCT and by Day 42 following UCBT, whereas secondary graft failure is defined as an ANC <500/mm² for three consecutive days with loss of donor chimerism after already engrafted, and the decrease in ANC cannot be attributed to an infection, medication, or disease progression.
- Poor graft function occurs when an HSCT recipient does not have adequate, sustained blood counts despite full or nearly full donor chimerism.
- Every effort should be made to minimize the risk of graft failure.
- Interventions for graft failure include administration of DLI and the use of a CD34+ donor stem cell boost followed by G-CSF mobilization.
- If a patient with graft failure does not reconstitute with autologous HSCs, then the patient needs a second HSCT to be performed as soon as possible.

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Engraftment Syndrome and Associated Cytokine Storm and Capillary Leak Syndrome

12

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Abstract

While engraftment is the desired end point of hematopoietic stem cell transplantation (HSCT), the phase of neutrophil recovery can be complicated by two acute entities termed engraftment syndrome (ES) and capillary leak syndrome (CLS). Both ES and CLS result from the release of pro-inflammatory cytokines (e.g., IL-1, TNFα, and IFNγ) from donor cells in response to the recipient's (or host's) tissues. ES and CLS can be seen in both autologous and allogeneic HSCT settings. The incidence of ES has been reported to be 7–59% of autologous and 6–82% of allogeneic HSCTs. CLS is much more common than ES and is seen in myeloablative allogeneic HSCT. The clinical manifestations of ES range from mild, self-limited erythroderma, and high fevers to a more severe (and sometimes life-threatening) entity of acute weight gain, pulmonary edema, and hepatic and renal dysfunction with or without encephalopathy. CLS is characterized by weight gain, generalized edema, hypotension, prerenal failure, ascites, intravascular depletion, and pericardial and/or pleural effusions. CLS typically first manifests 1–3 days *prior* to neutrophil recovery as measured by a detectable ANC in the blood, whereas ES typically occurs shortly after the onset of neutrophil recovery. ES is treated with a short course (usually

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<7 days) of IV corticosteroids and supportive care including close management of fluid balance and use of diuretics. In contrast, CLS is managed with the aforementioned supportive care but not with corticosteroids. Early recognition and institution of careful fluid management is key to achieving a positive outcome from ES and CLS.

Introduction

Hematopoietic stem cell transplantation (HSCT), particularly allogeneic, is associated with fluctuations in the normal steady state of circulating cytokines [1]. Post-HSCT neutrophil recovery, although the desired indicator of hematopoietic engraftment, is frequently marred by the sequelae of interaction between pro- and anti-inflammatory cytokines and tissue responsiveness. This often results in a well-recognized clinical syndrome known as engraftment syndrome (ES) [2, 3]. The spectrum of ES can range from a mild self-limiting episode with erythroderma (>/=25% BSA) and high fevers not attributable to an infectious etiology to a more severe course manifested by significant, acute weight gain, pulmonary edema, liver and renal dysfunction, and/or encephalopathy which can potentially be fatal [4–8]. The reported incidence of ES has varied from 6 to 82% in allogeneic HSCT and approximately 7 to 59% in autologous HSCT. This has been due, in part, to heterogeneity in clinical criteria and nomenclature and due to the overlapping clinical scenarios of ES and acute graft-versus-host disease (GvHD) in the allogeneic setting [9, 10].

Pathophysiology of Engraftment Syndrome

Limited data are available about the effector cells and cytokine profile in ES. Although the pathophysiology of ES is not well characterized, it has been established that an inflammatory cytokine cascade, aptly termed "cytokine storm," with mediators such as interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) and their interactions with the recovering cellular milieu during hematopoietic reconstitution play a major role in the development of the clinical syndrome [1, 11]. It is controversial as to whether ES and GvHD are clinical variants of a single pathology versus simply arising from two separate pathological processes [12]. Given the similarities in clinical presentation and similar management strategies (such as corticosteroids for ES and GVHD), ES has sometimes also been described as "hyperacute" GVHD [9, 13].

Cytokine Profile of Engraftment Syndrome

In healthy subjects, pro- and anti-inflammatory cytokines exist in a dynamic equilibrium or homeostasis [14]. The plasma concentration of each cytokine, the timing of cytokine release, cytokine receptor density, local homoeostatic milieu, and the tissue responsiveness to each cytokine determines the clinical implications of the "cytokine storm" [2, 15]. The profile of cytokine secretion in patients undergoing HSCT has been found to differ markedly from that in healthy controls [16]. The post-HSCT immune recovery and the production of cytokines from the engrafting immunologically active cells of the donor are both thought to contribute to the cytokine storm [1, 16]. Other factors suspected to contribute to the cytokine fluctuations are types of conditioning regimens, infections, and acute GvHD. Animal studies have shown that tissue damage and cellular toxicity induced by conditioning regimens (radiation and/or chemotherapy) lead to elevations in levels of circulating inflammatory cytokines (particularly IL-1 and TNF-α) and subsequently establish a cycle of lymphocyte activation that potentiates the development of GvHD [1, 17]. Mucosal injury secondary to myeloablative conditioning regimens leads to release of endotoxins or lipopolysaccharides from normal

bowel flora triggering an inflammatory cascade and cytokine fluctuations, particularly stimulating TNF-α, IL-1, and IL-12 which have been associated with mediation of GvHD [17]. Other studies of post-HSCT humoral cytokine profiling have identified elevated levels of IL-7, IL-15, and granulocyte colony-stimulating factor (G-CSF) [18–21]. In a prospective study of 56 consecutive pediatric allogeneic HSCT patients, Khandelwal et al. demonstrated a pattern of an initial surge in plasma levels of pro-inflammatory cytokines (IL-12 and IL-1β) in patients with engraftment syndrome in Week 4 post-HSCT followed by elevation in levels of anti-inflammatory cytokines (IL-4, IL-6, and IL-13) a week or so later [2]. In another retrospective study of 988 consecutive HSCT recipients, Chang et al. reported significant elevation in plasma levels of Interleukin 2 receptor alpha 2 (IL2Rα) and tumor necrosis factor receptor 1 (TNFR1) in patients with ES [9]. DiCarlo et al. mapped the cytokine profile of 51 children undergoing HSCT (allogeneic and autologous) by assaying 51 cytokines and chemokines weekly for 100 days post-HSCT [16]. They reported that global cytokine secretion in HSCT recipients was significantly lower than in concurrent healthy control subjects in Week 2 and 3 post-HSCT coincident with WBC nadir [16].

These observations suggest that, although ES is an inflammatory phenomenon that explains its clinical constellation, a unique signature pattern of cytokine abnormalities still needs to be determined.

Capillary Leak Syndrome

Capillary leak syndrome (CLS) is characterized by weight gain, generalized edema, hypotension, prerenal failure, ascites, intravascular depletion, and serous (pericardial and/or pleural) effusions. Of note, not all of these features need to be present. In general, allogeneic HSCTs (vs. autologous) and those with conditioning regimens resulting in greater toxicity and myeloablation are more associated with the development of CLS. CLS is much more common than ES. The manifestations of vascular leak occur during neutrophil recovery in both auto- and allo-HSCT settings and coincide with the inflammatory cytokine cascade as described above. In addition, soluble thrombomodulin and plasminogen activator type 1 (markers of endothelial injury) have been noted to be elevated in conjunction with the elevation in cytokine levels and are considered potential triggers of vascular leak [22].

Management of Engraftment Syndrome and Capillary Leak Syndrome

The frontline therapy for ES is the initiation of corticosteroids which dampens the cytokine levels and, in turn, leads to improvement in the clinical manifestations. In contrast, CLS alone is typically managed with supportive care, i.e., close management of fluid balance and judicious use of diuretics; corticosteroids are rarely used in the setting of CLS alone. Despite the evident role of pro-inflammatory cytokines in ES, targeted anticytokine therapy (such as etanercept or tocilizumab) has not proven to be of any benefit [23]. Recombinant human soluble thrombomodulin has been shown to ameliorate symptoms of vascular leak in ES [24] but is not widely used. Supportive care (e.g., antipyretics, oxygen, topical therapy of rash, diuretics) alone has been shown to lead to resolution of symptoms in approximately 20% of the patients [23]. There is also no literature supporting interventions to prevent ES. Whether the cytokine storm of ES contributes to initiation of GVHD or whether graft versus host alloreactivity contributes to severity of ES is yet to be determined [23]. Future studies are needed to further characterize the cytokine profile and delineate management strategies for this potentially fatal syndrome.

Key Points

- Engraftment syndrome (ES) is a well-recognized constellation of clinical symptoms and signs that can be seen during the post-HSCT neutrophil recovery phase. The

spectrum of ES can range from a mild self-limiting course with erythroderma and high fevers to a more severe episode manifested by significant, acute weight gain, pulmonary edema, liver and renal dysfunction, and/or encephalopathy which can potentially be fatal.

- Capillary leak syndrome (CLS) is seen more often with transplant protocols utilizing myeloablation and conditioning regimens with greater toxicity and is characterized by weight gain, generalized edema, hypotension, prerenal failure, ascites, intravascular depletion, and serous (pericardial and/or pleural) effusions.
- Both ES and CLS occur as a result of interplay between variations in the levels of pro- and anti-inflammatory cytokines in the homeostatic milieu and the recipient tissue responsiveness during immune recovery post-HSCT.
- Recognition of the manifestations of ES and CLS is the key to management. Supportive care has been the mainstay of treatment in addition to immune suppression.

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Nutrition in the Peri-HSCT Period 13

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Abstract

Nutrition is an integral part of the supportive care of hematopoietic stem cell transplantation (HSCT) patients, and good nutritional status has been shown to improve outcomes in HSCT. However, providing proper nutritional support over the course of HSCT can prove to be challenging because many side effects associated with HSCT transplant negatively impact nutrition. This chapter details the factors that can lead to inadequate intake, poor GI absorption, and metabolic alterations. It also describes how the use of anthropometric assessments and energy need estimations play a key role in monitoring nutritional status and determining when further interventions are needed. Specific nutritional considerations in SOS/VOD and GvHD are discussed herein. Enteral and parenteral nutrition are compared: While enteral nutrition offers several advantages over total parenteral nutrition, there are situations in which full enteral nutrition is not feasible and parenteral nutrition is required to fulfill the patient's caloric demands.

Importance of Nutrition in the HSCT Setting

Hematopoietic stem cell transplantation (HSCT) places a major strain on the body, and providing proper nutrition plays a key role in allowing patients to endure and recover from the process. During conditioning, patients who are adequately nourished have improved tolerance to conditioning chemotherapy and radiation therapy [1]. After HSCT, better nourished patients have a shorter time to engraftment and have more robust immune reconstitution, reducing the risk of infectious complications [2]. Nutrition support

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also aids in recovery from post-HSCT complications such as graft-versus-host disease (GvHD) or hepatic sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD) [1]. Adequate nutrition improves quality of life and, especially when the patient and family feel involved in the nutrition plan, provides psychosocial boosts [3]. Given these benefits, it is not surprising that being malnourished has been shown to be a negative prognostic factor for outcome after HSCT [1]. Unfortunately, many patients undergoing HSCT have diagnoses that directly impair their nutritional status or have received therapies that affect their nutrition and therefore enter the HSCT process relatively malnourished. A thoughtful regimen that combines multiple modes of nutrition support can prevent further worsening or even result in improvement of nutritional status.

HSCT-Associated Side Effects and Their Impact on Nutritional Status

Regardless of a patient's pre-HSCT nutritional status, nutrition support is frequently required during the peri-HSCT period, especially in the allogeneic HSCT setting. HSCT has a profound impact on the patient's ability to take in, absorb, and process nutrients. Table 13.1 summarizes HSCT-associated side effects that can impact nutrition.

Table 13.1 Treatment side effects impacting nutrition

Impaired intake	Poor GI absorption	Metabolic alterations
• Pain • Altered taste • Fluid restriction • Nausea/vomiting	• Diarrhea • GI inflammation	• Negative nitrogen balance • Impaired carbohydrate metabolism • Abnormal lipid metabolism

Impaired Intake

Most pediatric patients experience a significant reduction in appetite and oral intake during HSCT. One factor negatively affecting intake is pain from mucositis, the inflammation and ulceration of the oral and gastrointestinal mucosae due to chemotherapy and/or radiation therapy. Chemotherapy can also alter patients' taste receptors, affecting their desire for food. Abdominal pain from GvHD or SOS/VOD can prevent patients from eating and drinking. Additionally, the presence of SOS/VOD can increase fluid and sodium retention and therefore necessitate fluid limits.

Two of the biggest factors impairing intake are nausea and vomiting. There are multiple potential etiologies of nausea and vomiting in the HSCT setting. Sloughing of mucosal tissue during mucositis can induce an emetogenic response. Total body irradiation (TBI), which is used in several HSCT conditioning regimens, can cause significant nausea and vomiting, as can many of the chemotherapy agents used in conditioning especially since they are generally given in high doses [4]. Opioids play a key role in pain management, but their side effects can include nausea. Finally, patients are susceptible to GI infections during HSCT, which can be a significant source of nausea and vomiting.

There are multiple pharmacologic options for the management of nausea and vomiting in the HSCT setting. First-line therapy generally involves a serotonin (5HT-3) antagonist such as ondansetron or the longer-acting granisetron. Second-line options include benzodiazepines (generally lorazepam), promethazine in patients ≥2 years of age (typically given with diphenhydramine to prevent extrapyramidal effect), dexamethasone, and aprepitant (generally used with 5HT-3 antagonist ± dexamethasone) [4]. Additional options for older children include scopolamine patch and dronabinol; dronabinol also has the benefit of being an appetite stimulant.

Poor GI Absorption

Damage to the GI tract from factors including mucositis, GI infections, and GVHD often causes diarrhea and inflammation. This damage alters transport of nutrients across the luminal surface and can lead to decreased nutrient absorption and nutrient loss from the gut [5].

Metabolic Alterations

HSCT dramatically affects energy metabolism. Negative nitrogen balance is common in HSCT patients due to protein losses through the gut as well as catabolic effects on skeletal muscle during conditioning and subsequently if the HSCT course is complicated by sepsis and/or GvHD. Carbohydrate metabolism may also be affected due to impaired glucose tolerance from GvHD therapy (steroids and/or cyclosporine) or from complications from sepsis. Additionally, HSCT may negatively affect pancreatic β-cell function. Finally, complications such as VOD/SOS or liver GvHD can lead to abnormalities in lipid metabolism.

Nutrition Assessment in HSCT Patients

Introduction

Given the known benefits of proper nourishment and the myriad of nutritional complications HSCT recipients face, ongoing assessment of nutritional status of children and adolescents undergoing HSCT throughout the entire HSCT course is necessary. This necessity extends from the pre- to the post-HSCT transition to outpatient care. Pediatric malnutrition (undernutrition) related to chronic illness was ill defined prior to 2014 [6]. Formalized criteria were developed in 2014 to identify indicators of malnutrition and set thresholds to categorize malnutrition as either mild, moderate, or severe (see Table 13.2). These criteria can be used universally in pediatric critical illness, including HSCT, as well as for research purposes.

Historically, various biochemical markers and methods of assessing anthropometric measurements were used to indicate a state of malnutrition. Hepatic proteins (e.g., albumin, prealbumin, and transferrin) have limited value in defining malnutrition in critical illness [7]. Synthesis and serum levels of these proteins are significantly influenced by factors other than nutrition. Factors that impact serum levels of albumin, prealbumin, and transferrin are summarized in Table 13.3. However, hepatic protein levels are indictors of morbidity and mortality and can guide the clinician to identify patients at greatest risk of developing malnutrition.

Table 13.2 Criteria for diagnosing pediatric malnutrition (undernutrition) in children ages 1 month to 18 years (Adapted from Becker et al. 2014) [6]

Indicator	Mild malnutrition	Moderate malnutrition	Severe malnutrition
Weight for length Z score	−1.0 to −1.99[a]	−2.0 to 2.99	−3 z score or worse
BMI for age Z score	−1.0 to −1.99[a]	−2.0 to 2.99	−3 z score or worse
Length/height Z score	No data available	No data available	−3 z score or worse
Mid-upper arm circumference (≤60 months of age)	−1.0 to −1.99	−2.0 to 2.99	−3 z score or worse
Weight gain velocity (≤2 years of age)	51–75% of the World Health Organization (WHO) norm[b]	26–50% of the WHO norm[b]	≤25% of the WHO norm[b]
Weight loss (2–20 years of age)	≥5% usual body weight	≥7.5% usual body weight	≥10% usual body weight
Deceleration in weight for length/height or BMI for age	Decline of 1 Z score	Decline of 2 Z scores	Decline of 3 Z scores
Inadequate nutrient intake	51–75% of estimated energy/protein need	26–50% of estimated energy/protein need	≤25% of estimated energy/protein need

[a]Needs additional criteria to support diagnosis (i.e., weight loss, inadequate intake)

[b]Weight gain increments at the median of the WHO growth velocity standards for the time span between the two data points

Table 13.3 Factors that impact serum levels of albumin, prealbumin, and transferrin (Reprinted with permission from Fuhrman et al. 2004) [7]

Increase	Decrease
• Intravascular volume deficit • Exogenous albumin infusion • Renal failure • Iron deficiency[a]	• Intravascular volume excess • Recumbent posture • Extraneous loss of albumin • Liver disease • Pregnancy • Hypothyroid • Alcohol abuse • Uremia • Corticosteroids • Malignancy • Trauma (including surgery) • Inflammation

[a]*Transferrin only*

Anthropometric Assessment

Height/length, weight, weight-for-length, and body mass index (BMI) are used to assess nutritional status [6]. Daily weight and monthly height/length measurements are used during the peri-HSCT period to assess adequacy of nutrition provision as well as fluid status when intake versus output is considered. Appropriate growth charts should be maintained to assess changes and trends [8]. Weekly measurements of mid-upper arm circumference (MUAC) can be useful in nutrition assessment as it is less affected by fluid status than weight [9].

Energy and Protein Needs in HSCT

Introduction

Providing adequate nutrition can be challenging prior to, during, and after HSCT. Accurate determination of calorie needs can minimize the risk of under and over feeding. Resting energy expenditure (REE) in children undergoing allogeneic HSCT was found to nadir at 80% by week three post-HSCT, with a gradual increase by week four [10, 11]. Interestingly, while REE declines, energy requirements are higher than expected, and some reports suggest that needs are as high as 130–180% of basal energy expenditure [12, 13]. This wide range makes ongoing estimation of energy needs and adjustment to nutrition care plans in HSCT patients essential.

Protein Needs

Protein needs are elevated due to decreased protein synthesis, increased protein breakdown, and nitrogen loss [14]. Age-based estimations of protein needs in children undergoing HSCT are appropriate and can be as high as two times the recommended dietary allowance [13, 15].

Hydration

Fluid provision must be closely monitored. Fluid, including nutrition support, medications, transfusions, transducers and carriers, and oral intake, contributes to a patient's total intake. Clinical status, weight, intake and output, and laboratory values (e.g., sodium, blood urea nitrogen, creatinine, and albumin) are considerations in determining a HSCT patient's appropriate volume requirements. SOS/VOD is a complication that is usually accompanied by weight gain with sodium and fluid retention. This condition may warrant restriction to 80–90% maintenance fluid and minimal sodium intake to prevent third spacing [12]. Some have reported decreased energy needs during this condition.

Nutrition Support Modalities

Multimodal nutrition delivery is almost universal in pediatric patients undergoing HSCT. Enteral nutrition, including oral and tube feedings, is the preferred route of administration to maintain gut integrity, reduce bacterial translocation, and provide other protective factors [16]. Most institutions provide a reduced bacteria or neutropenic diet during hospitalization for HSCT. Restriction of foods with potential to introduce harmful bacteria such as *Listeria monocytogenes*, *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Cryptosporidium* is the goal [13]. Foods often restricted with this diet include fresh vegetables

and fruits, non-pasteurized dairy products, deli meats and cheese, and prepared salads such as potato salad and pasta salad. Undercooked meats, poultry and eggs, and salad dressing made with raw egg and aged cheeses are often restricted. A meta-analysis of neutropenic diet versus regular diet found no advantage to providing the neutropenic diet to cancer patients [17]. The American Society of Parenteral and Enteral Nutrition guidelines for adult patients undergoing HSCT currently state that it is prudent to restrict such foods during neutropenia [18]; therefore, most institutions continue to adhere to neutropenic diets.

In children undergoing HSCT, enteral nutrition is often contraindicated due to medical, gastrointestinal, and psychosocial aspects [16]. Use of parenteral nutrition may be the method of choice of nutrition support in the hospitalized phase of HSCT. Parenteral nutrition is not without risks, including volume overload, thrombosis, and infection [19].

The choice of nutrition support is controversial and requires assessment on an ongoing basis. Oral intake, enteral nutrition, and parenteral nutrition as nutrition support modalities are a continuum that can be used in conjunction with one another. Oral intake, using a reduced bacteria diet, may be provided throughout the hospitalization. Gastrointestinal tubes placed prior to hospitalization for HSCT may be used to provide nutrition throughout the HSCT course. Nasogastric tubes placed prior to the development of mucositis [5] or prior to stem cell infusion [20] have been found to be successful by some. Trophic enteral nutrition can provide protective benefits, and the advantages of enteral nutrition over parenteral nutrition are listed in Table 13.4, and Table 13.5 lists the potential barriers to enteral nutrition.

Enteral nutrition may be limited by gastrointestinal (GI) toxicities. Parenteral nutrition can be provided during the conditioning regimen phase of the peri-HSCT period but with great care due to electrolyte derangements that can arise as a side effect of the chemotherapy commonly used in conditioning regimens. Parenteral nutrition is often started immediately after HSC infusion and continued throughout the engraftment period.

Table 13.4 Advantages of enteral nutrition compared with parenteral nutrition (Used with Permission, Thompson & Duffy, 2008) [16]

• More efficient use of nutrients through first-pass metabolism in the liver
• Able to provide nutrients that are not available through parenteral nutrition, that is, fiber
• Helps maintain normal gut function
• Maintain gall bladder function
• Promotes IgA, which helps prevent bacterial adherence and translocation
• Helps maintain gut-associated immune function, GALT, and MALT
• Costs less than parenteral nutrition
• Decreased risk of infection

Table 13.5 Potential barriers to enteral nutrition in HSCT (Used with Permission, Thompson & Duffy, 2008) [16]

• Thrombocytopenia
• GI complications (nausea/vomiting/diarrhea)
• Mucositis/esophagitis
• Lack of enteral access
• Patient acceptance

Changes in taste and a lack of appetite can negatively impact oral intake that often necessitate use of enteral nutrition after engraftment and continued on after discharge. The continually evolving clinical status of the patient dictates which mode of nutrition support to employ. Individualized nutrition care plans need to be revised throughout hospitalization and beyond. Including a registered dietitian on a multidisciplinary team for HSCT patients is ideal.

Nutrition Considerations in the Context of Gut and Liver GvHD

Gut and liver GvHD has profound effects on body composition and nutrient utilization. Unfortunately, studies on nutrition support of children with GvHD are scant, and recommendations for monitoring nutritional status post-allogeneic HSCT are drawn from adults [21]. Recommendations include monitoring of

body weight and mid-upper arm circumference (MUAC), assessment of fluid retention, promotion of oral or enteral nutrition, and serum vitamin and mineral level surveillance (e.g., magnesium, 25 OH Vitamin D, B 12, and zinc) with appropriate supplementation as needed. Additionally, many recommend the avoidance of prebiotics and probiotics because of the lack of evidence for efficacy and the probable risk of infectious complications. In cases of severe gastrointestinal failure defined as >500 mL diarrhea per day, parenteral nutrition is the recommended mode of nutritional support. Consideration of pancreatic enzyme therapy in exocrine pancreatic insufficiency is an option. A multidisciplinary approach to the treatment of these children is prudent.

Nutritional Status Monitoring Following HSCT

The goal during the peri-HSCT period (from the start of conditioning through early engraftment) is to maintain an adequate nutrient status throughout this time course. A large proportion of pediatric patients have impaired nutritional status up to 12 months post-HSCT and may require oral nutritional supplements, including enteral and/or parenteral nutrition at the time of discharge. Ongoing monitoring and assessment by the physician and a registered dietitian are necessary as an outpatient.

Key Points

- Proper nutritional support improves outcomes in HSCT.
- HSCT side effects negatively impact nutrition in multiple ways, relating to inadequate intake, poor GI absorption, and metabolic alterations.
- A variety of anthropometric assessments are used to assess nutritional status.
- Hepatic proteins such as albumin, prealbumin, and transferrin have limited value in defining malnutrition since they can be significantly influenced by factors other than nutrition.
- Ongoing estimation of energy needs and adjustments based on monitoring of nutritional status is essential as resting energy expenditure, and energy requirements can vary greatly from patient to patient and be affected by what phase of HSCT the patient is in.
- Enteral nutrition offers several advantages compared to parenteral nutrition, but there are aspects of HSCT that can be barriers to full enteral nutrition in which case use of parenteral nutrition may be temporarily necessary as a supplementary or sole source of nutrition.
- Because a pediatric HSCT patient's nutritional status is such an important yet potentially complex, component of the HSCT process, many institutions have a registered dietician who specializes in this patient population as a part of the multidisciplinary pediatric HSCT team.

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14 Mucositis and Pain in the Peri-HSCT Period

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Abstract

Diagnosis and proper management of pain is a critical part of the necessary supportive care for patients undergoing HSCT but can be a significant clinical challenge for the healthcare team due to the multifactorial nature of pain in the HSCT setting. This chapter details the many causes of pain in HSCT. HSCT-associated pain is often due to the conditioning regimen (which consists of high-dose chemotherapy and/or total body irradiation) and graft-versus-host disease (GvHD) prophylaxis regimens as well as potential post-HSCT complications. Given that mucositis is one of the largest sources of pain and infection in patients undergoing HSCT, special attention is paid to mucositis-specific strategies. These strategies include oral care and topical therapies such as glutamine and palifermin which are under investigation. Systemic non-opioid pain medications are also discussed. The remainder of this chapter focuses on opioid options and discusses the principles of conversion between opioids, use of caregiver-/patient-controlled analgesia (which can facilitate better pain control and result in a lower total amount of opioids needed), management of opioid side effects, and guidelines for weaning opioids to avoid withdrawal symptoms.

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Pain in HSCT

One of the inherent difficulties of managing pain during HSCT is that there are multiple potential causes of pain in the HSCT setting. Potential causes include mucositis, acute graft-versus-host disease (GvHD), chronic GvHD, diarrhea, sinusoidal obstructive syndrome (SOS) (also known as veno-occlusive disease, VOD), neurotoxicity, and

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Table 14.1 Common etiologies of pain during and after HSCT

Mucositis
GvHD
Infection
Sinusoidal obstructive syndrome (veno-occlusive disease)
Neurotoxicity
Hemorrhagic cystitis

hemorrhagic cystitis, and it can be hard to accurately determine the etiology. Table 14.1 lists the most common causes of pain. Because different types of pain (e.g., postsurgical, musculoskeletal, neuropathic) may be managed differently, providers must pay close attention to the etiology of the pain to help optimize pain control management.

Mucositis

Mucositis is a common cause of pain, secondary to effects of chemotherapy and radiation therapy used as part of the HSCT conditioning or preparative regimens. Figure 14.1 shows a child with chemotherapy-induced mucositis [1]. The incidence of mucositis in pediatric patients undergoing HSCT is over 90% and is up to 99% in myeloablative regimens. The prevalence of mucositis seems to be greater in children than adults, which may be due to more rapid cell division in children. In particular, conditioning regimens containing etoposide, melphalan, and total body irradiation and immunosuppressive regimens containing methotrexate are associated with severe mucositis [2]. The inflammation of oral and gastrointestinal mucosae often leads to oral erythema, painful ulcerations, bleeding, and eventual sloughing of the mucosa. The natural course of mucositis is that it will persist until neutrophil recovery has occurred, and often it will get worse just around the time of engraftment. It is manifested by increased perioral edema, mucosal swelling, and pain due to increased cytokine release. In some cases, the edema and bleeding along with the thickening of oral secretions become so significant that the patient needs to be intubated in order to protect his/her airway while awaiting engraftment.

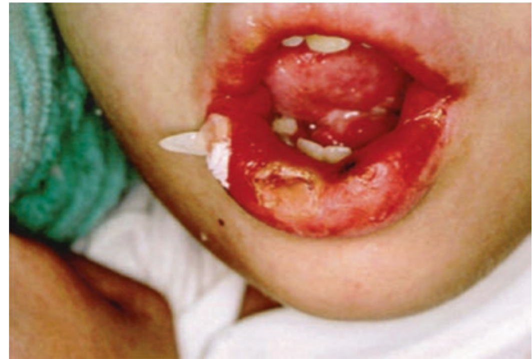

Fig. 14.1 Chemotherapy-induced mucositis. This photograph depicts a child with chemotherapy-induced mucositis. It represents the classic findings of friable, inflamed, beefy red mucosa with perioral edema and blood and saliva pooling in the oral cavity. (Used from Figliolia et al. 2008) [1]

Graft-Versus-Host Disease (GvHD)

GvHD occurs when immune cells (predominantly T cells) transplanted from an allogeneic donor (graft) recognize the transplant recipient (host) as “foreign,” which initiates an immune reaction that causes damage to the recipient’s body (see Chaps. 18 and 19).

Acute GvHD: In the acute setting, the damage is most frequently to the recipient’s skin, gut, and/or liver. Acute GvHD which can occur in 40–60% of allogeneic HSCT patients can be a significant source of pain [3, 4].

Chronic GvHD: Chronic GvHD can occur after previous acute GvHD, with ongoing acute GvHD, or arise de novo. It can affect multiple organs with skin the most commonly involved organ. Chronic GvHD of the skin tends to be sclerotic/fibrotic in nature. Chronic GvHD can cause pain of the skin, eyes, oral cavity, gut, nerves, joints, and muscles.

Diarrhea

In a study of 142 children (100 post-allogeneic HSCT and 42 post-autologous HSCT), diarrhea and associated abdominal pain derived from multiple etiologies (mucositis, gut GvHD, and/or gastrointestinal infection) were reported in two-thirds of patients [2].

Sinusoidal Obstruction Syndrome (SOS)

Sinusoidal obstruction syndrome (SOS), also known as veno-occlusive disease (VOD), ascites, and hepatomegaly often invoke stretch of the liver capsule, which can produce severe pain and respiratory compromise (see Chap. 15 for a detailed discussion of SOS).

Neurotoxicity

Potential etiologies of nerve pain include herpes simplex virus (HSV) and varicella zoster virus (VZV) and side effects of tacrolimus and cyclosporine used in allogeneic HSCT patients for the prevention and treatment of GvHD (see Chaps. 18, 19, and 24).

Hemorrhagic Cystitis

Painful hemorrhagic cystitis with associated dysuria can be due to chemotherapy or infection (most often BK viruria) and occurs in up to 25% of pediatric transplant patients (see Chaps. 16 and 22 for further discussion of hemorrhagic cystitis) [2].

Supportive Therapies for Mucositis

In addition to causing pain and decreased oral intake, the mucosal disruption weakens one of the body's remaining defenses against microbial invasion and fosters an environment in which bacteria thrive [5]. Mucositis increases the risk of febrile neutropenia, bacteremia, and need for prolonged total parenteral nutrition (TPN) [6]. For these reasons, mucositis-specific strategies and therapies to prevent mucositis or at least limit its impact are extremely important.

Oral Care Protocol: Good oral hygiene helps to minimize the extent and severity of mucositis. However, in many centers, patients are instructed to not brush or floss during the peri-HSCT period due to concern for bleeding or bacterial translocation complications. Instead, chlorhexidine rinses are often used to decontaminate the oral flora and are prescribed up to four times a day.

Topical Therapies: Upon development of oral lesions, mouthwashes containing a combination of antacid, antihistamine, and local anesthetic are often used. Throat sprays and lozenges that combine analgesic and anesthetic agents are also options. However, these agents must be used judiciously because mucosal breakdown can lead to increased absorption of local anesthetics into the body.

Glutamine: Glutamine is a primary fuel for enterocytes and gut-associated lymphoid tissue. During times of stress, glutamine stores can decrease by over 50%. This depletion is thought to contribute to mucositis [5, 7]. Therefore, glutamine can be administered in enteral and parenteral forms. Results from studies of glutamine are inconclusive: Some studies have demonstrated a decrease in duration of mucositis, opiate requirement, and TPN requirement, but other studies have shown no benefit [5, 8, 9].

Palifermin: Palifermin is a recombinant keratinocyte growth factor that stimulates the differentiation and proliferation of GI tract epithelial cells. Multiple studies have clearly indicated a significant reduction in incidence and duration of severe mucositis primarily in adult patients undergoing allogeneic HSCT. Data on use of palifermin is limited in children less than 15 years of age [10]. Though the cost of this medication is large, cost-benefit ratio may potentially be offset as the financial impact and resource utilization in treating mucositis and its complications are large. Elting et al. showed that the cost per patient receiving palifermin was not significantly different from the cost per patient receiving placebo even after the cost of palifermin was factored in with the per diem hospital costs and the avoidance of adverse outcomes of HSCT [6]. Studies in children are ongoing, with a recent phase 1 study showing that palifermin was tolerated at 90 μg/kg/day with no dose-limiting toxicity [11].

Management of Pain

Non-opioid Agents

While many HSCT patients require opioid medications at some point, non-opioids are often used prior to or in conjunction with opioids in an attempt to at least limit the patient's opioid use. Of note, increasing non-opioid doses above their recommended limits produces a "ceiling" effect whereupon there is little increase in analgesia but pronounced increase in side effects [12]. Thus, the use of non-opioid agents must be closely monitored.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs): Most NSAIDs are not used during the peri-HSCT period given their inhibition of platelet aggregation augments the bleeding risk already posed by the thrombocytopenia and mucosal inflammation that occurs during HSCT. However, in some cases, choline magnesium trisalicylate may be utilized as it does not inhibit platelet aggregation [13]. However, this medication is not available on the market in the United States as of 2017 due to manufacturer discontinuation.

Acetaminophen: Acetaminophen does not affect platelets, and it has a synergistic effect with opioids [14]. However, it should be used with caution in patients with hepatic impairment. Scheduled acetaminophen should be avoided because it may blunt a patient's ability to mount a fever.

Opioid Agents

For many of the etiologies of pain discussed above, systemic analgesia with opioids is frequently required with the use of parenteral opioids, specifically morphine, hydromorphone, or fentanyl. These agents require a starting dose that likely needs to be further titrated to provide adequate analgesia due to patient-specific factors and tolerance. When switching patients between opioids due to tolerance or side effect management, the equianalgesic dose must be calculated. Due to incomplete cross-tolerance that can result in a higher unanticipated potency, the calculated dose for the new opioid may require a reduction by 30–50% to limit side effects. Table 14.2 summarizes the equianalgesic dosing of opioids [15].

Patient-controlled analgesia (PCA) helps facilitate optimal pain control with a lower total amount of opioid usage [16]. Table 14.3 shows dosing guidelines for the initial dosing of opioids for PCA [17–19]. This modality also allows for patient-specific pain management *modality* in children that are developmentally appropriate and cognitively able to understand the use of PCA. Children less than 6 years of age likely require a surrogate, such as parent or nurse, to help effectively manage pain by operating the PCA module. In this scenario in which parents are trained to facilitate analgesia, they are identified as caregiver controlled analgesia (CCA) [2]. Patients on PCA or CCA require continual pain assessments that are integral in the titration of the basal and bolus doses, especially as patients develop tolerance to the dose of their opioid. Furthermore, opioid side effects may require the implementation of adjunct medications. Table 14.4 lists the most common side effects of opioids and how to manage them.

As the patient's pain subsides and sources of pain resolve, weaning of opioids is critical to prevent withdrawal symptoms. The basal and bolus infusion doses are typically weaned by 10–20% depending on the duration of PCA use

Table 14.2 Equianalgesic dosing of opioids (not accounting for incomplete cross-tolerance) [15]

Drug	Enteral dose	Parenteral dose
Oxycodone	20 mg	N/A
Morphine	30 mg	10 mg
Hydromorphone	7.5 mg	1.5 mg
Fentanyl	N/A	0.1 mg

Table 14.3 Dosing guidelines for initial PCA dosing [17–19]

Drug	Basal dose IV (mcg/kg/h)	Bolus dose IV (mcg/kg/dose)
Morphine	0–20	15–20
Hydromorphone	0–4	3–4
Fentanyl	0–1	0.25

Table 14.4 Medications used in managing opioid side effects

Potential side effect of opioid	Medication
• Nausea/vomiting	• Ondansetron
	• Lorazepam
	• Promethazine in combination with diphenhydramine
	• Scopolamine patch
• Pruritus	• Diphenhydramine
	• Nalbuphine
• Myoclonus	• Diazepam
• Constipation	• Sennosides
	• Docusate
	• Miralax
	• Polyethylene glycol
	• Lactulose
	• Mineral oil
• Urinary retention	• Nalbuphine

and patient response to initial weans. Enteral conversion of opioids can be completed when patients can tolerate this route; options for enteral opioids include oxycodone, morphine, hydromorphone, or methadone. The equianalgesic dose, duration of action, and incomplete cross-tolerance should be accounted for when calculating the enteral opioid dose. In some scenarios, weaning may be continued when patients are discharged with close follow-up to titrate patients safely off opioids when the pain source is no longer present.

Key Points

- There are many causes of pain in the HSCT setting, largely relating to side effects of the HSCT conditioning and GVHD prophylaxis regimens as well as to potential post-HSCT complications.
- Mucositis is one of the largest sources of pain and infection in patients undergoing HSCT. Mucositis-specific strategies include oral care and topical symptom-directed therapies; glutamine and palifermin are being studied.
- A variety of systemic non-opioid and opioid pain medications have been used in patients with HSCT-related pain.
- When switching patients between opioids due to tolerance or side effect management, the equianalgesic dose must be calculated and the final dose should take into account incomplete cross-tolerance.
- In patients requiring significant amounts of opioids, patient-controlled analgesia can help to facilitate optimal pain control with a lower total amount of opioid usage.
- As pain subsides, careful weaning of opioids is critical to prevent withdrawal symptoms.

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Hepatotoxicity in the Peri-HSCT Period

15

Valerie I. Brown

Abstract

Hepatotoxicity in the peri-hematopoietic stem cell transplantation (HSCT) period is relatively common with an incidence of approximately 80%. Because the liver plays such a central role in the metabolism of drugs; elimination of toxins; detoxification of metabolic waste products; synthesis of key proteins, such as albumin and clotting factors; bile production; the storage of vitamins A, D, E, and K; as well as the synthesis, metabolism, and/or storage of carbohydrates, proteins, and fats, liver damage during any phase of HSCT can cause a significant degree of morbidity and mortality. The most common causes of HSCT-related hepatotoxicity that occur during the peri-HSCT period include the chemotherapy and/or irradiation used in conditioning regimens, medications commonly used during the peri-HSCT period (e.g., immunosuppressants and antibiotics), total parental nutrition (TPN), iron overload, and infection/sepsis. The most common hepatic complications during the peri-HSCT period are transaminitis, sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD), acute graft versus host disease (GvHD) of the liver, and infections involving the liver. Because infections and acute GvHD are addressed in detail in other chapters (see Chaps. 17 and 18, respectively), this chapter will focus primarily on transaminitis and SOS.

Introduction

Hepatotoxicity that occurs during the peri-hematopoietic stem cell transplantation (HSCT) period which spans from the beginning of conditioning through the early post-HSCT period (< day 100 post-HSCT) is common with an incidence of approximately 80%. These hepatic

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complications are most often related to the toxic effects of the conditioning regimen (both chemotherapy and radiation), medications, infections, total parental nutrition (TPN), and graft versus host disease (GvHD). The most common hepatic complications seen during the peri-HSCT period include transaminitis, sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD), infections of the liver, and acute GvHD of the liver. Often, HSCT-induced liver injury is ultimately reversible, such as transaminitis, but certain etiologies, such as SOS, account for a significant percentage of transplant-related morbidity and mortality (TRM). Thus, close monitoring of liver function, frequent viral surveillance, and prompt intervention are the keys to the successful management of hepatic complications in the early HSCT setting. Because infections (see Chap. 17) and acute GvHD (see Chap. 18) are addressed in depth elsewhere, this chapter will concentrate on the topics of transaminitis and SOS.

Transaminitis

Introduction

Transaminitis is defined as the elevation of liver transaminases as a result of liver injury. Liver enzymes include alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AP). ALT is best at detecting hepatitis (i.e., inflammation of the liver) and is more specific than AST which is found in the heart and muscles in addition to the liver. AP is increased when bile duct obstruction is present. Direct bilirubin is also a measure of liver function because direct bilirubin arises from conjugation in the liver. Transaminitis may be asymptomatic and self-limited, but it can also represent significant liver damage causing severe hepatic dysfunction.

Risk Factors and Etiologies

Many drugs used during HSCT (e.g., antibiotics, antifungal agents, and immunosuppressants) as well as total parenteral nutrition (TPN) are frequently the cause of asymptomatic elevation of hepatic enzymes (see Chap. 28 for a comprehensive list of drugs and agents used in HSCT). In addition to drugs and TPN, iron overload and infections/sepsis can cause transaminitis. Acute GvHD can also be the etiology of transaminitis during the peri-HSCT period and is discussed elsewhere (see Chap. 18).

Diagnostic Studies

The diagnostic workup for transaminitis that arises during the peri-HSCT period includes ascertaining the characteristics of any signs or symptoms from the history or physical examination, such jaundice or abdominal pain, in the context of the timing of the HSCT process. Laboratory evaluation includes liver function tests (LFTs), serum chemistries, PT/INR, albumin, CBC, iron and total iron-binding capacity (TIBC), ferritin, and quantitative PCR for adenovirus, CMV, and EBV. Imaging studies, including abdominal ultrasound, CT, or MRI, may be warranted.

Management and Outcomes

The overarching management of transaminitis in the context of the peri-HSCT period is to address the potential etiologies of the transaminitis. For example, if a drug(s) is suspected, then every effort should be made to discontinue the causative agents and replace it with another that is potentially less hepatotoxic, such as the substitution of voriconazole with caspofungin for fungal prophylaxis. In some cases, it is not feasible to discontinue the hepatotoxic agent, such as TPN in a HSCT patient with severe mucositis. In cases such as these, strategies to limit the exposure are attempted. Another strategy is to monitor drug levels, as supratherapeutic levels of some drugs correlate with an increased degree of worsening hepatic function [1].

Iron Overload

Introduction: Secondary iron overload is caused by multiple transfusions of packed red blood cells (pRBCs). One unit of pRBCs contains

approximately 200 mg heme iron. This iron is deposited in organs, particularly the heart and liver, and can cause life-threatening damage. Many patients, such as those with AML, thalassemia, severe aplastic anemia, and sickle cell disease, who come to HSCT have received a significant number of pRBC transfusions. Iron overload can manifest itself early or late in the HSCT course. The sequelae from iron overload have been associated with other complications seen with HSCT. These include acute and chronic GvHD, infections (particularly fungal), SOS, late cardiotoxicity, and transaminitis [2].

Clinical manifestations: Most often, patients with iron overload are asymptomatic early on despite damage to tissues that has already begun [3]. Iron overload may lead to hair loss, skin color changes, heart arrhythmias, joint pain, osteoporosis, hepatosplenomegaly, abdominal pain, cirrhosis, amenorrhea, hypothyroidism, hypogonadism, adrenal insufficiency, hypopituitarism, hyperglycemia, and depression.

Diagnostic studies: Ferritin, iron, and TIBC are frequently checked pre-HSCT. While ferritin is an acute-phase reactant and is elevated with any inflammatory process, serum ferritin reflects the amount of stored iron in the body, and a serum ferritin >1000 μg/L is indicative of significant iron overload [4]. An MRI is often used to estimate iron levels in the liver. Superconducting quantum interference device (SQUID) uses a low-power magnetic field with detectors that measure the interference of iron within the field and can be a noninvasive method to measure liver iron overload, but it is still considered investigational [5]. The gold standard to diagnose liver iron overload is a liver biopsy because it provides a direct assessment of iron deposition in the hepatic tissue and gives an accurate measurement of liver iron concentration (LIC). However, a liver biopsy, even when using a transjugular approach, is invasive and carries a significant risk for bleeding and infection. Thus, it is rarely performed in the HSCT setting.

Management and outcomes: Traditionally, the management of iron overload is scheduled, intermittent phlebotomy. However, this approach is contraindicated in patients with anemia which is often the case for the potential HSCT patients. Thus, alternative treatments should be instigated such as the use of iron-chelating agents. Deferoxamine is an iron chelator that is administered as a continuous subcutaneous infusion, and so compliance can be an issue even though it has proven efficacy [6, 7]. Oral iron chelators, such as deferasirox, are efficacious in this setting and are commonly used [8].

Sinusoidal Obstruction Syndrome (SOS)

Introduction

Sinusoidal obstruction syndrome (SOS) (previously known as veno-occlusive disease, VOD) has been recognized for decades as a potentially life-threatening complication of HSCT. SOS ranges from a mild, reversible process to a life-threatening, severe syndrome with multi-organ failure. Historically, severe SOS had been associated with a high mortality rate (before the advent of defibrotide). SOS arises from damage to the epithelial cells of the sinusoids and to hepatocytes. It can also occur after hepatotoxic chemotherapies in a non-HSCT setting, such as 6-thioguanine. This syndrome was first described in 1920 following the administration of Senecio tea that contained pyrrolizidine alkaloid. The name veno-occlusive disease was first attributed in the late 1950s to describe a hepatotoxic syndrome of painful hepatomegaly, fluid retention, hyperbilirubinemia, and ascites that occurred after the ingestion of Senecio tea [9]. It was first described in a myeloablative SCT setting in 1979 by Jacobs et al. [10]. Further work in the 1980s showed that partial to complete obstruction of the terminal hepatic venules with central necrosis and damage to the sinusoids was present in the liver upon autopsy [11–13]. Work in the 1990s noted the presence of hypercoagulability as well as fibrin and factor VIII deposition in the terminal hepatic venules in patients with SOS [14, 15]. These observations were the basis of the initial prophylaxis and treatment strategies using anticoagulation for SOS, but they were generally unsuccessful ([16–21] and reviewed in Dignan et al. [22]). Subsequent work suggested that the initial damage occurs in the sinusoids with occlusion of venules as a consequence, rather than a

cause, of this syndrome and, thus, was more appropriately termed sinusoidal obstruction syndrome instead of VOD [11, 23].

Pathophysiology

In normal hepatic microanatomy, the major functional unit is the portal triad which is composed of branches of the (1) portal vein, (2) hepatic artery, and (3) bile duct. Each triad is further divided into a hexagonal "lobule" centered around a terminal hepatic vein, i.e., the central vein. Each lobule is segmented into acini. Each acinus is subdivided into zones that represent different metabolic regions that move blood from the afferent blood flow toward the central vein. Zone 3 of the acinus is the area that is typically found to be damaged in SOS (see Fig. 15.1) [11]. Blood from the portal vein and hepatic artery mixes in the sinusoids which are narrow cavities lined by fragile sinusoidal endothelial cells and then empties through the central vein. Sinusoids are fenestrated, and so plasma normally flows freely into the space of Disse.

The severity of SOS is proportional to the extent of injury to the liver. Conditioning regimen agents damage sinusoidal endothelial cells and hepatocytes, triggering multiple pathways that result in inflammation and destruction of the cytoskeletal structure of these cells with the cells occupying Zone 3 of the hepatic acinus being the most vulnerable to damage [11]. Activation of these pathways leads to sinusoidal narrowing. Along with deposition of fibrinogen and factor VIII as well as erythrocyte congestion, the damage to the sinusoidal endothelial cells leads to the loss of endothelial cell fenestrations. Red blood cells can then enter into the space of Disse and cause sinusoidal cell damage and denudation of the sinusoidal lining. The sloughed sinusoidal endothelial cells, erythrocytes, hepatocytes, and other debris embolize downstream, causing venous congestion. This congestion leads to venous occlusion that often progresses to disruption of the normal liver architecture with centrilobular necrosis. This

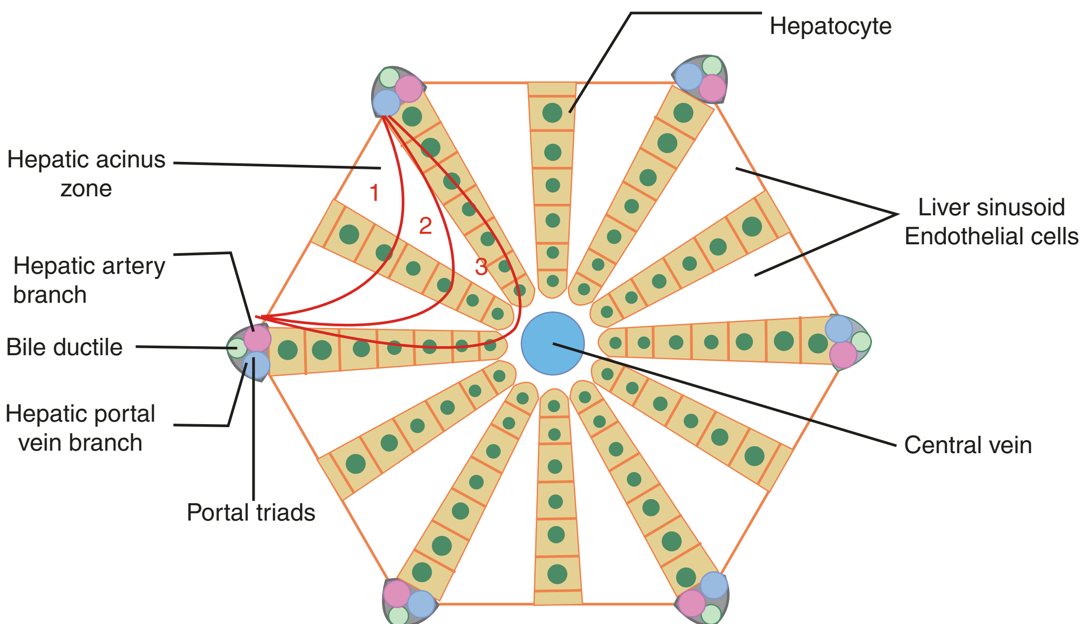

Fig. 15.1 Schematic representation of the hepatic acinus. In sinusoidal obstruction syndrome, obstruction of the hepatic sinusoids occurs in zone 3 of the hepatic acinus (Reprinted by permission from Macmillan Publishers Ltd.: Mohty M, Malard F, Abecassis M, Aerts E, Alaskar AS, Aljurf M, et al. Sinusoidal obstruction syndrome/veno-occlusive disease: current situation and perspectives—a position statement from the European Society for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplantation. 50(6):781–9, 2015 [84])

state can then progress to occlusion of the terminal venules and liver fibrosis, ultimately leading to liver failure and often death.

Damage to the endothelial cells and hepatocytes is mediated or directly caused by cytokines, such as TNF-α, IL-1β, and IL-6. Increased expression of adhesion molecules ICAM-1 and VCAM-1 on endothelial cell surfaces also occurs in response to the damage. Activation of leukocytes that release additional inflammatory cytokines causes digestion of the extracellular matrix resulting in portal vein hypertension and hepatic venous outflow obstruction that leads to hepatic enlargement with capsular distension. The end result is clinically manifested as SOS. In the late stages of this process, portal venous flow reversal occurs, and hepatorenal syndrome frequently develops in severe SOS that will progress ultimately to multi-organ failure and death if early recognition and intervention do not occur.

In addition to the mechanical damage to the liver caused by this inflammatory state, SOS is also characterized by having a procoagulant state. SOS is associated with low levels of antithrombin 3, factor VII, and protein C as well as a high level of plasminogen activator inhibitor (PAI-)1, which is an important inhibitor of fibrinolysis [24–27]. Tumor growth factor (TGF)-beta which is a cytokine directly involved in fibrinogenesis and released from platelets is associated with an increased risk of developing SOS [28]. The illustration in Fig. 15.2 represents the steps that lead to the clinical manifestation(s) of SOS.

Incidence

The incidence of SOS in HSCT patients is reportedly variable, ranging from 11% to 60%, and has been reported to be up to 55% of patients who received high-dose alkylator therapy as part of their conditioning regimen (reviewed in [29]). This wide range is most likely due to different diagnostic criteria that have been used over time. The mean incidence of SOS in pediatric patients has been reported to be 25% as compared to 13.7% in adult patients [30]. One hypothesis addressing this difference between pediatric and adult patients is that the underlying indications for HSCT in pediatric patients are associated with a substantial increased risk of SOS, including neuroblastoma, familial hemophagocytic lymphohistiocytosis (HLH), and osteopetrosis. Regardless, the incidence of SOS depends upon the proportion of patients who are at high risk for developing SOS pre-HSCT and their exposure to high-dose chemotherapy with or without irradiation used in their conditioning regimen. Overall, the reported incidence of SOS is declining due to the increased use of reduced-intensity and non-myeloablative conditioning regimens, pharmacologic dosing of busulfan, generally "healthier" patients pre-HSCT, and a decreased delay in recognition and initiation of treatment of SOS.

Risk Factors

Risk factors for SOS can be divided into two groups: pre-HSCT and HSCT related. In general, one cannot alter the pretransplant risk factors, only recognize them, whereas one can minimize the risk by selecting a conditioning regimen that minimizes risk for SOS without compromising the "benefits" from the conditioning regimen. Table 15.1 summarizes the risk factors for developing SOS. One of the major risk factors for developing SOS is pre-existing liver disease and/or dysfunction, including a history of viral hepatitis, iron overload, liver fibrosis and/or cirrhosis, prior liver transplantation, transaminitis, hyperbilirubinemia, abdominal irradiation, or hepatotoxic chemotherapy. Drugs, such as gemtuzumab ozogamicin and inotuzumab ozogamicin, are associated with a very high risk of developing SOS if given proximal to the start of the HSCT conditioning regimen [31, 32]. The age of the patient at the time of HSCT contributes significantly to a patient's risk for developing SOS. Younger children are at higher risk because of immature liver function. Cesaro et al. [33] showed that there was an increased incidence of SOS in patients <6.7 years versus ≥6.7 years (17% vs. 4%). Another study of 138 children undergoing a total of 144 HSCTs found the risk to be 30.4% in patients less than 2 years old, 12.5% in patients aged 2–8 years old, and

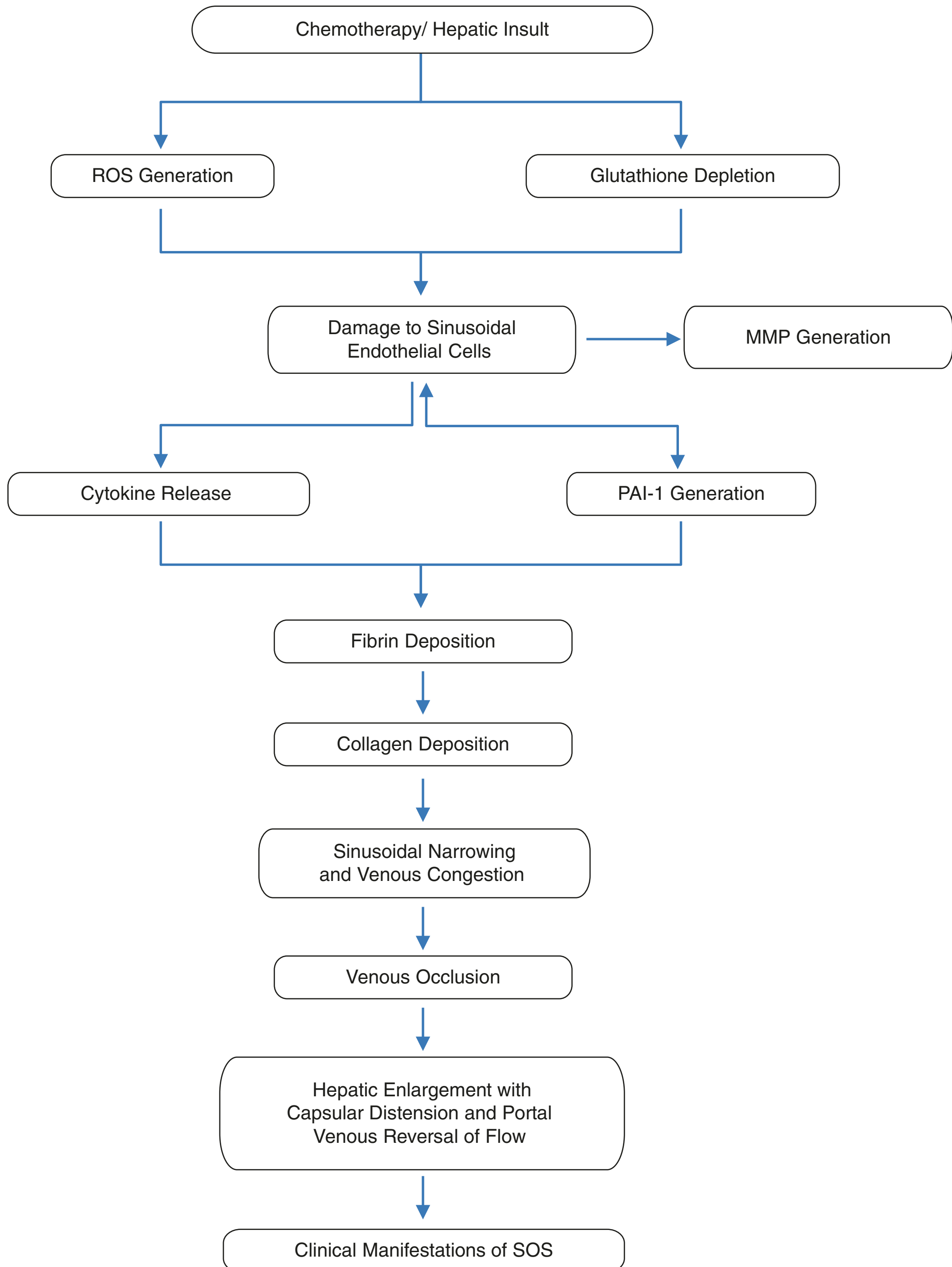

Fig. 15.2 Illustration of SOS pathogenesis. The pathogenesis is a complex process that involves glutathione depletion, inflammation, procoagulant environment, and fibronogenesis that leads to sinusoidal narrowing and venous congestion in the liver that can evolve into venous occlusion resulting in hepatic enlargement with capsular distension and venous reversal of flow which is manifested as severe sinusoidal obstruction syndrome (SOS). ROS, Reactive oxygen species; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor-1

Table 15.1 Compilation of risk factors of sinusoidal obstruction syndrome (SOS)

Pre-HSCT-related risk factors	HSCT-related risk factors
• Liver dysfunction – Elevated transaminases – Elevated bilirubin	• Allogeneic > autologous – Parental > sibling – Haploidentical > sibling
• Pre-existing liver disease – Viral hepatitis – CMV positivity	• Grade of HLA compatibility – Major mismatch > minor mismatch > match
• Age (particularly <1–2 years old)	• Myeloablative conditioning > reduced-intensity conditioning (RIC) – Busulfan + cyclophosphamide > cyclophosphamide + busulfan > busulfan alone > fludarabine > TBI (>12 Gy) + cyclophosphamide or BCNU + cyclophosphamide + etoposide
• Prior exposure to gemtuzumab ozogamicin or inotuzumab ozogamicin (particularly in proximal to HSCT)	• GvHD prophylaxis – CSA + MTX > CSA > sirolimus + MTX + tacrolimus
• Liver transplantation	• T-cell replete grafts
• Ferritin levels >1000 ng/mL	• Acute GVHD of the gut and/or liver
• Need for parenteral nutrition prior to HSCT	• Peripheral blood stem cells > bone marrow
• Prior abdominal radiation	–
• Poor performance status (<90%, Karnofsky score)	–
• Prior HSCT	–
• Underlying disease – Advanced leukemia – Immunodeficiency – Myelodysplasia – Inborn errors of metabolism – Thalassemia – JMML	–
• Prior life-threatening infections, e.g., sepsis	–
• Genetic factors – Glutathione s-transferase (GSTM1)-null genotype	–

7.7% in patients older than 8 years old [34]. Patients with advanced malignancies, HLH, adrenoleukodystrophy, osteopetrosis, and thalassemia are at a higher risk of developing SOS. The patient's health status at the time of HSCT plays a role. The presence of comorbidities and/or Karnofsky performance score <90% is associated with a higher risk of developing SOS [35]. A history of serious infections, such as CMV or sepsis, is associated with a higher risk of developing SOS as well. Patients who received total parenteral nutrition (TPN) prior to HSCT have a higher risk for developing SOS [36]. A genetic variant of the glutathione s-transferase gene has been associated with an increased risk for SOS [30, 37]. Because glutathione is essential in the metabolism of a number of chemotherapeutic agents that are utilized in HSCT, polymorphisms of the glutathione s-transferase gene can affect tissue glutathione levels that can lead to increased endothelial cell toxicity.

HSCT-related factors include the type of HSCT (syngeneic/autologous is at less risk than allogeneic HSCT), the degree of HLA compatibility (major mismatch > minor mismatch > match), the HSC source (peripheral blood < bone marrow or T-cell depleted HSC products < T-cell replete HSC products), the type of GvHD prophylaxis used, concurrent hepatotoxic drugs, and the conditioning regimen used.

Patients receiving myeloablative conditioning regimens are at higher risk for developing SOS than those receiving reduced-intensity conditioning. Both cyclophosphamide and busulfan alone are associated with SOS [38]. Cesaro et al. [33] reported that busulfan itself was associated with an increased risk of SOS (25% vs 13%), as confirmed in other studies. This risk is dependent upon the exposure/dose of busulfan because pharmacokinetic-targeted dosing of IV busulfan is associated with a decreased risk of developing SOS as compared to oral dose adjusted [39]. The agents used in conditioning regimens and the sequence in which they are given impact the HSCT patient's risk for developing SOS. The risk is higher in patients who were conditioned with the combination of busulfan and cyclophosphamide rather than busulfan alone, and the administration of busulfan followed by cyclophosphamide is associated with a higher risk of SOS than cyclophosphamide followed by busulfan [40]. Busulfan depletes glutathione stores which leave sinusoidal cells of the liver more vulnerable to damage caused by cyclophosphamide metabolites. While total body irradiation (TBI) alone (12–15 Gy) does not usually cause SOS, TBI can be synergistic with high-dose alkylator therapy to induce SOS ([24, 35] and reviewed in [41]). Combinations of GvHD drugs, particularly sirolimus with methotrexate and/or tacrolimus, are associated with a higher incidence of SOS as compared to these drugs given individually [42]. In addition, having undergone a previous HSCT places the patient at higher risk for developing SOS during the peri-HSCT period [24].

Clinical Features

SOS is characterized by fluid retention, rapid weight gain, right upper quadrant abdominal pain, hepatomegaly, ascites, and hyperbilirubinemia with no other identifiable cause for the liver dysfunction that typically develops over a relatively short period of time. Most commonly, SOS occurs from the first day of conditioning to day +30 post-HSCT, with a mean of day +12. However, SOS can occur after day +30 post-HSCT and can be more difficult to recognize resulting in a delay in the initiation of treatment.

Very often, severe SOS is associated with multi-organ failure (respiratory, renal, and/or cardiac). In addition, patients can develop confusion, encephalopathy, renal insufficiency, renal failure, pleural effusion or infiltration, and hypoxia. These manifestations are discussed in depth elsewhere in this chapter (see "Complications" section below).

Bearman [43] related the risk of developing severe SOS to the degree and rate of weight gain and increase in bilirubin to the day post-HSCT. While the specifics of this relationship may not be as relevant today with better supportive care and prophylaxis, the rule of thumb remains that the likelihood of severe SOS is increased if the patient experiences a quick weight gain and jaundice early on in the HSCT course. However, it needs to be noted that approximately one-fourth of pediatric patients with moderate to severe SOS will not have hyperbilirubinemia [44].

Differential Diagnosis

In general, the classic triad of SOS is rapid weight gain with or without ascites, an elevated bilirubin with or without jaundice, and right upper quadrant pain and/or hepatomegaly. All of these findings are nonspecific and can be seen during the HSCT course without developing SOS. Because the diagnosis of SOS is one of exclusion, one must consider the differential diagnosis of this constellation of signs and symptoms. In addition to SOS, capillary leak syndrome, drug toxicity, sepsis/infection, renal failure, congestive heart failure, acute GvHD, TPN-induced hepatic injury, cholestasis, hemolysis, as well as others listed in Table 15.2 should be considered when a patient develops one or more of these findings in the peri-HSCT period.

SOS Surveillance, Diagnostic Studies, and Diagnostic Criteria

SOS surveillance: The diagnosis of SOS is one of exclusion and is based upon a patient meeting a set of established clinic criteria. Because early

Table 15.2 Summary of the differential diagnosis of SOS based on signs and symptoms

Rapid weight gain	Jaundice/elevated bilirubin	RUQ pain/hepatomegaly/ascites
• Congestive heart failure	• Biliary obstruction or infection	• Congestive heart failure
• Renal failure	• Acute hepatic GVHD	• Fungal infections
• Sepsis	• Cholestasis	• EBV lymphoproliferative disease
• Capillary leak syndrome	• Cyclosporine	• Pancreatitis
–	• Hemolysis	• Portal vein thrombosis
–	• Autoimmune hepatitis	–
–	• Drug- or TPN-induced injury/toxicity	–
–	• Fungal abscess or viral hepatitis	–

recognition with initiation of treatment without delay is associated with a better outcome in patients with SOS, close monitoring is essential in patients at high risk for developing SOS. Patients need to be evaluated on an ongoing basis because the diagnosis of SOS is iterative rather than one point in time. Furthermore, patients may quickly escalate from mild or moderate to severe SOS very quickly, typically with a more than doubling of bilirubin over only 24 h [43]. Patients require daily assessment for signs of fluid overload, ascites, and hepatomegaly. Strict intake/output (I/O) needs to be recorded. Serum bilirubin, ALT, and AP should be monitored daily. One needs to pay attention to platelet consumption and transfusion needs, as rapid platelet consumption is often seen with SOS [45, 46]. This increased need for platelet transfusions is thought to be due to progressive endothelial damage.

Diagnostic imaging: Ultrasound with Doppler may be helpful in excluding other disorders in patients suspected to have SOS. However, this imaging modality is neither very sensitive nor specific for the diagnosis of SOS but may contribute valuable information when used in conjunction with clinical and laboratory findings. Findings of ascites, reversal of flow in the portal vein, hepatic artery resistance index (0.75), and abnormal portal vein waveform are suggestive of VOD [47]. Reported abnormalities seen with ultrasound include thickening of the gallbladder wall, reversal of portal vein blood flow, ascites, hepatomegaly, and splenomegaly; prospective studies have failed to show consistent results [48]. Other imaging measurements for predicting SOS or for diagnosis prior to the clinical manifestations of SOS include transient elastography (TE) measurement which is a noninvasive, indirect method to evaluate liver fibrosis by assessing liver stiffness. However, results using TE measurements to act as an indirect marker of SOS in adult HSCT patients have been mixed [49, 50]. The usefulness of liver stiffness and other measurements as ascertained by ultrasound and other imagining techniques are under investigation.

Liver biopsy: While a definitive diagnosis of SOS can be made via the use of invasive testing, such as percutaneous or transjugular liver biopsy, these procedures are rarely performed because of the very high risk of bleeding associated with these procedures in an already thrombocytopenic patient.

Biomarkers: While no reliable biomarker(s) of SOS has been identified thus far, it is an area of active investigation. Predictive biomarkers of endothelial injury, such as von Willebrand factor, thrombomodulin, E-selectin, and soluble intracellular adhesion molecule-1 (sICAM-1), have been investigated but are not currently incorporated into standard of care [51]. One study identified ST2, ANG2, L-Ficolin, hyaluronic acid (HA), and vascular cell adhesion molecule-1 (VCAM1) together to be a biomarker panel that can be used to make the diagnosis of SOS early on in its course [52]. Using a biomarker panel of L-Ficolin, HA, and VCAM1, Akil et al. [52] were also able to stratify patients at risk for SOS as early as the day of HSCT infusion. While these studies are promising, the use of a biomarker panel to identify patients at risk or to diagnose SOS early on in its course is still investigational.

Diagnostic criteria: Because the associated findings of SOS are nonspecific, criteria were devised and then modified to provide clinicians guidelines for making the diagnosis of SOS [12, 15, 53, 54]. Table 15.3 summarizes three sets of these criteria. In general, the Baltimore criteria are more indicative of severe SOS and are used more often in adult HSCT patients, but because of the stringent criteria, cases of SOS may be excluded when using the Baltimore criteria. In comparison, the Seattle criteria are considered to be more liberal or inclusive. Therefore, use of these criteria may result in the misidentification of patients having SOS and thus be overtreated. At this time, the modified Seattle criteria are used routinely in the pediatric HSCT patient population, as this set of criteria is the most relevant to this HSCT patient population [15].

A patient does not necessarily need to meet all of the criteria in order to be deemed appropriate to begin treatment for SOS. For example, the absence of an elevated bilirubin does NOT mean that SOS is absent if other criteria are present. Over one-fourth of pediatric patients with SOS will not have an elevated bilirubin at the time of diagnosis of SOS [44]. Furthermore, waiting for all of the criteria to manifest may delay initiation of therapy, resulting in a poorer outcome [55]. Performing an ultrasound with Doppler very early on in the HSCT course may lead to more timely diagnosis and thus therapeutic intervention. In some cases, SOS occurs after day 30 post-HSCT and is often under-recognized but should be considered in patients who have received high-dose busulfan.

Grading of SOS

The severity of SOS is based upon clinical criteria, but these criteria can only be assigned retrospectively. Table 15.4 summarizes the guidelines for assigning grade of SOS (mild versus moderate versus severe) as well as treatment by grade. Mild

Table 15.3 Criteria used to diagnose SOS

Baltimore criteria	Seattle criteria	Modified Seattle criteria
– More indicative of severe SOS	– More Inclusive	– Most relevant
Bilirubin must be >2 mg/dL before D + 21 post-HSCT, and two of the following criteria must be present	At least two of the following criteria within the first month post-HSCT	Two of the following criteria before day +20 post-HSCT must be present
• Hepatomegaly	• Jaundice	• Bilirubin >2 mg/dL
• Ascites	• Hepatomegaly and RUQ pain	• Hepatomegaly or RUQ pain
• Weight gain (>5% above pre-HSCT weight)	• Ascites and/or unexplained weight gain	• Weight gain (>5% above pre-HSCT weight) or ascites

Table 15.4 General guidelines for assigning grade and treatment of SOS by grade

Grade	Bilirubin (mg/dL)	Transaminases	Weight above baseline	Renal function (creatinine above baseline)	Rate of change of these factors	General treatment strategy
Mild	<5	<3× normal	<2%	Normal	Slow (over 6–7 days)	Observation → supportive care alone
Moderate	5.1–8	3–8× normal	2–5%	<2× normal	Moderate (over 4–5 days)	Judicious use of diuretics[a] ± paracentesis[b]
Severe	>8	>8× normal	>5%	>2× normal	Rapid (over 2–3 days)	Defibrotide (6.25 mg/kg IV q6h for a minimum of 21 days)

[a]Need to preserve renal blood flow and avoid prerenal azotemia
[b]Indicated if respiratory compromise or severe pain present

SOS is characterized by a serum bilirubin level <5, transaminases less than three times above the upper limit of normal, and weight <2% above baseline with a normal creatinine. The rate of change in these laboratory values is slow, over 6 and 7 days. In comparison, SOS is classified as moderate if the bilirubin is between five and eight, transaminases are 3–8 times above the upper limit of the normal range, and weight gain is 2–5% above baseline but with an abnormal creatinine that is no more than two times above the patient's baseline. Generally, the rate of change of these factors can occur over 4–5 days. In severe SOS, the patient's bilirubin peaks above eight in most cases with transaminases more than eight times above the upper limit of the normal range. Furthermore, a greater than 5% increase above the patient's baseline weight and a creatinine that is more than two times above the patient's baseline creatinine are associated with SOS [47]. Patients can escalate from mild or moderate to severe SOS as quickly as less than 24 h. Early recognition without a delay in instituting interventions is paramount to improving outcome [56].

Prevention and Prophylaxis

One of the most important interventions that can be done to prevent SOS is to avoid or at least minimize modifiable risk factors, such as reducing iron overload, as well as recognizing potential interactions of drugs prior to HSCT. For example, because the combination of busulfan/cyclophosphamide is associated with a higher risk of SOS as compared to busulfan alone, cyclophosphamide can be substituted with fludarabine, or busulfan can be substituted with treosulfan in the conditioning regimen [57]. Alternatively, the intensity of the conditioning regimen can be reduced (i.e., use of reduced-intensity conditioning in place of myeloablative conditioning) as long as the efficacy of the conditioning regimen is not compromised [58].

Many agents to prevent SOS have been tried. These include ursodeoxycholic acid (UDCA, ursodiol), defibrotide, low- and high-dose heparin (unfractionated and LMW), antithrombin III, and N-acetylcysteine. Tay et al. [59] performed a meta-analysis of six studies of ursodiol for the prevention of SOS. They concluded that their meta-analysis favored the use of ursodiol for SOS prophylaxis [60–62]. Furthermore, the evidence was inconclusive for low-dose sodium heparin, LMW heparin, PGE1, N-acetylcysteine, and antithrombin III. Another meta-analysis which included 12 studies of LMW or unfractionated heparin as SOS prophylaxis reported that these agents did NOT significantly decrease the risk of SOS [18, 19, 63]. Glutamine supplementation has been tested as prophylaxis for SOS in one study, but the data was difficult to interpret because the sample size was small and a variety of conditioning regimens were used [64]. Corbacioglu et al. [65] reported the results of a prospective, randomized controlled trial testing defibrotide as SOS prophylaxis. The design of this landmark study had two arms: the prophylaxis arm with defibrotide (25 mg/kg/day divided q6h) starting on the first day of conditioning through day +30 post-HSCT ($n = 180$) versus the control arm of no prophylaxis ($n = 176$). If patients developed SOS on either arm, they were treated with defibrotide until resolution. Of the total of 356 study participants, the median age was 6.6 years; 65% underwent allogeneic HSCT whereas 31% were autologous. The results of this European Society for Blood and Marrow Transplantation (ESBMT) study showed that the incidence of SOS by day +30 post-HSCT was significantly lower in the prophylaxis arm as compared to the control arm (12% versus 20%, $p = 0.05$), as was the incidence of SOS with renal failure (1% versus 6%, respectively, $p = 0.02$). Furthermore, the incidence of GvHD was lower in the defibrotide arm (45% versus 63%, respectively, $p = 0.004$). The transplant-related mortality (TRM) at day +100 post-HSCT was the same in both arms at 9%, and there was no difference in the number of patients with significant adverse events between the two arms (87% versus 88%). Although the numbers were small, patients with a prior exposure to gemtuzumab had a lower incidence of SOS (18%) as compared to those in the control arm (40%). The results for patients with pre-existing liver disease were 15% versus 22%

(prophylaxis versus control); for patients who underwent a second myeloablative HSCT, the rates were 8% versus 17% (prophylaxis versus control) and for those with HLH as the indication for SCT was 0% versus 40% (prophylaxis versus control). Since 2013, in England, defibrotide is recommended for the prevention of SOS in pediatric HSCT patients with the following risk factors: pre-existing liver disease, second myeloablative HSCT, allogenic HSCT for leukemia in >CR2, busulfan-containing conditioning regimens, prior treatment with gemtuzumab ozogamicin or inotuzumab ozogamicin, and a diagnosis of HLH, ALD, or osteopetrosis ([33, 34, 66, 67] and reviewed in [22, 41]). The use of defibrotide for SOS prevention is under investigation in the USA.

Complications

In addition to the liver, severe SOS affects the renal and pulmonary systems predominantly; however, SOS-associated complications of the cardiac, hematologic, integumentary, and neurologic systems can occur with varying degrees.

Renal dysfunction/failure: SOS is the primary cause of renal failure during the first 21 days post-HSCT and resembles hepatorenal syndrome. SOS-associated renal failure is the result of vasoconstriction or poor glomerular perfusion. Albumin exerts a strong oncotic pull by drawing fluids from the extravascular space to the intravascular compartment. SOS causes hepatic injury that leads to synthetic dysfunction with resultant hypoalbuminemia. Thus, hypoalbuminemia leads to ascites and peripheral edema. In the presence of ascites and peripheral edema, the kidneys attempt to correct this hypovolemic state by vasoconstriction which affects renal blood flow and thus worsens renal function. In addition, portal hypertension and hepatic venous outflow obstruction contributes to the accumulation of ascites. The patient develops prerenal azotemia with the BUN-creatinine ratio greater than 30:1 (normal is <20:1), and prerenal azotemia can cause tubular damage of the kidneys. Hyponatremia is common, but one wants to keep serum sodium low (in the 127–133 range) in order to decrease retention of water. Initially, patients with severe SOS have normal or slightly decreased urine output with a high urine specific gravity (USG) > 1.015. If uremia continues, then weakness, fatigue, and abnormal hemostasis may occur. As hepatic function deteriorates, so does the renal function, and patients may require hemodialysis.

Respiratory compromise/failure: Generally, SOS-induced respiratory compromise is caused by fluid overload and capillary leak. It may also be caused by aspiration pneumonia secondary to decreased mentation. Furthermore, abdominal distension may exert pressure on the diaphragm resulting in hypoventilation which is clinically evident by tachypnea and rales on auscultation.

Cardiovascular compromise/failure: SOS-associated cardiac complications are linked to renal function. Cardiac output increases when the arterial pressure is low. Patients may need inotropic agents to maintain a normal blood pressure. Also, pericardial effusions with or without tamponade may develop.

Hematologic: SOS typically develops while the post-HSCT patient is still profoundly thrombocytopenic; this thrombocytopenic state in combination with the coagulopathy that results from an elevated BUN and bilirubin which can interfere with coagulation pathways as well as the decreased liver synthetic function can result in spontaneous bleeding from the nares, oral mucosa, and GI tract. Patients may require fresh frozen plasma (FFP) and fibrinogen infusions in addition to platelet and pRBC transfusions in order to ameliorate the clinical manifestations of the SOS-induced coagulopathy.

Skin: Significant edema and ascites cause the skin to become very thin and taut that typically results in a very shiny appearance. This state increases the risk of skin breakdown and injury in the setting of poor wound-healing capacity. Pruritus often occurs, but scratching needs to be discouraged because of the increased risk of skin breakdown in the context of poor wound-healing capacity contributing to an increased risk for infection. Loose cotton clothing (as compared to synthetic fabrics) should be used because cotton

provides better air exchange and less sweating, as sweating can potentiate pruritus.

Neurologic: Neurologic complications as a consequence of SOS include mental status changes and encephalopathy. Lethargy is common. The etiology of mental status changes is multifactorial. It can be the result of renal or hepatic failure. Serum ammonia levels increase as liver dysfunction worsens and can no longer adequately detoxify nitrogenous waste products into urea. Hepatic encephalopathy can range from mild to severe and correlate with serum ammonia levels. While not effective at treating the underlying cause of the liver dysfunction (i.e., SOS), lactulose is often used to decrease ammonia levels and thus improve the encephalopathy. Hepatic clearance of medications is impaired with SOS and can result in half-life prolongation of hepatically cleared medications. Thus, patients with SOS who are receiving concurrent IV opiate or anxiolytics need to be monitored very closely for neurologic changes in order to avoid exacerbation of any neurologic changes.

Management

If SOS is suspected, early intervention is crucial in order to achieve a favorable outcome in patients with SOS. These interventions include supportive care and treatment with defibrotide, the only FDA-approved agent for the treatment of SOS in the HSCT setting. In addition, one should get critical care colleagues involved early on in the course of SOS.

Supportive care: Historically, management of SOS focused primarily on supportive care to minimize fluid overload while maintaining adequate renal perfusion and are still important today. Specific supportive care measures include diuresis, correction of coagulopathies, adequate analgesia, as well as paracentesis to relieve discomfort from ascites and to improve ventilation. Hemodialysis, hemofiltration, and mechanical ventilation are performed in patients with multi-organ failure as indicated. Management of hepatorenal syndrome includes fluid restriction, avoidance of crystalloid and any sodium-containing fluids, as well as the judicious use of diuretics. Furosemide is often used, but, because it is a loop diuretic, it should be used carefully, as furosemide can deplete intravascular volume resulting in decreased renal perfusion. Spironolactone is often used in this situation because it increases sodium and water excretion while sparing potassium excretion. Low-dose dopamine (1–2 μg/kg/h) has been used to improve renal perfusion but is not always effective. Nephrotoxic medications should be avoided as much as possible. Patients are hypovolemic but the effectiveness of repletion with pRBC transfusions has not been supported.

Management of SOS-associated respiratory compromise and/or failure may improve with paracentesis. Pulse oximetry monitoring may not be sufficient to monitor respiratory status because it will not detect O_2 retention or abnormal pH. Thus, arterial blood gas (ABG) monitoring may be needed. Supplemental oxygen should be delivered as needed. However, despite all of these supportive care maneuvers, patients may require mechanical ventilation to provide adequate respiratory support, and this forebodes a very poor prognosis.

Defibrotide: Although it has been used and tested since the 1990s, defibrotide was approved by the FDA in 2016, and it is the only FDA-approved agent for the treatment of severe SOS. Defibrotide is a polydeoxyribonucleotide derived from the mammalian mucosa or lung, and it is an adenosine receptor agonist [68]. The mechanism of defibrotide is not fully understood. However, it seems to exert protective effects on endothelial cells by decreasing the influx of inflammatory mediators (by decreasing intercellular adhesion molecule (ICAM)-1 and heparanase) and by restoring the thrombotic-fibrinolytic balance (by the activation of the fibrinolytic system by increasing tPA, tissue factor pathway inhibitor (TFPI), and thrombomodulin and by decreasing PAI-1, TF, and vWF) [69–76]. It also increases levels of prostaglandin I2 and E2 as well as prostacyclin. Defibrotide can promote endothelial cell proliferation in vitro and thus may contribute to the recovery of injured liver tissue. Defibrotide appears to work locally versus systemically,

accounting for its relatively benign toxicity profile with a minimal bleeding risk.

The first report of defibrotide used for post-HSCT SOS was a retrospective study in the USA of 19 patients (six were under 20 years old) with severe SOS (as diagnosed by the Baltimore criteria) and multi-organ failure and/or <30% risk by the Bearman model, who received defibrotide on a compassionate use basis [77]. Resolution of SOS occurred in 42% of patients with a trend of young patients (<20 years old) achieving a complete response (67% versus 31%, <20 years old versus ≥20 years old). In a phase II, multicenter, randomized, dose-finding trial of 149 patients with severe SOS (of which 48 were pediatric patients), patients were randomized to receive 25 or 40 mg/kg/day. Eligibility criteria included patients who had a 30% or greater chance of developing severe SOS according to the Bearman prognostic model [43] or had multi-organ failure. Overall, 57% of the pediatric patients had a complete response (versus 40% in the adult patients). Survival at day +100 post-HSCT was 52% versus 37% in the pediatric versus adult populations [78]. In a phase III, historically controlled clinical trial of defibrotide that included 44 pediatric patients in the treatment arm and 14 in the historical control group with severe SOS (as determined by Baltimore criteria) within 21 days post-HSCT and had developed multi-organ failure (as defined as significant renal and/or lung dysfunction) within 28 days of HSCT, the day +100 overall response (OR) was achieved in 36% of the pediatric patients receiving defibrotide as compared to only 7% in the historical control group. Hematologic adverse events were similar between the two groups [79]. Data of 303 pediatric patients from an international compassionate use program of defibrotide for the treatment of severe SOS (from December 1998 to March 2009) showed a 65% overall survival at day +100 post-HSCT which was superior to the OS of adult patients [80]. The dosing and schedule for defibrotide are 6.25 mg/kg q6 h to be infused over 2 h for 3 weeks.

Other options: Transjugular intrahepatic portosystemic shunt (TIPS) has been shown to relieve ascites in some but is only helpful if the patient has increased portal hypertension but still has liver synthetic function intact. It appears that this option is not as efficacious late in the course of severe SOS [81]. Liver transplant can be beneficial and lifesaving in some severe cases, but it is contraindicated in patients with cancer because of the high risk of disease relapse.

Other agents tested but no efficacy: Many agents have been investigated for the treatment of SOS but without demonstrating benefit. Heparin and tPA have been tested extensively for the treatment of SOS, but anticoagulant and thrombolytic therapies have been shown to be largely ineffective with no survival benefit; therefore, they have been found to be associated with significant, sometimes life-threatening, bleeding complications [21, 82]. N-Acetylcysteine (NAC) which is a thiol antioxidant that is thought to aid in glutathione synthesis has shown no significant benefit [22]. Methylprednisolone may be considered for treatment of SOS, but this approach places the patient at greater risk for side effects, including infection. Thus, it is not recommended for treatment of SOS [22].

Outcomes

Generally, the risk of mortality increases as the severity of SOS increases. Typically, mild SOS is self-limiting and is not treated beyond supportive care. The mortality rate for mild SOS has been reported to be 9%. Before the availability of defibrotide, moderate SOS, which is treated with supportive care including judicious fluid management, use of diuretics, and use of pain medications, had a pre-defibrotide era mortality rate of 23%. Associated morbidities include peripheral edema as well as renal insufficiency, pulmonary infiltrates, ascites, and hypoxia. Prior to the availability of defibrotide, the mortality rate of severe SOS by day +100 post-HSCT was >90%. The cause of death was due to heart and/or kidney failure much more often than of liver failure. With the advent of defibrotide, the overall survival for all HSCT patients with SOS is 65% and is 36% for those

with multi-organ failure [79, 80]. Multi-organ failure, as manifested by the development of a supplemental oxygen requirement, renal dysfunction, and encephalopathy, remains the best predictor for a poor outcome for severe SOS. The rate of rise of bilirubin and rate of weight gain are also useful prognostic markers. In a single-institution retrospective study, Cheuk et al. [34] found that the development of SOS is associated with a higher mortality rate before day +100 post-HSCT. They went on to report an overall survival of 62% in post-HSCT pediatric patients with SOS. They identified predictors of mortality, including non-sibling allogeneic donor or autologous HSCT, moderate to severe acute GvHD of the liver and/or skin concurrent with SOS, admission to the intensive care unit (ICU), presence of pleural effusion, and weight gain >9% above baseline. They also confirmed that the higher the peak bilirubin, the higher the risk of mortality (specifically with a peak bilirubin above 17.5 mg/dL (300 μmol/L)) as well as the rate of rise of the bilirubin. A bilirubin >11.7 mg/dL (200 μmol/L) by day +21 post-HSCT was associated with high mortality. More importantly, they found that the more predictors that a patient with SOS had, the higher the mortality rate, and patients with SOS who had four or more of these predictors had 100% mortality [34]. Thus, the best treatment for SOS is prevention (or at least the minimization of modifiable risk factors prior to HSCT), but early recognition and initiation of therapy (i.e., before it progresses to severe SOS) makes a positive difference in outcomes. In one study, a delay of more than 2 days in the initiation of therapy with defibrotide after making the diagnosis resulted in a decreased complete response rate which was particularly striking in patients ≤16 years old (41% versus 27%) as well as in survival (60% versus 49%) [83]. With a better understanding of the underlying pathophysiology of SOS, improved supportive care, and early recognition and institution of treatment with the only FDA-approved agent for SOS, defibrotide, the outcomes for patients with SOS, particularly severe SOS, have significantly improved.

Summary

Rapid, accurate diagnosis, early risk assessment, and initiation of treatment are the key components in halting the progression of severe SOS to multi-organ failure as well as in improving outcomes and overall survival. Defibrotide is the only agent approved in the USA and in Europe for the treatment of post-HSCT severe SOS in adult and pediatric patients. Supportive care also plays a critical role in the management of patients with SOS regardless of its grade. The institution of prophylaxis is also important in patients at high risk for developing severe SOS. Defibrotide is recommended in British Guidelines for HSCT but is still considered investigational as prophylaxis in the USA. However, clinical trials in adult and pediatric HSCT patients are ongoing in the USA.

Key Points

- Hepatotoxicity during the peri-HSCT is quite common with transaminitis, SOS, acute GvHD of the liver and infections of the liver most common.
- Transaminitis is most commonly asymptomatic and self-limiting but can represent significant liver injury. Patients must be assessed for risk factors for SOS prior to HSCT in order to minimize the risks as much as possible during the peri-HSCT period.
- The diagnosis of SOS is a clinical one and should be based on established clinical criteria (i.e., Modified Seattle or Baltimore criteria).
- Ultrasound imaging may be helpful in excluding other disorders in which the diagnosis of SOS is not clear.
- Escalation from mild to moderate and from moderate to severe SOS can occur very quickly (under 24 h), and thus, vigilant monitoring of the patient is warranted.
- Mild SOS does not require any intervention other than supportive care.
- Moderate SOS should be treated with aggressive supportive care including judicious fluid

management to strike a balance between diuresis and the maintenance of renal perfusion.

- Severe SOS should be treated with defibrotide without delay.
- Severe SOS is a major contributor to transplant-related mortality (TRM). Thus, the prevention of SOS or instituting treatment early in its course is crucial in lowering the TRM.
- Defibrotide is an effective treatment for SOS in both the allogeneic and autologous HSCT settings and is well tolerated with manageable toxicities.

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Renal Toxicities in the Peri-HSCT Period

16

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Abstract

Renal dysfunction within the first 100 days post-hematopoietic stem cell transplantation (HSCT) is common. Many HSCT patients have some degree of renal dysfunction prior to HSCT, and the degree of this dysfunction impacts the extent of renal dysfunction that can occur during the pre-engraftment and early post-engraftment periods of the HSCT course. Renal dysfunction may be due to renal tubule damage, compromised renal blood flow, and obstruction or irritation of post-renal structures. Renal dysfunction impairs the ability of the kidneys to maintain acid-base and electrolyte balance as well as maintain fluid balance and eliminate waste. The renal complications post-HSCT are discussed in Chap. 22. The most common renal toxicities are related to medications frequently used during the peri-HSCT period. These include calcineurin inhibitors, antifungal agents, antibiotics (particularly aminoglycosides), and antiviral agents. In addition, components of conditioning regimens such as alkylators and irradiation to the bladder can cause significant renal toxicity. An infrequent but very serious renal complication is hemorrhagic cystitis which is the focus of this chapter. The remainder of HSCT-associated renal complications is addressed in Chap. 22.

Hemorrhagic Cystitis

Introduction

Hemorrhagic cystitis (HC) is defined as the presence of bladder inflammation with urinary symptoms including increased frequency, urgency, dysuria, suprapubic pain, and hematuria with or without clots potentially leading to urinary obstruction in patients following hematopoietic

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stem cell transplant (HSCT). The etiology of HC is multifactorial [1–7] and is associated with cyclophosphamide- or busulfan-based conditioning regimens, acute or chronic graft-versus-host disease (GVHD), pelvic radiation or total body radiation (TBI), and viral reactivation such as cytomegalovirus, BK polyomavirus virus, and adenovirus (type 7, 11, 34, 35) [1, 8, 9].

Hemorrhagic cystitis (HC) can cause severe morbidity and mortality in the post-HSCT period, leading to prolonged hospitalization [1, 6]. The incidence of HC ranges from 7% to 70% [1–7]. It can be classified within multiple subcategories, such as early versus late onset, infectious versus noninfectious, and chemical versus radiation induced [1, 4, 9, 10]. Treatment and management for HC varies depending on the etiology, timing of onset, and its severity.

Pathophysiology

HC results from an initial insult to the bladder's transitional epithelium and vasculature. This insult leads to nonspecific bladder wall inflammation, sloughing, vascular damage, and subsequent hemorrhage [7, 9].

Etiologies

Chemotherapy-induced HC: Cyclophosphamide is used commonly in pre-HSCT conditioning regimens and occasionally used as post-HSCT GVHD prophylaxis [11]. Chemotherapy-induced HC is typically an early-onset complication and can occur within 24–48 hours following chemotherapy [1, 11, 12]. The incidence of cyclophosphamide-induced HC, without preventative measures, is 25–60% [11, 13, 14]. Cyclophosphamide itself is not harmful to the bladder; however, its metabolite, acrolein, is toxic to the urothelial epithelium [9, 11, 12, 15]. Acrolein results in proteolysis and damages DNA strands. It also causes pyroptotic reactions leading to ulceration of the bladder, thereby exposing the underlying bladder mucosa [6, 7, 9]. This process then further exposes deeper smooth muscles in the bladder (detrusor) and its vasculature leading to further damage that can cause fibrosis, necrosis, and ultimately cell death [9].

Acrolein typically does not cause *renal* injury because acrolein does not stay in contact with the renal epithelium to cause damage. In contrast, the bladder is quite vulnerable to acrolein damage due to the prolonged duration of contact in the bladder prior to voiding. This results in bladder wall and urinary tract inflammation, hemorrhage, and thrombosis. This significant damage to the bladder can lead to severe urinary obstruction that can eventually cause secondary renal damage and ultimately to renal impairment [11, 12].

Radiation-induced HC: Radiation-induced HC is relatively rare, but it is associated with pelvic radiotherapy. It can occur as early as during treatment or as late as 6 months to 10 years after treatment [6, 16, 17]. The pathophysiology is microscopic progressive obliterative endarteritis due to fibrosis leading to hypoxia and ischemic cell death. This resultant ischemia then leads to ulceration and hemorrhage of the bladder [6, 17].

Viral-induced HC: Viral-induced HC is likely due to reactivation of the virus due to the HSCT recipient's immunocompromised state; this viral reactivation can result in nephritis, urethritis, and cystitis. The most common etiologies are BK polyomavirus, adenovirus (specifically types 7, 11, 34, 35), cytomegalovirus (CMV), JC virus, and herpesvirus [3, 4, 7, 18]. Development of viral-induced HC typically occurs more than 30 days after allogeneic HSCT [3].

Primary BK viral infections usually occur in childhood. This virus is a double-stranded DNA virus that remains latent in the gastro-urinary tract after primary infection [7]. Reactivation occurs only if the host is in an immunocompromised state as a result of prolonged corticosteroid use, HIV/AIDS, or post-HSCT [3, 6]. There are two mechanisms by which BK virus is thought to cause HC: The first is related to impaired immunity of the HSCT recipient. Impaired immunity results in proinflammatory cytokines to stimulate BK viral replication post-HSCT, which ultimately leads to a cytopathic effect on the urothe-

lial epithelium. In the second mechanism, replicating BK virus triggers the recovering immune system (specifically natural killer cells and neutrophils) to attack the bladder. This activation leads to bladder wall injury and HC [6, 19–21]. It is important to note that most post-HSCT recipients have BK viruria but never develop HC. However, there appears to be a direct relationship between progressive BK viruria leading to BK viremia and the incidence of HC [3, 4, 6, 7, 10, 18]. Further studies are needed to elucidate this observation, as it is clear that the presence of BK viruria is not sufficient to cause HC in the majority of post-HSCT patients.

Risk Factors

The incidence and prevalence of HC is multifactorial. However, certain risk factors place patients at a higher risk of developing HC. Table 16.1 lists the common risk factors associated with HC and further delineates factors associated with greater severity HC [2, 3, 6, 8, 10]. This is not a comprehensive list and other factors may apply clinically [2, 22–24].

Table 16.1 Common factors associated with greater severity of hemorrhagic cystitis

Risk factor associated with HC	Risk factor associated with greater severity
Age >5 years old	Older age
Male > female	Late onset of HC
Allogeneic HSCT > auto HSCT	Positive BK virus
Cyclophosphamide and busulfan	
Intensity of condition (MAC > RIC)	
GVHD (grades III–IV)	
Pelvic radiation	
Donor source (UCB > MSD/HLA mismatch > HLA matched)	
Viral reactivation (CMV, BK, adenovirus, JC, and herpesvirus)	

MAC myeloablative conditioning, *RIC* reduced intensity conditioning, *GVHD* graft-versus-host disease, *UCB* umbilical cord blood, *MSD* match sibling donor. *CMV* cytomegalovirus

Grading System

Table 16.2 delineates the grading system by which HC severity is measured. At times, Grade I HC can resolve spontaneously, while Grade IV can be very difficult to control [6, 8, 12, 25, 26]. There are no clear guidelines or gold standards regarding treatment. The best approach is prevention with supportive care that includes hyperhydration prior to the administration of cyclophosphamide, the use of mesna (2-mercaptoethane sulfonate Na (sodium)) as a bladder protectant, and forced dieresis with diuretics, for example, when urine output decreases [7–9, 11, 12, 25, 26].

The mechanism of action of mesna is as follows: The sulfhydryl group of mesna binds to the acrolein and detoxifies it before it can harm the bladder wall [1, 7, 11, 26]. Mesna should be administered concurrently with the cyclophosphamide infusion and at scheduled times following the infusion until cyclophosphamide is fully metabolized.

Treatment

Initial treatment for HC is conservative management, i.e., hyperhydration, lowering the threshold for transfusing platelets, forced diuresis, and pain management [1, 7]. These interventions increase voiding frequency, thereby decreasing urinary contact of acrolein with the bladder epithelium while promoting clot formation of any friable, bleeding bladder epithelium with increased number of platelets. With most cases of Grade I and II HC, conservative management has proven to be effective [1, 5, 7, 17]. If conser-

Table 16.2 Grading system by which HC severity is measured

	Hemorrhagic cystitis grade
Grade I	Microscopic hematuria
Grade II	Gross hematuria with small clots
Grade III	Gross hematuria with clots and urethral obstruction
Grade IV	Gross hematuria with clots, urethral obstruction leading to impaired renal function

vative management is ineffective, then one should consider three-way catheter continuous bladder irrigation (CBI) [1, 7, 27]. This intervention involves large catheter insertion into the bladder via the urethra and continuous irrigation of the bladder with either normal saline (NS) or sterile water. In pediatric patients, there is a potential risk for bladder distention and rupture. As a result, CBI should be performed with caution in pediatrics [7, 27, 28].

Cystoscopy is also a consideration for refractory HC. Although a relatively noninvasive procedure, it must be pursued with great caution given that patients with HC are usually very immunocompromised. Cystoscopy should be considered as the first-line surgical intervention when all other conservative measures have failed [1, 7, 29]. It is essential that vigorous attempts have been made to remove clots prior to cystoscopy with intravesical irrigation and fulguration for better success rates. Fulguration should be used with caution as it can result in contractions and shrinkage [7, 27, 29].

Other therapies have demonstrated some success in adults, although more studies are needed to prove their safety and efficacy in pediatric patients. One percent alum has minimal side effects and exerts its effect by causing protein precipitation over bleeding vessels, resulting in clot formation. It can produce elevated levels of aluminum and should be avoided in patient with renal impairment. Prostaglandin and Amicar are also used. However, prostaglandin can cause vasospasms, and Amicar can lead to thrombosis [7, 27].

Cidofovir is an antiviral used often in the post-HSCT period to treat viral infections such as adenovirus, CMV, and BK virus. Cidofovir can occasionally be used for refractory HC, especially when the etiology is viral. Cidofovir is associated with renal toxicity which can limit its use in practice. However, there is evidence that lower systemic doses or intravesical doses can be used to treat HC [7, 30–32]. There is also some evidence that estrogen therapy is another option for treatment of HC. However, there are limited data in pediatrics [7, 28].

Since the 1980s, hyperbaric oxygen therapy (HBO) has been associated with successful treatment of HC. Initially, it was used for radiation-induced HC, but now it is thought to have potential efficacy in all forms of refractory HC when combined with other supportive care measures [7, 17]. The mechanism of action of HBO is thought to induce healing of damaged tissues, decrease edema, and promote neovascularization. HBO has shown promise in adult trials, but there are limited data in pediatrics [17].

Finally, major surgical intervention can be considered but only in the most refractory cases of HC. These interventions include the use of fibrin glue that is inserted into the bladder via the suprapubic tract with the guidance of urethral cystoscope [5, 7]. Unfortunately, further studies are needed to validate its safety and efficacy in pediatric HSCT recipients. The most invasive procedure is supravesical urinary diversion (SUD) and cystectomy. SUD is thought to be effective by reducing the harmful effects of urokinases. They compose a group of proteolytic enzymes found in the urine, which is thought to contribute to HC due to its fibrinolytic properties. When urokinases are eliminated from the bladder via SUD, the bladder mucosa can then heal and form proper clots [1, 7, 29].

In summary, renal dysfunction during the peri-HSCT period is common, with the majority of patients having some degree of dysfunction. Hemorrhagic cystitis is a rare complication that can cause severe morbidity and, at times, mortality. This complication may cause physical pain and discomfort and can extend the patient's hospital stay leading to emotional and mental distress. Hemorrhagic cystitis can have many etiologies and can occur at different times during the posttransplant period. The timing is related to the underlying cause of HC. It is important to make every attempt to diagnose the cause of HC, as treating the underlying etiology in addition to supportive therapy will result in the best outcomes.

Key Points

- Hemorrhagic cystitis (HC) is a rare complication during peri-HCST period.
- HC is multifactorial and has numerous etiologies including infectious, chemical, or radiation.
- Clinical manifestations of HC arise from bladder inflammation leading to increased urinary frequency and dysuria as well as suprapubic pain and hematuria that can range from microscopic bleeding to large, frank blood clots in the urine.
- The best treatment is prevention, followed by supportive treatment, including avoidance or treatment of initial insult resulting in HC as well as hyperhydration, bladder irrigation, and invasive interventions.

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17 Infectious Complications and HSCT

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Abstract

The incidence of life-threatening and/or opportunistic infections in the hematopoietic stem cell transplantation (HSCT) setting varies widely. Despite improved microbial detection methods and supportive care, infection remains one of the leading causes of morbidity and mortality in HSCT recipients. Furthermore, infection can confound the management of other HSCT-related complications such as graft-versus-host disease (GvHD). Thus, prevention and management of infection remain significant focuses of supportive care of the HSCT patient. This chapter discusses the relevant factors that influence infection risk as well as the most common or significant pathogens associated with HCST. Preventative measures and management of these HSCT-associated infectious complications are discussed herein.

Introduction

Infectious complications account for a major cause of morbidity and mortality in pediatric and adult patients undergoing hematopoietic stem cell transplant (HSCT) [1–4]. In addition to its direct impact on post-HSCT health, infections can confound the management of other complications, including GvHD. Despite improved strategies for prevention, particularly prophylactic antimicrobials, fatal infections still occur. The incidence of fatal infections varies widely but accounts for the most common primary and secondary causes of death in HSCT patients. A recent review of infections in children undergoing HSCT reports that infections account for 13% of deaths after matched sibling donor HSCT,

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17% after unrelated donor HSCT, and 7% after autologous HSCT [5]. Therefore, although survival rates for HSCT have improved over the years, infection remains a leading cause of death and a major case of morbidity significantly hindering the success of HSCT. Recipients should be carefully followed with appropriate prophylactic measures in the post-HSCT period, while new treatment options should be considered to reduce the infection-related mortality rates.

Both prevention and management of infection are major focal points of supportive care in the post-HSCT period. No one factor but rather a complex interplay of factors, including patient demographics, duration of neutropenia, indication for HSCT, GvHD, HSCT modality (i.e., autologous, allogeneic, partially matched, type of conditioning), total body irradiation, hematopoietic stem cell (HSC) source, CMV donor/recipient (D/R) status, and era of HSCT, contributes to infection risk. For practical purposes, the risk group of infections after HSCT can be divided with respect to the type of transplantation. See Table 17.1 and Fig. 17.1 that outline the relevant factors influencing infection risk in terms of the type of transplant and the common infections seen in the various phases of HSCT.

The risk for infections parallels the pattern of bone marrow reconstitution yielding periods of immune system deficiency and recovery, which begins with the chemotherapy and/or radiation therapy used in conditioning [6]. For example, prolonged neutropenia increases the risk for bacterial and fungal infections, while deficits in cel-

Table 17.1 Infection risk related to HSCT type

Type of HSCT	Source of hematopoietic stem cells	Risk of early infection: typical time for neutrophil recovery	Risk of late infection: impaired T- and B-cell function	Risk of ongoing infection: GvHD and iatrogenic immunosuppression	Graft-versus-tumor effect
Autologous	Self (recipient)	High risk: neutrophil recovery at times prolonged	~1 year	Minimal to no risk of GvHD and late-onset severe infection	None (−)
Syngeneic (genetic twin)	Identical twin	Low risk: 1–2 weeks for recovery	~1 year	Minimal risk of GvHD and late-onset severe infection	+/−
Allogeneic: related	Sibling	Low risk: 1–2 weeks for recovery	~1 year	Minimal to moderate risk of GvHD and late-onset severe infection	++
Allogeneic: related (haploidentical)	Child/parent	Intermediate risk: 2–3 weeks for neutrophil recovery	1–2 years	Moderate risk of GvHD and late-onset severe infection	++++
Allogeneic: unrelated adult	Unrelated donor	Intermediate risk: 2–3 weeks for neutrophil recovery	1–2 years	High risk of GvHD and late-onset severe infection	++++
Allogeneic: unrelated cord blood	Unrelated cord blood units (×2)	Intermediate to high risk: neutrophil recovery sometimes prolonged	Prolonged	Minimal to moderate risk of GvHD and late-onset severe infection	++++
Allogeneic: mini (nonmyeloablative)	Donor (transiently coexisting with recipient cells)	Low risk: neutrophil counts close to normal	1–2+ years	Variable risk of GvHD and late-onset severe infection	++++ (but develops slowly)

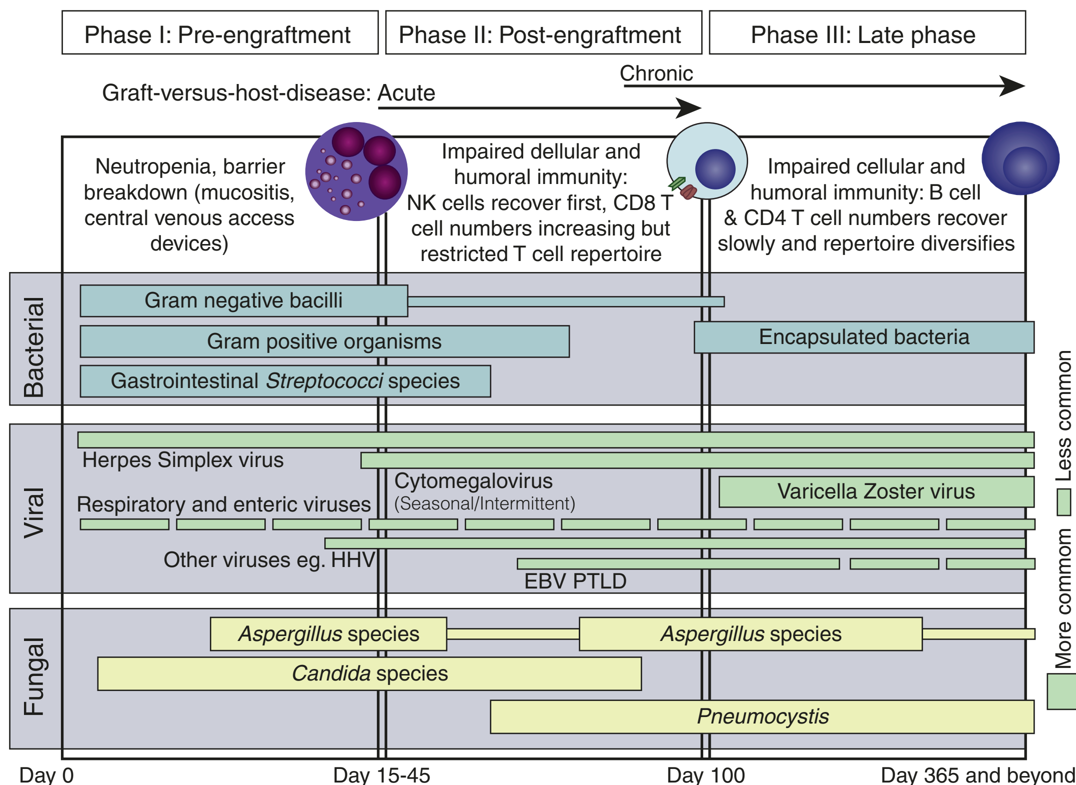

Fig. 17.1 The risk of bacterial, viral, and fungal infection at 0–30 days, 31–100 days, and 101 days and beyond HSCT [9]. This illustration depicts the relevant time line of the most common infections found in the HSCT setting. During phase I (pre-engraftment), bacterial infections are the most common. During phase II, bacterial infections become less common, but there is a rise in incidence of CMV reactivation and fungal infections. During phase III, PJP, *Aspergillus*, varicella-zoster viruses, and encapsulated bacteria are the most common pathogens seen, particularly in patients with chronic GvHD who have direct and indirect impaired immune function

lular adaptive immunity can increase the risk for bacterial, fungal, viral, protozoan, or helminthic infections. Thus, the rate and severity of infections are directly related to the degree of, rapidity of onset, and duration of neutropenia. Furthermore, conditioning regimens not only destroy normal hematopoiesis for neutrophils, monocytes, and macrophages but also damage mucosal progenitor cells, causing temporary loss of mucosal barrier integrity [6]. As a result, the gastrointestinal (GI) tract remains a reservoir of potential pathogens, which normally contains bacteria and commensal fungi. Virtually all HSCT patients lose immune memory in spite of a lifetime of exposure to infectious agents, environmental antigens, and vaccines, mostly through rapid loss of T- and B-cells after conditioning. While donor immunity provides some protection, this is temporary and variable and cannot provide long-term immunity against infectious diseases in HSCT recipients.

Recovery of the immune system following conditioning takes place broadly in three main phases beginning at day 0, the day of the HSC infusion. Phase I is the pre-engraftment phase leading up to day 0 and then from day 0 to 29 days after HSCT; phase II is the early post-engraftment phase, encompassing 30–100 days after HSCT; and phase III or the late post-engraftment phase is the time 100 days after HSCT lasting up to 1 year and potentially longer if exogenous immunosuppression persists [6].

In the pre-engraftment phase (I), the risk factors influencing the incidence of infection include the presence of prolonged neutropenia, the presence of a central venous catheter (CVC), and the translocation of bacteria through non-intact mucosa [7]. In fact, the breakdown in the mucosal

barrier has now been identified as a significant risk for the incidence of central line-associated bloodstream infections (CLABSI) such that the Centers for Disease Control and Prevention (CDC) recently defined a new term of "mucosal barrier injury laboratory-confirmed" BSI (MBI-LCBSI) [7]. A recent retrospective review of children undergoing HSCT identified that approximately half of BSIs more accurately meet this definition in the peri-HSCT period and lead to significant morbidity, mortality, and healthcare resource utilization in HSCT patients. Consequently, oral, gastrointestinal, and skin flora are primary sources of infection during this phase. Prevalent pathogens include *Candida* species and *Aspergillus* with extended neutropenia. Herpes simplex virus (HSV) reactivation can also occur during this phase. While conditioning can influence the risk of infection in broad terms, the risks are similar for autologous and allogeneic patients in the early pre-engraftment phase. Importantly, while the first fever during pre-engraftment is most often caused by a bacterial pathogen, the organism or site of infection is rarely identified. Therefore, most institutions favor treating infections preemptively or empirically during this time, at least until the neutropenia resolves.

Phase II, or the early post-engraftment phase, is dominated by impaired cell-mediated immunity. Unlike in phase I, phase II is impacted more by the extent of acute GvHD and the use of associated immunosuppressive therapy. Herpesviruses, particularly cytomegalovirus (CMV), are critical pathogens. The risks for CMV are also influenced by donor and recipient status: CMV D/R status (all R+ and R- with D+ donor) increases the risk for CMV viremia [4]. Other common pathogens during this phase include *Pneumocystis jirovecii* and *Aspergillus* species.

The risk for infection during phase III or the late post-engraftment phase reflects the extent of recovery in adaptive immune function and the severity of a patient's GvHD. Therefore, recipients of an autologous HSCT typically have more rapid recovery and subsequently lower risk for infections in comparison to allogeneic recipients. Similarly, recipients of reduced intensity conditioning (RIC) HSCTs are not necessarily at increased risk for bacterial, fungal, viral infections or bacteremia [4], which may reflect the rapidity of immune recovery. In contrast, allogeneic HSCT patients with chronic GvHD or recipients of alternative donor allogeneic transplant are at risk for infections that include CMV, varicella-zoster virus (VZV), EBV-related posttransplant lymphoproliferative disease (PTLD), community-acquired respiratory viruses, and infections with encapsulated bacteria (e.g., *Haemophilus influenza* and *Streptococcus pneumonia*). Recipients of mismatched haploidentical allogeneic transplant are at higher risk for GvHD and with higher severity; therefore, their risk for infection is higher. In contrast, patients undergoing autologous HSCT are most at risk for infection in phase I.

Other independent risk factors that influence infection include the type of conditioning regimen, HLA compatibility between the patient and the donor, and the type of infectious agents detected during the course of pre-HSCT treatment. The HSCT process and additional immunosuppressive therapy for GvHD also further exacerbates or prolongs the existing deficiencies in humoral and cellular immune functions.

Below is a compilation of the most common and/or significant infections seen in HSCT patients by pathogen class in terms of incidence, clinical and radiographic features, diagnostic studies, and management.

Bacteria

HSCT patients are at the highest risk for bacterial infections during the pre-engraftment period (days 0–30 post-HSCT) due to their prolonged period of profound neutropenia, loss of the mucosal barrier, and the presence of a CVC [6]. Mortality attributed to bacteremia has been described up to 6%, although the incidence was significantly higher in the early 1990s as compared to later eras [4]. This improvement has been attributed to earlier engraftment times, better supportive care, improved patient education, and rigorous hand hygiene. The most common bacteria include coagulase-negative staph, such as *Staph epidermidis*, *Strep viridans*, and *Staph aureus*, and Gram-negative organisms including *Escherichia coli*, *Pseudomonas* spp., and *Klebsiella* [6].

Gram-Positive (GP) Organisms (Most Commonly *Staph epidermidis*, *Viridans Streptococci*, *Enterococcus* spp.)

Incidence: The incidence varies by location. One retrospective review documented that 82% of infections were GP infections [3, 4]. GP infections are more prevalent than Gram negative (GN) in all three post-HSCT phases.

Risk factors: Risk factors include prolonged neutropenia, the presence of CVC, and allogeneic HSCT.

Clinical and radiographic features: None are diagnostic for bacterial infections. However, patients may present with fever.

Diagnostic studies: Blood cultures are performed to isolate the pathogen and identify the antibiotic sensitivity pattern.

Gram-Negative Organisms (Most Commonly *Klebsiella* spp., *Escherichia coli*, *Pseudomonas*)

Incidence: The incidence varies by location.

Risk factors: Risk factors include prolonged neutropenia, the presence of CVC, and allogeneic HSCT.

Clinical and radiographic features: None are diagnostic for bacterial infections; however, HSCT patients may present with fever, with or without hypotension.

Diagnostic studies: Blood cultures are performed to isolate the pathogen and to identify the antibiotic sensitivity pattern.

Viruses

HSCT patients are susceptible to viral infections, including both de novo infection and reactivation. Given the risk for reactivation, the viruses that are most prevalent vary based upon the patient's immune status. Therefore, HSCT candidates should be tested for the presence of serum anti-CMV, anti-HSV, and anti-VZV IgG antibodies before undergoing HSCT to determine the risks for primary viral infection and reactivation after HSCT [6]. In the pre-engraftment phase, HSV, respiratory and enteric viruses, and HHV6 are the most prevalent, whereas CMV, EBV, and varicella (in addition to respiratory and enteric viruses) are most prevalent during phase II (30–100 days post-HSCT) and phase III (>100 days post-HSCT) engraftment periods.

Required medications depend on recipient serostatus and prior exposure to viruses of interest (e.g., HSV, VZV, CMV, EBV). For viruses such as CMV, it is crucial to also take the donor exposure status and type of graft into account when determining the need for prophylaxis.

Herpes Simplex Virus (HSV1/2)

Incidence: HSV 1/2 is most commonly seen in the first 30 days post-HST and can occur up to 2 years post-HSCT [4].

Risk factors: Risk factors include HSV serology-positive recipient and HSC graft manipulation (i.e., T-cell depletion).

Clinical and radiographic features: HSV most often presents with mucocutaneous lesions in the orofacial region (85–90%) and genital area (10–15%). However, other manifestations can include pneumonia, hepatitis, meningitis, encephalitis, and bone marrow suppression.

Diagnostic studies: Diagnostic studies include viral culture and HSV 1/2 PCR from serum or CSF.

Management and outcomes: Acyclovir prophylaxis should be included in all HSV-seropositive allogeneic HSCT recipients, starting during the early posttransplant period or at the start of conditioning therapy and continuing through engraftment [6]. HSV prophylaxis is not indicated for HSV-seronegative HSCT recipients even if donors are HSV seropositive. Other agents used to treat HSV 1/2 infections include valacyclovir, foscarnet, cidofovir, and famciclovir.

Cytomegalovirus (CMV)

Incidence: In one retrospective review, CMV viremia was seen in 5% of patients pre-

engraftment and 9% in phase II [4]. Other documented ranges of incidence vary from 16% to 28%. CMV pneumonia is the most common post-HSCT CMV *disease* (ranging from 1% to 6% in autologous HSCT recipients and from 10% to 30% in allogeneic HSCT recipients) [8].

Risk factors: CMV serostatus of donor (D) and recipient (R) most strongly influences the risk for CMV disease. The risk is highest in CMV R+/D− with a reactivation rate of 60–70%, while the risk for reactivation in a R−/D+ patient is 30% [9]. CMV R−/D+ has a higher risk of all viral infections from day 0 to 30, while CMV R+/D+ has a higher risk of viremia in days 31 to 100. Risk factors for the development of late CMV disease include allogeneic HSCT accompanied by chronic GvHD, steroid use, low CD4 counts, delay in high-avidity anti-CMV antibody, and recipients of matched unrelated or T-cell-depleted HSCTs.

Clinical and radiographic features: There are no specific findings; however, maintaining a high suspicion for the disease particularly in the previously mentioned risk groups is highly recommended, and preemptive strategies with prophylaxis should be considered. CMV can affect nearly any organ of the body causing pneumonia, retinitis, hepatitis, colitis, uveitis, pericarditis, and encephalitis.

Diagnostic studies: Direct detection of CMV DNA by PCR is the most useful and sensitive detection method. Viral culture of urine, saliva, blood, or bronchoalveolar washings by rapid shell vial culture or routine culture are also potential sources, but these are less sensitive than CMV DNA PCR testing. CMV pp65 antigen detection in leukocytes is the preferred screening since it is more rapid and sensitive than culture with a good positive predictive value [6]. However, given the reliance on the presence of leukocytes, testing for CMV pp65 is less useful especially during periods of lymphopenia. Therefore, CMV DNA detection by PCR is the preferred method during period(s) of neutropenia. In the absence of prophylaxis, routine surveillance performed at least one to twice a week is recommended for evidence of CMV reactivation as long as a substantial immunocompromised state persists. Most practices favor routine weekly CMV surveillance for at least 3 months after allogeneic HSCT.

Management and outcomes: Recipients at high risk for CMV disease, CMV R+ and all CMV R- with CMV D+ (allogeneic HSCT), should use prophylactic or preemptive treatment with ganciclovir. Alternatively, acyclovir is often used in the early pre-engraftment phase to avoid the myelosuppressive side effect of ganciclovir until cell counts improve. IVIG is often added for treatment while undergoing resistance testing to ensure sensitivity to the agent chosen. For first-line preemptive treatment, foscarnet is often used. See Table 17.2 for additional treatment options.

Unfortunately, the outcomes for CMV *disease* are poor. CMV pneumonia is the most serious manifestation with the highest mortality rate, described up to 31% survival at 1 month after diagnosis (see Chap. 21 for further discussion) [10].

Varicella-Zoster Virus (VZV)

Incidence: VZV is a late complication after allogeneic HSCT and is typically due to viral reactivation. Primary infection is rare and usually severe and may occur in seronegative patients.

Risk factors: While HSCT candidates should be tested for the presence of serum anti-VZV IgG antibodies, these tests are not 100% reliable, particularly in immunosuppressed patients. All HSCT candidates and recipients should avoid exposure to persons with active VZV infections. All those in contact with the patient (HSW, family members, household contacts, and visitors) without a reported history of varicella infection or who are VZV seronegative should receive VZV vaccination before being allowed to visit or have direct contact with an HSCT recipient.

Diagnostic studies: PCR testing of material collected from an unroofed vesicle when there is a suspicious rash is performed.

Management and outcomes: All HSCT recipients with VZV disease should be placed under airborne and contact precautions to prevent trans-

Table 17.2 Recommendations for anti-infective prophylaxis and treatment of common opportunistic viral and fungal infections

Pathogen	Prophylaxis medication and typical dosing	Treatment medication and typical dosing	Surveillance and diagnosis	Special circumstances
Bacteria				
Gram-positive bacteria	Consult institutional standards based on local antibiograms to determine appropriate Gram-positive and Gram-negative coverage, both for prophylaxis and treatment			
Gram-negative bacteria	Consult institutional standards based on local antibiograms to determine appropriate Gram-positive and Gram-negative coverage, both for prophylaxis and treatment			
Viruses				
CMV	Ganciclovir 5 mg/kg IV BID[a] Foscarnet 90 mg/kg IV daily[a] Valganciclovir 7 × BSA (mg/m^2) × Cr clearance[a] Immune globulin (IVIg) Ganciclovir is first line and however is associated with severe myelosuppression. Therefore, consider foscarnet (second line) if counts remaining low while awaiting engraftment[b]	Foscarnet 90 mg/kg IV q12 h[a] Ganciclovir 5 mg/kg IV BID[a] *Adoptive immunotherapy with CMV-specific cytotoxic T-cell therapy*	Routine weekly serum evaluation for quantitative PCR Consider measuring CMV titers during the pretransplant period to identify high-risk patients Send resistance testing if suspected infection to guide antiviral management	Consider preemptive treatment in "high-risk patients" – highest risk in recipient-positive, donor-negative (R+/D-) patients; lowest risk (R−/D-) or (R+/D+) Renal toxicity and cleared renally, therefore requires drug monitoring with compromised renal function and dose adjustment with compromised renal function. Use with caution in patients receiving concomitant nephrotoxic agents[a]
EBV	None	Rituximab 375 mg/m^2 weekly up to 4 weeks	Routine weekly serum evaluation for quantitative PCR	Monitor B-cell function and quantitative IgGs – replace with IVIg Consider limiting dosages to prevent excessive B-cell depletion
HHV6	None	Foscarnet 90 mg/kg IV q12hrs[a]		Renal toxicity[a]
HSV	Acyclovir 250 mg/m^2/dose q8 h[a] Valacyclovir for oral lesions	Acyclovir: $<$12 years: 10 mg/kg/dose every 8 h IV ≥12 years: 5 mg/kg/dose every hours IV		Renal toxicity[a]
Adenovirus	None	Cidofovir *high* dose 5 mg/kg weekly with probenecid and IVF[a]	Routine weekly serum evaluation for quantitative PCR	Renal toxicity[a]

(continued)

Table 17.2 (continued)

Pathogen	Prophylaxis medication and typical dosing	Treatment medication and typical dosing	Surveillance and diagnosis	Special circumstances
BK	None	Cidofovir *low* dose 0.25 mg/kg IV q 2 weeks[a]	Detect viral PCR in urine and blood with symptoms of new onset hematuria not explained by other etiology	Renal toxicity, consider hyperhydration up to 1.5–2× maintenance IV fluids to avoid excess discomfort from passing blood clots[a]
Fungus				
Pneumocystis jirovecii	Trimethoprim/sulfamethoxazole load 2.5 mg/kg BID (starting with conditioning through D + 2 posttransplant) Pentamidine monthly	High-dose trimethoprim/sulfamethoxazole Pentamidine inhaled or IV Atovaquone Dapsone		If sulfa allergy, use pentamidine (IV or INH) or atovaquone. Trimethoprim/sulfamethoxazole associated with myelosuppression, therefore consider switch to atovaquone or pentamidine posttransplant
Candida spp.	Fluconazole 6–12 mg/kg daily	Fluconazole 12 mg/kg daily (maximum 600 mg/dose) Caspofungin 70 mg/m^2 on day 1, 50 mg/m^2 daily thereafter Voriconazole (depends on age)[b] Posaconazole 4 mg/kg/dose (maximum 200 mg) three times a day with a full meal or carbonated beverage		If known history, continue all prior treatments, and escalate treatments posttransplant. Add antifungal coverage if on steroids for GvHD prophylaxis or treatment
Aspergillus spp.	Voriconazole (depends on age) Posaconazole 4 mg/kg/dose (maximum 200 mg) three times a day with a full meal	Voriconazole (depends on age)[b] Liposomal amphotericin B (Ambisome[c]) 5 mg/kg daily Posaconazole 4 mg/kg/dose (maximum 200 mg) three times a day with a full meal or carbonated beverage		Significant interactions of azoles with tacrolimus, cyclosporine, and sirolimus, leading to increased levels of these immunosuppressants. Careful dose adjustments and monitoring of serum concentrations of the immunosuppressant agent is necessary
Mucormycosis (*Zygomycetes*)	Voriconazole (depends on age) Posaconazole 4 mg/kg/dose (maximum 200 mg) three times a day with a full meal	Liposomal amphotericin B (Ambisome[c]) Aggressive surgical debridement of involved tissues as soon as diagnosis is suspected Posaconazole 4 mg/kg/dose (maximum 200 mg) three times a day with a full meal or carbonated beverage		

[a]Precautions for renal toxicity associated with foscarnet and ganciclovir
[b]Voriconazole IV: 2–11 years, 7 mg/kg/dose every 12 h; ≥12 years and adults, 6 mg/kg every 12 h for two doses and then 4 mg/kg/dose every 12 h. Voriconazole PO: 2–11 years, 9 mg/kg/dose (maximum 350 mg) every 12 h; ≥12 years and adults, 200 mg every 12 h
[c]Titrate doses based on therapeutic drug monitoring, goal trough level: 1–6 μg/mL

mission to other HSCT recipients. Contact precautions should remain until all skin lesions are crusted. Airborne precautions should be instituted up to 10 days after exposure to VZV and continued through 21 days after the last exposure or 28 days post-exposure if the patient received varicella-zoster immunoglobulin (VZIG).

VZIG should be administered as soon as possible or at least within 96 h after close or household contact with a person with chickenpox or shingles if the HSCT recipient is immunocompromised.

Any HSCT recipient undergoing conditioning whom experiences a VZV-like rash should receive intravenous acyclovir until a minimum of 2 days after the lesions have crusted. Long-term prophylaxis with acyclovir is not recommended to prevent recurrent VZV. Other agents used for prophylaxis include foscarnet (especially if acyclovir resistance is suspected or documented).

Adenovirus

Incidence: Adenovirus infection causes a wide spectrum of disease states ranging from asymptomatic viremia to severe disseminated disease. The incidence has been estimated to be between 5% and 47% [11], with most cases occurring in the first 100 days post-HSCT.

Risk factors: The main risk factors for adenoviral infection are delayed immune reconstitution, including umbilical cord blood and other unrelated donors, severe GvHD, HLA-mismatched HSCT and T-cell depletion of the donor graft in vivo or in vitro, and higher adenovirus viremia in plasma [11].

Diagnostic studies: Adenovirus real-time PCR from serum, urine, or stool is performed.

Management and outcomes: There is no approved antiviral agent for the treatment of adenovirus infection. Current strategies may include reduction in immune suppression and off-label use of IVIg or IV cidofovir. Oral brincidofovir is emerging as another agent with antiviral efficacy against adenoviruses and is under investigation [11]. Survival of HSCT recipients with disseminated disease is very poor.

RSV

Incidence: In a cohort of 759 children who underwent allogeneic HSCT, there was a 1% incidence in the first 30–100 days post-HSCT. There was a minimal increase to 2% in days 101–730 post-HSCT [4].

Risk factors: Risk factors include T-cell-depleted donor graft and respiratory/viral season.

Diagnostic studies: Any recipient who experiences signs or symptoms of a respiratory viral infection should have nasopharyngeal swab (or nasopharyngeal wash, endotracheal tube aspirate, bronchoalveolar lavage (BAL) sampling) for antigen detection, typically rapid diagnostic assays performed on respiratory specimens. There are RT-PCR assays now commercially available. BAL testing is advised when no respiratory pathogen has been identified despite persistence of respiratory symptoms.

Management and outcomes: No definitive, uniform effective preemptive therapy has been identified for RSV, although strategies including aerosolized ribavirin and RSV antibody therapy in combination with aerosolized ribavirin has been used for lower tract respiratory disease. The monoclonal anti-RSV antibody, palivizumab, has been administered as immunoprophylaxis as IM injections during peak seasons for patient at highest risk (<2 years).

Other Respiratory Viruses (Rhinovirus, Influenza, Parainfluenza, and Human Metapneumovirus)

Incidence: One study describes the incidence of parainfluenza and influenza in patients post-HSCT at 2% and 1%, respectively, in the first 30 days after HSCT [4]. In that same study, there was an increase in both viruses by days 100–730, with 5% of patients developing parainfluenza and 4% developing influenza.

Risk factors: Risk factors include T-cell-depleted donor grafts and respiratory viral season.

Diagnostic studies: Direct detection of viral antigens in respiratory secretions using immunofluorescence (IF) or enzyme immunoassay (EIA)

is preferred; direct detection by RT-PCR; rapid diagnostic assays performed on nasopharyngeal or other respiratory specimens for antigen detection are also done.

Management and outcomes: The management involves respiratory supportive care; Tamiflu has been used in HSCT patients with influenza.

BK Virus

Incidence: Hemorrhagic cystitis is a well-recognized BK virus-associated complication in HSCT patients (see Chaps. 16 and 22) [12]. The frequency of BK hemorrhagic cystitis is broad with an incidence ranging from 7% to 70% from various reports [13]. The major clinical manifestations include BK-associated nephropathy or ureteric stenosis, although also implicated in pneumonia, nephritis, and encephalitis.

Risk factors: A major driver of BK virus reactivation is immunosuppression. BK virus hemorrhagic cystitis is more prevalent in matched unrelated donors and unrelated umbilical cord blood HSCT recipients in comparison to matched related donor HSCT recipients.

Diagnostic studies: Quantitative BK PCR from serum and urine is performed. A threshold of 1×10^4 copies/ml of BK virus in the serum, or $>1 \times 10^7$ copies/ml in the urine, has been proposed as a threshold level most associated with a high risk of BK hemorrhagic cystitis [12]. Kidney biopsy is the gold standard for determining disease progression although is rarely needed.

Management and outcomes: Supportive measures including bladder irrigation, blood transfusion, and symptom relief are the standard of care for hemorrhagic cystitis. No antiviral drug has proven efficacy against BK viral replication. Many treatments including ganciclovir, leflunomide, and long-term ciprofloxacin have been trialed. Case reports support the use of cidofovir; however, a major limitation is its significant risk for nephrotoxicity [12, 13]. Brincidofovir is an orally administered prodrug of cidofovir and is associated with a lower incidence of nephrotoxicity compared to cidofovir. It is currently under investigation in phase III clinical trials.

HHV-6

Incidence: Infections due to HHV-6 are generally encountered earlier than CMV. HHV-6 may lead to engraftment delays or graft failure after HSCT. Significantly high incidence of HHV-6 viremia has been noted following unrelated umbilical cord blood recipients [14].

Risk factors: Higher plasma HHV6 DNA levels increase the risk for the development of HHV-6 encephalitis. The use of umbilical cord blood as the HSC source is also a risk factor for HHV-6.

Diagnostic studies: A small number of patients without clinical signs exhibit HHV-6 persistence and present with high viral loads in the blood as well as in other body fluids and tissues due to genomic integration of the virus. Therefore, HHV-6 PCR should only be performed if there is a clinical suspicion.

Management and outcomes: The impact of HHV-6 viremia on outcomes in children is not well described, but its prevalence has increasingly been recognized [14]. Foscarnet 90 mg/kg IV q12 h is used to treat viremia.

Epstein-Barr Virus (EBV) and EBV-Posttransplant Lymphoproliferative Disease (PTLD)

Incidence: The incidence of EBV viremia and PTLD varies across HSCT centers. However, the incidence has been reported between 0.1% and 63% for viremia, with the median time to development of EBV-PTLD of 2–4 months after HSCT [5, 15].

Risk factors: EBV infection often occurs secondary to endogenous reactivation or graft-originated contamination with posttransplant lymphoproliferative disease (PTLD) as the most significant clinical syndrome. The risk for the development of EBV-PTLD is less clearly related to specific malignancy, HSCT procedure, or source but is predominantly related to the degree of depletion or impairment of T-cells. Therefore, strategies that deplete T-cells from the donor graft increase the risk for EBV-PTLD [15]; the use of alemtuzumab or antithymocyte globulin

(ATG) has been associated with an increased risk. Umbilical cord blood HSCT is also thought to confer an intrinsic risk due to T-cell naivety related to the HSC source.

Diagnostic studies: Detection of EBV DNA by PCR in tissue or blood (whole blood, plasma, or serum) is preferred. EBV-PTLD can be diagnosed as probable or proven with associated significant lymphadenopathy, hepatosplenomegaly, or other end-organ manifestations without a tissue biopsy. The detection of EBV nucleic acid in blood is not sufficient for diagnosis; EBV-PTLD can be definitively proven by the presence of at least two of the following histological features: (1) disruption of underlying cellular architecture by a lymphoproliferative process; (2) the presence of monoclonal or oligoclonal cell populations as revealed by cellular and/or viral markers; and (3) evidence of EBV infection in many of the cells, e.g., DNA, RNA, or protein [15].

Management and outcomes: No prophylaxis or preemptive therapy is recommended; however, data are emerging for the use of donor-derived, EBV-specific cytotoxic T-lymphocytes either as prophylaxis or treatment. First-line therapy in the case of proven or probable EBV-PTLD includes rituximab once weekly for up to four doses while monitoring EBV viral load. Reduction of immunosuppression is rarely successful as the sole intervention in post-HSCT PTLD.

Fungus

Fungal pathogens are one of the leading causes of mortality and morbidity after allogeneic HSCT with a typical frequency distribution [16]. Considering cohort studies published in the past decade, incidences of fungal disease are about 10% or higher and are consistently reported in populations of patients with acute myeloid leukemia and recurrent acute leukemia and after allogeneic HSCT [16]. However, the assessment of natural incidence in pediatric patients and HSCT is greatly limited by the prophylactic and empiric use of systemic antifungal agents in most contemporary series and by differences in the use of diagnostic procedures. Regardless, the overall consensus is that early recognition and prompt antifungal treatment are key to the control of invasive fungal disease.

Candida spp. are seen more frequently in the early pre-engraftment phase. However, the vast majority of fungal infections are observed during the early post-engraftment period and late phase, particularly due to GvHD and the use of high-dose steroids. In contrast to yeasts that generally enter the body via translocation from a CVC or impaired intestinal mucosa, a mold infection occurs via inhalation of airborne spores [16]. Impaired cellular immunity increases their proliferation due to impaired phagocytosis and clearance.

Primary prevention is more often centered around factors influencing the environment including high efficiency particulate air (HEPA)-filtered HSCT rooms. Fluconazole prophylaxis is effective against yeasts; however, its protection against molds is lower. Prophylactic voriconazole has been shown to decrease the frequency of *Aspergillus* infections, while posaconazole prophylaxis provides a survival advantage particularly among patients who develop GvHD [16]. It is important to note that not all licensed antifungal agents are approved in pediatric patients, and for agents with a pediatric label, appropriate doses might not have been studied and established for all age groups and indications. In Table 17.2, we have attempted to include the appropriate dosing for those agents based on age.

Candida spp.

Incidence: *Candida albicans* is the predominant cause of invasive fungal disease in the early pre-engraftment phase and the second most common fungal infection (after *Aspergillus*) noted in a recent prospective study of HSCT recipients [16].

Risk factors: Risk factors include a prior history of candida infection, the use of T-cell-depleted donor grafts, and ongoing immunosuppressive therapy.

Diagnostic studies: Early recognition and prompt antifungal treatment are the key to control of invasive fungal infections. Blood cultures for yeasts are performed and cultures or microscopic

examination of appropriate liquid and solid diagnostic specimens. In vitro susceptibility testing should be performed from all isolates to determine the minimal inhibitory concentration (MIC) [17]. Detection of circulating 1,3-β-D-glucan (Fungitell assay) can be used in patients with fungal infections due to *Aspergillus* and *Candida*, *Fusarium*, *Trichosporum* or *Saccharomyces*, and *Pneumocystis*. Guidelines from the Infectious Diseases Society of America (IDSA) favor the removal of the CVC, performing a lumbar puncture and dilated retinal examination.

Management and outcomes: Allogeneic recipients should receive fluconazole prophylaxis to prevent invasive disease, particularly during phases of neutropenia. Potential treatments include caspofungin, fluconazole, and lipid formulations of amphotericin B. See Table 17.2 for further recommendations regarding prophylaxis and treatment. The IDSA recommends prompt initiation of antifungal treatment for at least 14 days after blood cultures are sterile and to remove or at least replace the CVC.

Aspergillus

Incidence: Nosocomial mold infections among HSCT recipients result primarily from respiratory exposure to and direct contact with fungal spores (see Chap. 21). Invasive aspergillosis is the leading fungal infection noted in a prospective study of 23 HSCT centers in the USA (range, 5–10%) [16].

Risk factors: Prior or preexisting invasive aspergillosis increases the risk for recurrence among allogeneic recipients.

Diagnostic studies: The primary means for detection for *Aspergillus* spp. relies on histopathological and microbiological examinations and imaging methods. However, microbiological methods for fungal detection are limited since their results take a long time to become available. *Aspergillus* galactomannan antigen and beta-D-glucan detection are often used for serum surveillance. However, false-positive results can be seen due to interference with some foods or enteral nutrition, administration of parenteral beta-lactam antibiotics, sodium gluconate, blood products, and the presence of other fungal infections. Among imaging techniques, chest X-ray and CT are frequently used.

Management and outcomes: Oral posaconazole confers a broad anti-mold activity with low breakthrough of invasive fungal infections, although it relies on enteral absorption. Voriconazole is an important antifungal agent for high-risk patients given the ability for intravenous administration and proven anti-mold activity. Both azoles also enable therapeutic drug monitoring during treatment, although voriconazole causes liver toxicity more frequently than fluconazole. Amphotericin B has been associated with lower rates of fungal infections and lower fungal infection-related mortality; however, the use of amphotericin B is limited by renal toxicity, electrolyte wasting, and overall tolerability.

Zygomycetes spp. (*Mucor, Rhizopus, Rhizomucor*)

Incidence: Nosocomial mold infections among HSCT recipients result primarily from respiratory exposure to and direct contact with fungal spores. Mucor is the third most common invasive fungal disease after aspergillosis and candidiasis [18]. Descriptions of up to 10% of allogeneic HSCT recipients suffering from invasive fungal disease have been reported [19].

Risk factors: HSCT recipients, who remain immunocompromised, should avoid hospital construction or renovation areas since these have been associated with an increased risk for nosocomial mold infection.

Diagnostic studies: Biopsy of a suspected lesion yielding microscopic, culture, and/or histopathological examination of relevant samples. High suspicion from characteristic radiologic findings such as the reversed halo sign, while not diagnostic, is highly suggestive of infection and should prompt further evaluation and initiation of treatment.

Management and outcomes: Mortality rates of mucormycosis have been reported to reach

up to 90% [19]. Successful management of mucor depends on early diagnosis, emergent surgical debridement of devitalized tissues, and management of underlying risk factors [16] Typically, administration of an empirical treatment with caspofungin or liposomal amphotericin B is done.

Prevention of Opportunistic and Life-Threatening Infections in the HSCT Patient

The most important management strategy in treating infection in a HSCT patient is to *prevent* or administer preemptive prophylactic therapy as soon as possible. The information below is intended to guide prevention and treatment of commonly seen infections in pediatric patients undergoing HSCT. These are not necessarily exhaustive. Contraindications, drug-drug interactions, and specific warnings for each compound have to be considered. Therefore, the information herein should be integrated into algorithms that are tailored to the specific population of patients and the infectious epidemiology of each institution. See Chap. 28 for additional information.

General Infection Control in the Hospital Environment

There exists a number of sources of infectious agents both in hospitals and homes, including water, air, dust, the ventilation system, potted plants, flowers, cereals, nuts, spices, carpets, and construction areas [20]. As a result, the general recommendations for the prevention of opportunistic infections in HSCT recipients include a wide range of interventions related to management of ventilation systems, HSCT unit construction and cleaning, isolation and barrier precautions, interactions with healthcare workers and visitors, skin and oral care, infection surveillance, and the prevention of specific nosocomial and seasonal infections. Infection control procedures include (1) preventing dust accumulation by cleaning surfaces, isolating patient wards from outside air, and maintaining room positive pressure; (2) providing rooms with HEPA filters; (3) avoiding patient exposure to tap water during severe immunosuppression; and (4) cleaning the showering facility before each use. *Measures to reduce hospital-acquired candidal and bacterial infections rely on good hand hygiene, an important, simple, and inexpensive infection control strategy*.

Pharmacological Preventive Strategies

Consult your institutional guidelines for specific recommendations regarding institution antibiograms that will influence the choice of prophylaxis and treatment strategies.

Antibacterial prophylaxis: Many centers place patients on prophylactic antibiotics to decrease the risk of bacteremia during times of profound myelosuppression. Some centers do this routinely for all allogeneic and autologous HSCT patients, while others reserve this for those who have had a prior documented history of infections with *Strep viridans* species or with resistant organisms. Additionally, some centers use oral antibiotics for purposes of gastrointestinal decontamination. For example, studies are ongoing through the Children's Oncology Group (COG) to determine the efficacy of prophylactic antibiotics for the prevention of bacteremia in HSCT patients.

Antiviral prophylaxis: Antiviral prophylaxis can be a critical component of circumventing viral infection during HSCT. Required medications depend on recipient serostatus and prior exposure to relevant viruses (e.g., HSV, VZV, CMV, EBV). For viruses such as CMV, it is also crucial to take into account the donor exposure status and type of graft when determining the need for prophylaxis. High-risk patients for viral transmission include donors who have had no prior exposure (D-) and recipients who had had exposure (R+), as well as those receiving umbilical cord blood transplants and heavily T-cell-depleted products or conditioning regimens including alemtuzumab or ATG. In many of the aforementioned condi-

tions, T-cell recovery will be significantly delayed and, therefore, associated with an increased risk for viral reactivation. While the choice of medication varies greatly by center, Table 17.2 provides general guidelines.

Antifungal prophylaxis: HSCT patients are at high risk for developing fungal infections, and allogeneic HSCT should be on antifungal prophylaxis. The choice of antifungal agents is based on the patient's prior history of fungal infection, the type of donor, the HSC source, and the manipulation of the donor graft. Also, the choice of antifungal agent is institution dependent. In addition, to the use of antifungal prophylaxis, many institutions enforce prospective monitoring once to twice weekly with galactomannan detection. Galactomannan is a cell-wall component released by all *Aspergillus* spp. that can be detected by an enzyme immunoassay with high specificity. The optimal cutoff value in children is not well defined, although the threshold of an optical density index of 0.5 or higher is considered a positive test result according to the Infectious Diseases Society of America panel [21]. However, false-positive test results however are common and occur for various reasons.

Key Points

- Infection is a major cause of morbidity and mortality in all patients undergoing hematopoietic stem cell transplant (HSCT).
- Infection risks are influenced by a complex interplay of factors including patient demographics, duration of neutropenia, indication for HSCT, incidence of GvHD, HSCT modality, total body irradiation, HSC source, viral donor and recipient status, and era of transplantation.
- Measures to reduce hospital-acquired infection rely on good hand hygiene, an important, simple, and inexpensive infection control strategy.
- HSCT recipients should be carefully followed with appropriate antibiotic prophylactic measures based on institutional guidelines while taking into account the factors that can influence infection risk.

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Acute Graft-Versus-Host Disease: Diagnosis, Prophylaxis, and Treatment

18

Karen L. Bride, Neil S. Patel, and Jason L. Freedman

Abstract

Graft-versus-host disease (GvHD) results from the recognition of the hematopoietic stem cell transplantation (HSCT) recipient's tissues as being foreign by immunocompetent donor T cells. GvHD remains a major cause of HSCT-related morbidity and mortality. Historically, acute GvHD is defined as GvHD that is evident prior to day 100 post-HSCT. However, acute GvHD can occur after this arbitrary HSCT milestone, and the diagnosis of acute GvHD is made based on the clinical presentation and other factors and not necessarily on the timing. The incidence of acute GvHD has been reported from 20% to 70%. Acute GvHD most commonly affects the skin ranging from mild erythema confined to the palms and soles to full-body erythroderma with bullae. The GI tract and liver are common target organs of acute GvHD but are rarely affected without skin involvement. Because acute GvHD is associated with high morbidity and mortality rates, patients receive GvHD prophylaxis during the peri-HSCT period in an effort to prevent, or at least ameliorate, acute GvHD. The choice of medications for GvHD prophylaxis is based on the type of HSCT, the degree of histoincompatibility between the donor and the recipient, the hematopoietic stem cell (HSC) source, and the age of the donor and recipient at the time of

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HSCT. The diagnosis is most often made on a clinical basis and treated with immunosuppression. Mortality risk corresponds to the stage and grade of acute GvHD. The identification of biomarkers of acute GvHD is an active area of investigation. This chapter addresses the risk factors, clinical features, diagnostic studies, and management of acute GvHD as well as strategies for GvHD prophylaxis. Chapter 19 focuses on chronic GvHD.

Introduction

Graft-versus-host disease (GvHD) occurs when immunocompetent T cells from the donor recognize and damage the tissues in the immunocompromised recipient. It can occur after allogeneic HSCT or after donor lymphocyte infusion (DLI). Despite advances in supportive care, immunosuppressive therapy, DNA-based human leukocyte antigen (HLA) typing, and the emergence of donor manipulation, GvHD continues to be a major cause of morbidity and mortality after allogeneic HSCT. By convention, acute GvHD occurs within the first 100 days post-HSCT, whereas chronic GvHD occurs after 100 days from HSCT (see Chap. 19). However, acute GvHD is more specifically a systemic disorder driven by donor T cells with varying clinical presentations involving multiple target organs, including the skin, liver, and the gastrointestinal (GI) tract. Acute GvHD is not seen until there are signs of donor engraftment. Occasionally, acute GvHD is the first sign of engraftment. In addition, there may not be a clear distinction between acute and chronic GvHD outside of these delineated periods. Clinical manifestations are varied and can include specific derangements in the skin, liver, and GI tract, occasionally the eyes, oral mucosa, and lungs. Most often, recipients present with skin rash, but they may have diarrhea, elevated direct bilirubin, and recurrent infections as well. It is unusual to have other manifestations of acute GvHD without skin involvement. The mortality risk depends on the stage and grade of acute GvHD (see Table 18.1) [1]. The most widely used acute GvHD grading scheme was originally proposed by Glusckberg et al. in 1974 [2]. While the grading systems have evolved over the years, broadly, aGvHD is clinically graded and staged in severity from grades I to IV depending on the extent (or stage) of skin, liver, and upper and lower GI tract involvement. Therefore, the diagnosis therefore is made on clinical grounds and is not always straightforward. More evidence is emerging for plasma biomarkers including IL-2Rα, TNFR-1, IL-8, and hepatocyte growth factor that may aid in the diagnosis of acute GvHD at the onset of symptoms and provide prognostic information independent of GvHD severity [3]. However, these biomark-

Table 18.1 Current staging system, devised in 1994, reflects the number and extent of organ development [1, 11]

	Skin (% maculopapular rash of body surface area (BSA))	Liver (bilirubin level in mg/dL)	Lower GI tract (stool output/day)
Stage[a]			
0	No GvHD Rash	<2	<500 mL/day or persistent nausea
1	25%	2–3	500–999 mL/day
2	25–50%	3.1–6	1000–1500 mL/Day
3	>50%	6.1–15	Adult: >1500 mL/Day
4	Generalized erythroderma bullae/desquamation	>15	Severe abdominal pain with or without ileus or stool with frank blood or melena
Grade			
I	Stage 1–2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	–	Stage 2–3 or	Stage 2–4
IV	Stage 4 or	Stage 4	–

[a]Stage is assigned based on maximum involvement in an individual organ system

ers have not yet been validated. Depending on the patient and donor cohorts, incidences vary from 20% to 50% or 35 to 70% for grade II or more acute GvHD, with 30% developing grade III or IV, despite immunosuppressive prophylaxis; acute GvHD remains the second leading cause of death following allogeneic HSCT with the response rate of steroid-refractory acute GvHD of only 30–50% and poor overall survival [4].

Risk Factors

The extent of HLA disparity is the major risk factor of acute GvHD. The greater the disparity between the donor and recipient, the higher the risk of acute GvHD. Other risk factors for the development of acute GvHD include increased age of both the recipient and donor, gender disparity, multiparous female donors (due to alloimmunization), ineffective GvHD prophylaxis, the intensity of the HSCT-conditioning regimen, and the HSC source of the donor graft [3]. Therefore, GvHD is more common with grafts from unrelated donors as compared to sibling donors as well as older donors. Higher T-cell content and associated donor T-cell activation in the graft are also associated with higher risk of significant acute GvHD [5]. Sex-mismatched HSCTs, especially a female donor for a male recipient, have been found to be significantly associated with an increased risk of acute GvHD. Reduced intensity regimens are generally associated with less GvHD as compared to myeloablative regimens, given that more intensive conditioning leads to more tissue damage, subsequent cytokine secretion, and resultant inflammation [5]. This mechanism is similar in total body irradiation, as well as direct epithelial damage, and why TBI-containing regimens are also associated with higher rates of acute GvHD [5]. Graft source also factors into the risk for GvHD [6]. Studies have consistently shown an association with the use of peripheral blood stem cells and increased chronic GvHD [7], whereas the risk of acute GvHD with PBSCs is more controversial [6]. Infection in the recipient escalates the risk for acute GvHD. CMV seropositivity of the donor or recipient increases the risk of acute GvHD but not CMV reactivation.

Pathophysiology of Acute GvHD

The pathogenesis of acute GvHD revolves around donor T-cell alloreactivity and is thought to occur in three broad phases. During phase I (host APC activation), tissue damage of the host/recipient occurs as a result of the conditioning regimen. In response to this damage, damaged recipient cells secrete pro-inflammatory cytokines (TNF alpha and IL-1). These cytokines attract and retain white blood cells to the affected areas causing inflammation. In addition, host antigen-presenting cells (APCs) are activated and begin to present host ("self") antigens. Concurrently, innate immunity is activated after leakage of pathogens and their associated endotoxins (e.g., LPS) from the GI lumen to the circulation occurs. During phase III (donor T-cell activation), host APVs present host antigens to donor T cells that were infused along with the HSCs contained in the donor graft product. The donor T cells recognize the presented host antigens as foreign and mount an immune response (i.e., T-cell activation). This alloreactivity gets amplified and results in a "cytokine storm." In phase III, these activated CD8+ cytotoxic T cells go to the sites of tissue damage which are predisposed to an inflammatory response to the donor T cells. This inflammatory reaction causes apoptosis of the targeted tissues and is manifested as acute GvHD. The more extensive the tissue damage, the greater the immune and inflammatory response which results clinically in more severe acute GvHD.

Prevention/Prophylaxis

The optimal approach to GvHD management is focused on prevention, as results with treatment have been disappointing. Overall, current acute GvHD prophylaxis and treatment are only partially effective, with an increased risk for infections, disease relapse, and long-term adverse effects. Given the primary role of T cells in GvHD pathogenesis, the prophylaxis and treatment focus on immunosuppressive agents that interfere with T-cell activation, signaling, and function [4, 8].

The choice of agents used as GvHD prophylaxis is institution specific. Table 18.2 lists commonly used agents for acute GvHD prophylaxis,

Table 18.2 Approaches to post-HSCT GvHD prophylaxis and treatment in children according to class [7–9, 12, 13, 17, 18]

Name	Rationale	Mechanism of action	Dosages ideal goal range if able to measure	Adverse effects (common or significant)	Special considerations
Immune modulators					
Antithymocyte globulin (ATG)	• Eliminates residual recipient immune cells	• Polyclonal antibody that acts on T-cell surface antigens to deplete $CD4^{+}$ T-cells	• IV Rabbit: – 3–10 mg/kg/day over 3–4 consecutive days • IV Equine: – 60–100 mg/kg/day over 3–4 consecutive days	• Infection (bacterial and viral) • Infusion-related reactions • Post-transplant lymphoproliferative disease (PTLD)	• Premedications necessary for the prevention of infusion related reactions • Dosing differences between rabbit and equine are present
Methotrexate (MTX)	–	• Antimetabolite (precise mechanism unknown)	• IV: – 15 mg/m^2/dose on day 1; 10 mg/m^2/dose on days 3, 6, 11 – May omit day 11 if ≥grade 2 toxicity	• Mucositis • Nephrotoxicity	• Use of leucovorin rescue is necessary in patients with severe side effects of MTX
Mycophenolate mofetil (MMF)	–	• Inhibits inosine monophosphate dehydrogenase (IMPDH), which is required for de novo guanosine nucleotide synthesis, resulting in T and B lymphocyte inhibition	• IV/PO: • Children: – 15 mg/kg every 8 h • Adults: – 600 mg/m^2 ever 12 h	• Gastrointestinal toxicity: – Nausea – Vomiting – Diarrhea • Dose dependent leukopenia and neutropenia • Cellcept® and Myfortic® are not easily interchangeable due to differences in absorption	• Levels do not always correlate with efficacy due to intra- and interpatient variability with free mycophenolic acid levels

Sirolimus (Rapamycin)	–	• Inhibits the mechanistic target of rapamycin (mTOR), a regulatory kinase, resulting in suppressed cytokine mediated T-cell activation and proliferation	• PO: – Dosing range varies • Goal level: 3–12 ng/mL	• Edema • Hyperlipidemia • Mucositis • Wound dehiscence or delayed wound healing	• CYP3A4 inhibitors (fluconazole, voriconazole, posaconazole) increase sirolimus concentrations – Empirically reduce sirolimus dose or avoid combination • CYP3A4 inducers (rifampin) decrease sirolimus concentrations: – Avoid CYP3A4 inducers or increase sirolimus dose
Calcineurin inhibitors					
Cyclosporine (CSA)	• Inhibits T-cell expansion	• Inhibits production and release of interleukin 2 (IL-2) • Inhibits IL-2 induced T-lymphocyte activation	• IV: – 2–3 mg/kg/day as a continuous infusion or divided every 12 h • PO: – 5–10 mg/kg/day divided every 12 h • Goal CSA level: 200–400 ng/mL	• Gingival hyperplasia • Hirsutism • Hyperglycemia • Hypertension • Nephrotoxicity	• CYP3A4 inhibitors (fluconazole, voriconazole, posaconazole) increase cyclosporine concentrations: – Empirically reduce CSA dose • CYP3A4 inducers (rifampin) decrease cyclosporine concentrations: – Avoid CYP3A4 inducers or increase CSA dose

(continued)

Table 18.2 (continued)

Name	Rationale	Mechanism of action	Dosages ideal goal range if able to measure	Adverse effects (common or significant)	Special considerations
Tacrolimus (Prograf®)	• Inhibits T-cell expansion	• Binds to FKBP-12 • Inhibits production and release of interleukin II (IL-2) • Inhibits IL-2 induced T-lymphocyte activation	• IV: – 0.03 mg/kg/day as a continuous infusion • PO: – Convert IV to PO with a 1:4 ratio • Goal serum level: 5–15 ng/mL	• Hyperglycemia • Hypertension • Nephrotoxicity • Neurotoxicity – Tremors – Headache	• CYP3A4 inhibitors (fluconazole, voriconazole, posaconazole) increase tacrolimus concentrations: – Empirically reduce Tacrolimus dose or avoid combination • CYP3A4 inducers (rifampin) decrease tacrolimus concentrations: – Avoid CYP3A4 inducers or increase tacrolimus dose

Alternative regimens [11]

General approach	*Mechanism and rationale*	*Potential shortcomings*
Ex-vivo T-cell depletion	• Most effective GvHD preventive method	• Increased risk of graft rejection and non-relapse mortality – Possible relapse incidence due to delayed immune reconstitution
High-dose post-HSCT cyclophosphamide	• Eliminates allo-activated T-cells early after HSCT without affecting HSCs • T-cell preservation permits lower intensity conditioning • Low incidence of chronic GvHD	• GvHD incidence higher than after ex vivo T-cell depletion • However similar GvHD incidence with matched HSCT • Higher leukemia relapse incidence after non-myeloablative conditioning
Post-HSCT donor lymphocyte infusion (DLI) after ex vivo T-cell depleted HSCT	• To treat disease relapse through graft versus malignancy (GVM) effect	• Limited efficacy

many of which may be used for treatment as well. The backbone of most T-cell replete conventional acute GvHD prophylaxis regimens includes a combination of a calcineurin inhibitor (cyclosporine or tacrolimus) with a short-course methotrexate (MTX) as prophylaxis [4, 8, 9]. This particular regimen has been repeatedly shown to result in a reasonable balance between GvHD prophylaxis and graft-versus-malignancy benefit in matched sibling donor HSCTs after myeloablative conditioning regimens. The choice of prophylactic regimen also depends on the type of HSCT (related versus unrelated), the underlying disease, and the HSC source since medications for rejection prophylaxis may also be used for GvHD prevention.

Medications for GvHD prophylaxis are not benign, and they can increase the risk of severe complications including viral reactivation and invasive fungal infections. As a result, alternative regimens are being explored. For example, recent insights into intestinal homeostasis and the discovery of new pathways and targets have greatly improved our understanding of GvHD pathophysiology and will likely influence contemporary GvHD prophylaxis and treatment [10]. Further, the ex vivo depletion of T cells contained within the donor HSC product has been explored as an acute GvHD prophylaxis alternative; however, complete T-cell depletion can result in a higher incidence of graft failure and relapse. Although T cells in the donor graft are the primary factor in the development of GvHD, they do facilitate engraftment, play a significant role in post-HSCT immune reconstitution, and eliminate residual disease through the HLA incompatibility with the recipient malignant cells. As a result, complete T-cell depletion, while met with initial success in terms of GvHD incidence, still carries an estimated treatment-related mortality (TRM) in excess of 40%, related to a significant delay in recovery of the adaptive immune system. Newer understanding the specific T-cell subsets that contribute more to the development of GvHD (e.g., alpha-beta as opposed to gamma-delta T-cell subsets) will help future efforts to further manipulate and engineer a graft to optimize the immune cell content toward graft-versus-malignancy effect and immunologic reconstitution without increasing the risk for GvHD [11].

Clinical Features

Acute GvHD includes specific derangements in the skin (81% of patients), liver (50%), and gastrointestinal (GI) tract (54%), occasionally the eyes, oral mucosa, and lungs [12]. One or more organs may be involved. It often presents with skin rash at the time of neutrophil engraftment, manifested as a maculopapular rash starting at the back of the neck and shoulders and often involves the palms, soles, and ears, with sparing of the scalp. As the rash progresses, it can become confluent, reminiscent of a generalized sunburn. In severe cases, it may occur as generalized erythroderma and blisters (bullae) followed by desquamation.

GI manifestations include abdominal cramping and pain, diarrhea, malabsorption, hematochezia, and ileus (lower GI), as well as anorexia, food intolerance, nausea, and vomiting (upper GI). The most common GI tract manifestation is diarrhea which is characteristically voluminous, watery, mucoid, and often guaiac positive. Patients with lower GI tract acute GvHD can develop a paralytic ileus directly due to acute GvHD or indirectly due to the use of narcotics to treat the associated abdominal pain.

Liver acute GvHD is due to damage to bile canaliculi, leading to cholestasis with hyperbilirubinemia and elevated alkaline phosphatase. Furthermore, jaundice from hyperbilirubinemia (elevation of direct bilirubin) is the hallmark of liver GvHD. They may also have right upper quadrant abdominal pain and hepatomegaly. In severe cases, patients may also develop ascites and encephalopathy.

Differential Diagnosis

The differential diagnosis of acute GvHD of the skin includes drug rash, allergic reaction, viral exanthem, and chemotherapy- and radiation-induced toxicity. Infectious causes can mimic acute

GvHD of the GI tract and should be considered and eliminated before starting treatment with immunosuppression. The differential diagnosis of acute GvHD involving the liver includes sinusoidal obstructive syndrome (SOS)/veno-occlusive disease (VOD), chemotherapy-induced hepatotoxicity, drug-induced hepatotoxicity, and infection.

Diagnostic Studies

The diagnosis of acute GvHD can be made mostly on clinical grounds in patients presenting with a rash, diarrhea, and elevation of bilirubin within the first several weeks of transplant. The reason to pursue a tissue biopsy is to help differentiate from other diagnoses which may mimic GvHD, such as viral infection or drug reaction.

Skin: The role of skin biopsies may be helpful in making the diagnosis but is still controversial. Skin biopsies can show epidermal cell apoptosis, dyskeratotic keratinocytes, lymphocyte exocytosis, basal cell necrosis, depletion of Langerhans cells, and satellite lymphocytes next to the dyskeratotic keratinocytes [13]. All findings are not necessarily present nor is histology always pathopneumonic.

GI: Endoscopy and flex sigmoidoscopy with or without biopsy may be necessary to confirm the diagnosis. Upper and lower endoscopy have similar diagnostic yield in patients with GI tract GvHD even for patients presenting only with diarrhea [14]. Due to ease and safety of upper endoscopies, gastroenterologists favor upper endoscopy for an initial endoscopic approach [14]. Typically endoscopy of the GI tract reveals edema, mucosal sloughing, and possibly bleeding. Most often these findings would be found in the cecum, ileum, and colon but also may involve the upper intestinal tract [13]. Other etiologies, such as infectious diarrhea, may be eliminated with flex sigmoidoscopy with biopsy.

Liver: LFTs typically demonstrate elevation of direct bilirubin. The role of a liver biopsy may be helpful but is also controversial. Histopathology shows crypt cell necrosis and dropout with crypt abscess [13]. Pathology of liver acute GvHD can demonstrate early cytotoxic lymphocyte attack on bile ducts to irregular bile ducts, depending on the timing of the biopsy and the duration of liver acute GvHD prior to the biopsy. Bile duct apoptosis and endothelialitis can also be seen.

A panel of plasma biomarkers have recently been suggested to provide confirmatory tool for the diagnosis of acute GvHD, including IL-2 receptor alpha, TNF receptor-1, IL-8, and hepatocyte growth factor. The use of these biomarkers remains an active area of investigation.

Management/Prognosis

Despite adequate prophylactic regimens and new innovations in graft engineering, GvHD remains a significant issue. Treatment with high-dose corticosteroids, usually methylprednisolone, is the standard first-line choice, and the majority of patients will respond [5]. For patients who do not respond or only respond partially (classified as "steroid refractory"), additional immunosuppression is added including agents that can be used as prophylaxis to suppress T-cell function (MMF, calcineurin inhibitors, ATG, sirolimus) or to inhibit T-cell signaling by inhibition of IL-2 secretion (daclizumab, basiliximab) or by TNF alpha inhibition (infliximab, etanercept) (see Table 18.2). Extracorporeal photopheresis is a novel, non-immunosuppressive modality by which UV-A irradiation of T cells and subsequent inactivation are done via apheresis [15]. Each pheresis cycle takes approximately 3–4 h, but the schedule varies by institution. The treatment runs over several months altogether. Newer agents are also being investigated such as imatinib and JAK inhibitors such as ruxolitinib [16]. However, the majority of these newer treatment approaches have the best effect on skin acute GvHD with limited responses in acute GvHD of the lower GI tract and especially the liver. A proportion of patients with acute GvHD will go on to develop chronic GvHD. This progression is considered a poor prognostic indicator, contributing significantly to the morbidity and mortality of the allogeneic HSCT recipient.

Acute GvHD still remains a significant cause of morbidity and mortality in pediatric HSCT

and future graft engineering techniques, prophylaxis, and better treatments that are imperative and are active areas of research.

Key Points

- Acute GvHD is caused by donor T-cell activation in the skin, liver, and/or GI tract of the recipient.
- Acute GvHD usually occurs within the first 100 days post-HSCT, but it can occur later.
- Various, institution-specific prophylactic regimens exist and are based on the HSCT type and HSC source but often include a calcineurin inhibitor.
- Newer modalities to prevent GvHD rely on graft manipulation and selective removal of T cells from the graft.
- Acute GvHD treatment is aimed at suppressing the immune system in order to reduce T-cell activity, and the mainstay therapy is steroids.
- Acute GvHD is associated with significant morbidity and mortality, and the mortality risk depends on the stage and grade of the acute GvHD.

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Part IV

The Late Post- HSCT Period (> 100 Days)

Chronic GvHD

19

Terry Wikle Shapiro and Malika Kapadia

Abstract

Graft-versus-host disease (GvHD) is one of the most common complications seen after allogeneic hematopoietic stem cell transplantation (HSCT) and can be life-threatening. The risk of GvHD increases as HLA disparity between the donor and recipient increases. Historically, GvHD has been divided into two major groups, acute and chronic, based on the time in the HSCT course that it was diagnosed. GvHD was considered acute if diagnosed prior to 100 days post-HSCT, whereas patients were diagnosed with chronic GvHD after 100 days post-HSCT regardless of the presentation. With the advent of alternative donor HSCT approaches, including umbilical cord blood transplantation (UCBT), the use of reduced-intensity conditioning regimens, and donor lymphocyte infusion(s) after HSCT, it has become apparent that GvHD needs to be categorized based on its characteristics and not strictly by the timeline of presentation. While the management of both acute and chronic GvHD is based on immunosuppression mostly directed at T-cells, the approach to treatment and monitoring is different. Acute and chronic GvHD have overlap in their target organs (i.e., skin, liver, GI tract, and lungs). However, their effect on these target organs differs. In addition, chronic GvHD may affect every organ system, although most have 1–3 organ systems involved. Chronic GvHD acts like an autoimmune disease and often is treated as such, with corticosteroids considered first-line treatment. Because of the natural history

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as well as treatment with immunosuppression of chronic GvHD, patients with chronic GvHD are very vulnerable to infection, particularly opportunistic and life-threatening, and infection is the leading cause of death of patients with chronic GvHD. Management of chronic GvHD requires a multidisciplinary team approach; early recognition along with appropriate, comprehensive intervention and supportive care will help curtail long-term complications and disability. This chapter addresses the risk factors, clinical features, diagnostic criteria, classification, and the management of chronic GvHD. Chapter 18 discusses acute GvHD.

Overview

Graft-versus-host disease (GvHD) is a complication unique to allogeneic HSCT. GvHD results when the infused donor hematopoietic stem cells (i.e., graft) recognize the recipient (i.e., host) as foreign tissue. Donor-derived T-cells (T-lymphocytes) attack and damage recipient (host) tissues, sometimes irreversibly.

GvHD is classified as acute or chronic. Classically, this determination has been made on the basis of the time at which GvHD occurs after HSCT. Clinical manifestations that occur before day 100 post-HSCT are often designated as acute GvHD. Chronic GvHD is a set of clinical manifestations that occur 100 or more days post-HSCT. Historically, a clear distinction was drawn between an early acute form of GvHD and a delayed chronic form of GvHD. However, recent observations of patients receiving an unrelated umbilical cord blood transplant (UCBT), reduced-intensity conditioning regimen, and donor lymphocyte infusion after HSCT confirmed that acute GvHD can occur several months after allogeneic SCT and that the classic characteristics of chronic GvHD can occur as early as 2 months after HSCT [1, 2]. There is growing recognition that acute and chronic GvHD are best differentiated by their features rather than the time at which they occur because they are pathophysiologically different.

A new paradigm for identifying acute and chronic GvHD and for diagnosing and staging chronic GvHD [1, 2] includes classic acute GvHD (i.e., maculopapular rash, nausea, vomiting or diarrhea, and elevated liver function test results); persistent, recurrent, or late acute GvHD (i.e., features of acute GvHD occurring beyond 100 days, often during withdrawal of immunosuppression); classic chronic GvHD without features of acute GvHD; and an overlap syndrome that includes the diagnostic or distinctive features of chronic GvHD and acute GvHD [3].

In general, the incidence of acute and chronic GvHD is 30–60% in cases involving histocompatible, sibling-matched allografts, with more GvHD occurring with greater HLA mismatches between the donor and recipient. The mortality rate directly or indirectly related to GvHD may reach 50% [1]. The risk factors for chronic GvHD include histoincompatibility between the donor and recipient including sex mismatching, donor parity, older age at the time of HSCT, post-HSCT infection (i.e., viral infections), the use of donor lymphocyte infusions after transplantation, and the type of GvHD prophylaxis used and will be addressed in more detail below [4].

Chronic Graft-Versus-Host Disease (GvHD)

Introduction/Incidence

Chronic GvHD typically occurs 100–400 days after HSCT, although it can begin as early as 45 days post-HSCT. It can be a debilitating, chronic condition that mimics autoimmune disease. Chronic GvHD usually occurs in patients who have had acute GvHD (termed progressive chronic GvHD), although it can occur in the absence of acute GvHD (termed de novo chronic GvHD). Chronic GvHD can occur after the resolution of acute GvHD and is termed quiescent

chronic GvHD. Progressive chronic GvHD accounts for approximately 45% of all patients with chronic GvHD, whereas 12% have de novo and 43% have quiescent chronic GvHD. In patients who survived 150 days after allogeneic HSCT, chronic GvHD was observed in 33–49% of HLA-identical related donor HSCTs and in 64% of matched unrelated donor HSCTs [5]. The incidence of chronic GvHD is higher among recipients of peripheral blood stem cells (PBSCs) than recipients of bone marrow-derived hematopoietic stem cells.

Risk Factors

Risk factors for chronic GvHD include previous acute GvHD, older recipient age, and sex mismatching (i.e., female donor and male recipient). HLA histoincompatibility and HSC source contribute greatly to the risk of the development of chronic GvHD. The greater the HLA disparity between the donor and recipient, the more likely the patient will develop chronic GvHD. Patients receiving HSCs derived from G-CSF-mobilized peripheral blood are at higher risk for chronic GvHD as compared to bone marrow-derived HSCT recipients. Patients who receive donor lymphocyte infusions (DLI) are at higher risk for going on to develop chronic GvHD.

Differential Diagnosis

The differential diagnosis is broad and differs for each organ system that can be involved in chronic GvHD. Because other conditions, particularly infections, can mimic chronic GvHD, a systematic, multidisciplinary evaluation of the patient with chronic GvHD is essential.

Pathophysiology

Chronic GvHD results from donor T-cells' recognition of the recipient's antigens and cells as foreign (i.e. T-cell alloreactivity). Thus, alloreactive T-cells are thought to play a key role in chronic GvHD pathogenesis which is supported by the observation that chronic GvHD is extremely rare after autologous or identical twin HSCTs. In chronic GvHD, T-cells recognize not only the major human leukocyte antigens (HLA) (which are the major antigens to which the donor and recipient are typed and determined to "match" prior to HSCT) but also the minor HLA antigens that are not usually matched. Four theories regarding the pathophysiology of chronic GvHD have been derived from experimental data. These include (1) damage to the thymus that results in defective negative selection of T-cells; (2) deficiencies in regulatory T-cell number and function; (3) the presence of B-cells that produce autoantibodies, secrete aberrant cytokines, and present improper antigens; and (4) the formation of profibrotic lesions [6]. The activated immune response in chronic GvHD continues on without the normal tolerance-promoting mechanisms regulated by central (thymic) or peripheral elimination of allo- and autoreactive T-cells. This dysregulated, pathologic immune response leads to the direct attack of target tissues by cytotoxic T-cells as well as the secretion of pro-inflammatory and profibrotic cytokines that can eventually end in irreversible organ damage. Regulatory T-cells play an important role in dampening the immune response. It is thought that regulatory T-cell deficiencies thus play a role in the pathophysiology of chronic GvHD. Aberrant B-cells may contribute to chronic GvHD by cytokine production, antigen presentation, and immune regulation. Thus, chronic GvHD is a disease of immune dysregulation predominantly involving T-cells, but it involves other immune cell types as well.

Clinical Features

Essentially, every organ system can be affected by chronic GvHD. A list of the systems known to be involved in chronic GvHD along with a summary of the clinical manifestations, screening, and diagnostic studies and interventions by organ system is compiled in Table 19.1. Manifestations

Table 19.1 Clinical manifestations, screening, and interventions of chronic GvHD by organ system [1, 2]

Organ system	Clinical manifestations	Screening studies or evaluation	Interventions	Special considerations
Dermal	• Dyspigmentation • Xerosis (dryness) • Hyperkeratosis • Pruritus • Scleroderma • Lichenification • Onychodystrophy (nail ridging or nail loss) • Alopecia	• Thorough clinical examination • Skin biopsy: 3-mm punch biopsy	• Immunosuppressive therapy • Psoralen and ultraviolet A radiation (PUVA) • Topical with steroid creams, moisturizers or emollients, antibacterial ointments to prevent superinfection	• Avoid sunlight exposure • When outdoors, use sunblock and wear a large hat that shades the face
Oral	• Lichen planus • Xerostomia • Ulceration	• Oral biopsy	• Steroid mouth rinses • PUVA • Pilocarpine and anethole trithione for xerostomia • Fluoride gels or rinses	• Careful attention to oral hygiene • Regular dental evaluations
Ocular	• Keratoconjunctivitis • Sicca syndrome • Dry eyes • Blurry vision • Eye irritation • Eye pain • Photophobia	• Schirmer test • Regular ophthalmologic evaluation • Slit-lamp test	• Preservative-free artificial tears • Restasis (cyclosporine eye drops) • Temporary or permanent lacrimal duct occlusion	
Pulmonary	• Shortness of breath • Cough • Dyspnea • Wheezing • Fatigue • Hypoxia • Pleural effusion	• Pulmonary function tests • Peak flow monitoring • Arterial blood gas • High-resolution computed tomography of the chest	• Prevention and aggressive treatment of pulmonary infections	• Aggressively investigate changes in pulmonary function because they may represent GvHD of lung (bronchiolitis obliterans syndrome) or bronchiolitis obliterans organizing pneumonia
Hepatic	• Jaundice • Abdominal pain	• Liver function tests	• Actigall	

Gastrointestinal/ nutritional	• Nausea • Odynophagia • Dysphagia • Anorexia • Early satiety • Malabsorption • Diarrhea • Weight loss • Pancreatic insufficiency • Protein and calorie deficiency • Sensitivity to mint, spicy foods, or tomatoes • Dehydration • Muscle wasting	• Esophagogastroduodenoscopy • Colonoscopy • Nutritional assessment • Fecal studies • Fat store measurement • Prealbumin	• Referral to gastroenterologist • Nutritional monitoring and support • Pancreatic enzyme supplementation	• Need to exclude infections • Monitor weight closely
Genitourinary	• Vaginal sicca • Vaginal atrophy, stenosis, or inflammation	• Pelvic examination	• Intravaginal topical steroid cream (efficacy is being evaluated)	
Immunologic	• Hypogammaglobulinemia • Autoimmune syndromes • Cytopenias • Recurrent infections – Cytomegalovirus – Herpes simplex virus – Varicella-zoster virus – Fungi – *Pneumocystis jirovecii* – Encapsulated bacteria • Poor wound healing	• Quantitative immunoglobulin levels • CD4+/CD8+ T-lymphocyte subsets	• Intravenous immunoglobulin • Prophylactic antimicrobials for prophylaxis against PJP and encapsulated organisms • Surveillance for cytomegalovirus reactivation	• Immunologic complications are due to the pathophysiology of chronic GvHD (dysfunctional immune system) and due to treatment of chronic GvHD with immunosuppression (different aspect of immune dysfunction)
Musculoskeletal	• Joint stiffness • Contractures • Debility • Muscle cramps	• Performance status • Formal assessment of quality of life • Rehabilitation needs	• Physical therapy	• Close monitoring for contractures and early intervention with physical therapy are essential to halt progression of joint decreased range of motion

Data from Dhir, S., Slatter, M., & Skinner, R. (2014). Recent advances in the management of graft versus host disease. Archives of Diseases in Childhood, 99(12), 1150–1157; Mitchell, S. A. (2013). Graft versus host disease. In S. A. Ezzone (Ed.), Peripheral blood stem cell transplant: Guidelines for oncology nursing practice (pp. 103–157). Pittsburgh, PA: Oncology Nursing Society Press

are most commonly observed in the skin, mouth, and eyes with the liver, gastrointestinal (GI) tract, lungs, and musculoskeletal system often involved. While Table 19.1 provides a comprehensive summary of chronic GvHD, the sections below highlight the most notable clinical manifestations of chronic GvHD by organ system.

Skin: Up to 80% of patients with chronic GvHD have skin involvement. Patients may present with erythematous, itching, burning, dry, flaky skin with or without ulcerations, In general, chronic GvHD of the skin has two types: sclerodermatous (most common) and lichenoid. In many cases, patients have both types forming a puckered appearance. Initially, skin may be erythematous with areas of plaque formation and/or desquamation which often progresses to hyper- or hypopigmentation and a tightening of the skin with atrophy that results in a shiny, sclerotic, hide-like appearance. These fibrotic, sclerodermatous changes often lead to joint contractures which can be quite debilitating, particularly without early intervention with physical therapy. Figure 19.1 is an example of sclerodermatous cutaneous manifestation of chronic GvHD. The manifestations of chronic GvHD of the nails and hair are discussed in Chap. 25.

Mouth: Patients with chronic GvHD involving the mouth often develop xerostomia (dry mouth), taste disturbances including sensitivity to acidic and/or spicy foods, and oral pain. The mucosal changes range from erythema to lichenoid changes and ulcerations as depicted in Fig. 19.2. The oral mucosa may have a white, lacy striated pattern to plaque-like lesions on the buccal mucosa and/or tongue that mimics oral candidiasis. Patients may have a smooth tongue as well. Sclerodermatous fibrosis of the oral cavity can often limit the patient's ability to open his/her mouth fully. These findings interfere with the patient's ability to maintain his/her nutritional status, increase their risk for dental caries, and are associated with increased risk for infection.

Eyes: Ocular involvement usually begins with excessive tearing with progression to a burning or gritty sensation with or without photophobia. Over time, dry eye syndrome develops. Keratitis and scarring may occur that may lead to blind-

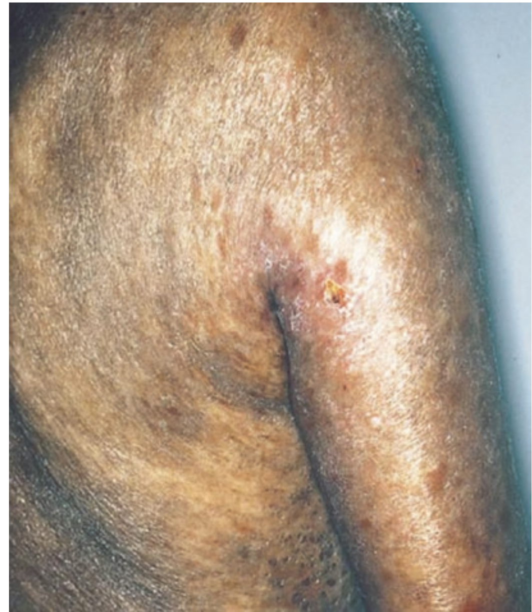

Fig. 19.1 Sclerodermatous skin manifestation of chronic GvHD. This is an example of sclerodermatous changes that can be seen with chronic GvHD of the skin. Notable is the leathery, shiny, dry, flaky appearance with areas of skin breakdown. Both hypo- and hyperpigmentation are present (Courtesy Terry Wikle Shapiro, Penn State Pediatric Stem Cell Transplant Program, Hershey, PA)

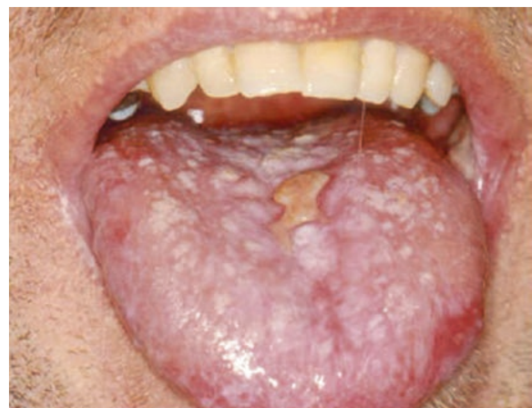

Fig. 19.2 Chronic GvHD of the mouth. Note the large patchy areas of white lichenoid hyperkeratosis with an area of ulceration on the tongue. Note the thick saliva consistent with salivary gland dysfunction due to chronic GvHD. Additionally, note the decrease oral range of motion (Courtesy Terry Wikle Shapiro, Penn State Pediatric Stem Cell Transplant Program, Hershey, PA)

ness. Complications of ocular chronic GvHD are also discussed in Chap. 24.

Liver: Most often, liver chronic GvHD does not become clinically apparent until the involvement becomes severe. It typically presents as a cholestatic obstructive pattern with jaundice, mild hepatomegaly, coagulopathy, elevated alkaline phosphatase, elevated transaminases, and elevated (direct) bilirubin. It is essential to eliminate infection as the etiology of hepatic inflammation prior to the initiation of immunosuppression to treat liver GvHD because treatment with immunosuppression in the context of an undiagnosed infection will most likely result in a potentially fatal infection.

GI tract: Both the upper and lower GI tracts can be affected by chronic GvHD. Overall, patients with lower GI (or gut) chronic GvHD have a wasting syndrome of malabsorption, weight loss, a poor performance score, and progressive GI symptoms. Upper GI tract involvement often is manifested by nausea, vomiting, early satiety, dysphasia, and anorexia, whereas lower GI tract involvement is associated with diarrhea, abdominal pain, and cramping. While diarrhea is the most common manifestation of lower GI tract chronic GvHD, patients with chronic GvHD may have pancreatic exocrine insufficiency with steatorrhea as well.

Sinopulmonary: Sinopulmonary manifestations are found in 5% to 10% of patients with chronic GvHD. Sinusitis is a common complication of chronic GvHD and results from sicca syndrome (dry, burning, itchy eyes). Bronchiolitis obliterans (BO) and bronchiolitis obliterans organizing pneumonia (BOOP) are thought to be pulmonary manifestations of chronic GvHD and are discussed in Chap. 21.

Musculoskeletal: Musculoskeletal involvement of chronic GvHD is directly caused by severe sclerodermatous skin chronic GvHD and is manifested by joint stiffness, contractures, and/or pain with limited range of motion. Patients can also have joint swelling, arthralgias, muscle cramps, muscle weakness, and carpal spasm. The degree of contractures can be very significant and debilitating. Patients can also develop fasciitis (as manifested by taut, irregularly thickened skin with depressed areas) and myositis (as manifested by moderate to severe proximal muscle weakness, myalgias, fever, skin contractures, and skin induration).

Diagnostic Studies and Grading

Chronic GvHD is graded as mild, moderate, or severe. Table 19.2 outlines the first National Institutes of Health (NIH) consensus publication with proposed diagnostic criteria and an improved classification for chronic GvHD [4, 7]. Clinical manifestations for making the clinical diagnosis of chronic GvHD are classified as diagnostic (which is sufficient to establish the diagnosis of chronic GvHD), distinctive (insufficient alone to establish a diagnosis of chronic GvHD), or common (seen in both acute and chronic GvHD). For example,

Table 19.2 Grading of chronic graft-versus-host disease severity

Severity	Definition[a]
Mild	Involves one or two organs or sites (except the lung), with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites)
Moderate	At least one organ or site with clinically significant impairment but no major disability (maximum score of 2 in any affected organ or site) or three or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites) or lung with a score of 1
Severe	Major disability caused by chronic graft-versus-host disease (score of 3 in any affected organ or site) or a lung score of ≥2

Data from Dhir, S., Slatter, M., & Skinner, R. (2014). Recent advances in the management of graft versus host disease. Archives of Diseases in Childhood, 99(12), 1150–1157; Flowers, M. E. D., & Martin, P. (2015). How we treat chronic graft versus host disease. Blood, 125(4), 606–615; Jacobsohn, D. (2010). Optimal management of chronic graft-versus-host disease in children. British Journal of Haematology, 150:278–292

[a]Each organ is scored between 0 and 3 depending on physical manifestations and disabilities caused by GvHD (0, no symptoms/signs; 1 to 3, increasingly severe symptoms, signs, or abnormal studies). These scores and the number of organs or sites involved are used to grade the overall severity

poikiloderma, lichen planus-like, sclerotic, morphea-like, and lichen sclerosis-like skin manifestations are all considered diagnostic, whereas depigmentation and papulosquamous lesions are distinctive skin findings of chronic GvHD. In contrast, because the histology and clinical manifestations of the liver are indistinguishable between acute and chronic GvHD, the diagnosis of chronic GvHD cannot be made by liver biopsy alone and requires a distinctive clinical manifestation in at least one other organ system. Other manifestations, such as sweat impairment, thrombocytopenia, eosinophilia, lymphopenia, pericardial or pleural effusions, thinning, or premature graying of the hair without another explanation, are considered manifestations of chronic GvHD as long as the diagnosis has already been confirmed. For assessing the severity of chronic GvHD, each designated organ or site (i.e., skin, mouth, eyes, GI tract, liver, lung, joints, genital tract, and performance score) is assigned a grade between 0 and 3 according to the clinical manifestations and resultant disability. Then, an overall grade of mild, moderate, or severe is assigned according to the extent of involvement of each organ. The grade has clinical relevance because moderate chronic GvHD implies at least one organ with clinically significant features but without major disability, whereas severe chronic GvHD (with a score of 3 in at least one organ) reflects major disability and thus increasing the risk of mortality [8].

Biopsies should be performed of the tissue if the diagnosis of chronic GvHD is in question or if another diagnosis, such as infection, needs to be eliminated. A typical biopsy of skin GvHD shows thinning or loss of the dermal layer with evidence of fibrosis. Also, there is significant loss of hair follicles and sweat glands which are normally contained within the dermis. Epidermal atrophy is also present. In patients with liver involvement of chronic GvHD, a liver biopsy shows features of degeneration with disorganized epithelial lining of the bile ducts, shrunken bile duct size, and generally cells ranging of abnormal size and shape. In biopsies of gut chronic GvHD, apoptosis of the glands is present or missing altogether.

Management and Outcome

Poor prognostic indicators for the outcome of chronic GvHD include persistent severe thrombocytopenia, lichenoid skin changes, serum bilirubin >1.2 mg/dL, progressive onset from acute to chronic GvHD, and a Karnofsky scale <70%. The major cause of chronic GvHD-associated mortality is related to infection with up to 60% dying from infectious complications. Early recognition and treatment of chronic GvHD before disability ensues is critical. The goal of the management of chronic GvHD is to maximize the benefits of treatment while minimizing the side effects. The treatment is also tailored to the affected organ(s). The mainstay treatment of chronic GvHD is the use of corticosteroids often in combination with other immunosuppression agents, including calcineurin inhibitors (e.g., cyclosporine, tacrolimus), azathioprine, sirolimus, mycophenolate mofetil (MMF), rituximab, pentostatin, hydroxychloroquine, methotrexate, and extracorporeal photopheresis [1–4, 9].

Primary therapy for mild chronic GvHD includes topical and oral corticosteroids, cyclosporine, or tacrolimus. Recognizing and treating chronic GvHD early is crucial. Prednisone at a dose of 1 mg/kg every other day as monotherapy decreases treatment-related mortality rates as compared to prednisone combined with azathioprine (21% vs 40%, respectively), which is associated with a survival rate of 61% in patients with chronic GvHD without thrombocytopenia. Patients refractory to first-line therapies or those with moderate to severe chronic GvHD may be placed on azathioprine alternating with cyclosporine, corticosteroids, or thalidomide [10, 11]. The addition of cyclosporine at a dose of 6 mg given every 12 h every other day in patients at with GvHD with thrombocytopenia may improve survival rates from 26% to 52%. It may also improve functional performance to near-normal in long-term survivors by significantly decreasing the incidence of disabling scleroderma. The addition of tacrolimus to prednisone was associated with a high response rate of 72% but led to a high chronic GvHD-

related mortality (34%) and a significant need for salvage therapy (47%). Thalidomide has been reported as effective primary treatment for chronic GvHD because of its TNF-modulating effect. The 3-year survival rate is about 48%, with a diminished incidence of infection in long-term survivors [4]. The most commonly used immunosuppressive agents for chronic GvHD and their associated specific considerations are presented in Table 19.3.

Treatment of Steroid-Refractory Chronic GvHD

Often alternative therapies are employed in cases of steroid-refractory chronic GvHD. These include mycophenolate mofetil, rituximab, pentostatin, hydroxychloroquine, methotrexate, and extracorporeal photopheresis. Specific interventions are used depending upon the organ system involved. For example, cyclosporine eye drops

Table 19.3 Selected immunosuppressants used for post-allogeneic HSCT chronic GvHD and their associated specific considerations [2, 4, 7, 12–14]

Agent	Specific considerations
• Calcineurin inhibitors – Cyclosporine – Tacrolimus	• Therapeutic drug window is relatively narrow, so the monitoring of drug trough levels is critical • Cyclosporine and tacrolimus trough levels should be drawn before administration of morning dose • Monitor calcineurin inhibitor levels carefully in patients with renal or hepatic dysfunction • Dosing should be adjusted for renal dysfunction • Monitor serum creatinine, blood urea nitrogen, potassium, magnesium, glucose, and triglyceride levels • Replete electrolytes as indicated • Drug-drug interactions can lead to subtherapeutic or toxic cyclosporine or tacrolimus levels; patients need to advise their healthcare providers of changes made in concurrent medications • Potassium-sparing diuretics should be avoided • Grapefruit juice or grapefruit-containing products should be avoided due to interference with calcineurin inhibitor pharmacokinetics • Bioavailability differs for the oral solution and capsule formulation; after a regimen is established, patients should be instructed not to change their formulation or brand • Patients need to notify the HSCT healthcare team immediately if unable to take because of gastrointestinal side effects • Can take with or without food but must be consistent • A calcineurin inhibitor should be discontinued for at least 24 hours before starting another calcineurin inhibitor
• Corticosteroids	• Monitor serum chemistries and glucose • Monitor closely for corticosteroid-induced hyperglycemia; instruct patients in strategies to prevent and treat hyperglycemia; may need to consult pediatric endocrine team for diabetes education • Patients treated with corticosteroids (>0.5 mg/ kg/day) need antiviral, antibacterial, and antifungal prophylaxis • Patients who have visual changes need to be referred to ophthalmology • Consult physical therapy for devising a proximal muscle strengthening exercise program • Patients on long-term steroids are at risk for osteopenia and need to undergo regular dual-energy x-ray absorptiometric (DEXA) scans, receive calcium and vitamin D supplementation, and may require specific treatment for osteopenia with antiresorptive agents, such as alendronate (Fosamax) • May increase tacrolimus or cyclosporine levels • Administer oral corticosteroids with food or milk to minimize gastrointestinal upset • Administer H2-blockers or proton pump inhibitors to decrease gastric acidity

(continued)

Table 19.3 (continued)

Agent	Specific considerations
• Mycophenolate mofetil	• Monitor complete blood cell count at regular intervals, and adjust dosage for pancytopenia accordingly • Monitor liver function tests (i.e., bilirubin and serum transaminases) at regular intervals, and adjust dosage for liver function abnormalities accordingly • Monitor plasma levels of mycophenolic acid (i.e., metabolite of mycophenolate mofetil) to guide treatment of patients with renal dysfunction • There may be decreased absorption of MMF when co-administered with magnesium oxide, aluminum- or magnesium-containing antacids, or cholestyramine • Should be taken on an empty stomach
• Azathioprine	• Use with caution in patients with hepatic or renal impairment • May lead to anemia and leukopenia when given with angiotensin-converting enzyme inhibitors; synergistic with other bone marrow suppressants • Teratogenic and so patients and their partners need to use effective forms of contraception
• Infliximab (Remicade)	• Used mostly for management of gut GvHD • Monitor patient for development of infusion-related toxicities and have medications (e.g., acetaminophen, antihistamines, corticosteroids, epinephrine) and supplemental oxygen for treating hypersensitivity reactions immediately available in the event of a reaction • Consider premedication with acetaminophen and diphenhydramine
• Anti-thymocyte globulin – Equine (Atgam) – Rabbit (Thymoglobulin)	• Monitor patient closely during and after infusion for signs of serum sickness and anaphylaxis • Medications for treating hypersensitivity reactions should be immediately available in the event of a reaction • Consider premedication with corticosteroids, acetaminophen, and H1- and H2-blockers • Medications for treating hypersensitivity reactions (e.g., acetaminophen, antihistamines, corticosteroids, epinephrine) and supplemental oxygen should be available for immediate use in the event of a reaction • Evaluate need for blood pressure support (e.g., fluid boluses, dopamine, dobutamine) • Because transient and sometimes severe thrombocytopenia may occur after antithymocyte globulin administration in patients with platelet counts less than 100,000/μL, the platelet count should be evaluated 1 hour after administration and as ordered and platelets transfused as indicated • Consider starting antifungal and antiviral prophylaxis because of the significant blunting of T-cell function
• Alemtuzumab (Campath-1)	• Monitor patient for development of infusion-related toxicities, and premedicate patient with acetaminophen and diphenhydramine • Consider treatment with meperidine to control infusion-related rigors • Administer fluid bolus as needed to treat hypotension • Because alemtuzumab reduces rapid and prolonged lymphopenia, patients need to be on broad antifungal, antibacterial, antiviral, and antiprotozoal prophylaxis for at least 4 months after treatment and undergo close surveillance for cytomegalovirus infection
• Sirolimus (Rapamycin)	• May suppress hematopoietic recovery if used in patients who have recently undergone allogeneic HSCT • Monitor sirolimus levels (typical target trough level is 5 to 15) • Like calcineurin inhibitors, sirolimus is metabolized through the cytochrome P450-3A system and so need to anticipate related drug-drug interactions • Oral bioavailability is variable and may be improved when administered with a high-fat meal

Table 19.3 (continued)

Agent	Specific considerations
• Thalidomide	• Thalidomide should not be started if the absolute neutrophil count is less than 750/mm^3, and therapy should be reevaluated if the absolute neutrophil count drops below this level • Thalidomide is a potent teratogen and is contraindicated in patients who are or who are likely to become pregnant. A systematic counseling and education program, written informed consent, and participation in a confidential survey program at the start of treatment and throughout treatment are required for all patients receiving thalidomide. Men and women who are of childbearing potential must practice protected sex while on this drug • Perform pregnancy test before initiating treatment and periodically throughout treatment course • Obtain baseline electrocardiogram before treatment • Avoid thalidomide with other drugs that can cause drowsiness or neuropathy • Administer doses in the evening to minimize impact of drowsiness on lifestyle and safety • May cause lightheadedness, peripheral neuropathy (including numbness or tingling in the hands or feet), and skin rashes or ulcerations that require immediate cessation of the drug until the patient can be evaluated • Often causes constipation; administer a stool softener or mild laxative • Avoid exposure to ultraviolet light or sunlight; use sunscreen liberally and wear protective clothing
• Methoxsalen (Oxsoralen)	• Toxicity increases with concurrent use of phenothiazines, thiazides, and sulfanilamides • Patients who have received cytotoxic chemotherapy or radiation therapy and who are taking methoxsalen are at increased risk for skin cancers • Severe burns may occur from sunlight or ultraviolet A exposure • Pretreatment eye examinations are indicated to evaluate for cataracts. Repeat eye examinations should be performed every 6 months while patients are undergoing psoralen and ultraviolet A therapy • Take methoxsalen with milk or food and to divide the dose into two portions, taken approximately one-half hour apart

Data from Appelbaum, F, Forman, S. J., Negrin, R. S., & Blume, K. G. (2009). Baird, K., Steinberg, S. M., Grkovic, L., Pulanic, D., Cowen, E. W., Mitchell, S. A.,. Deeg, H. J. (2009), Pavletic, S. Z. (2013). National Institutes of Health chronic graft-versus-host disease staging in severely affected patients: Organ and global scoring correlated with established indicators of disease severity and prognosis. Biology of Blood and Marrow Transplantation, 19(4), 632–639; Flowers, M. E. D., & Martin, P. (2015). How we treat chronic graft versus host disease. Blood, 125(4), 606–615; Mitchell, S. A. (2013). Graft versus host disease. In S. A. Ezzone (Ed.). Peripheral blood stem cell transplant: Guidelines for oncology nursing practice (pp. 103–157). Pittsburgh, PA: Oncology Nursing Society Press

are used for ocular involvement. Clofazimine, an anti-leprosy agent, has been effective in treating cutaneous and oral lesions of chronic GvHD and may be useful as a steroid-sparing agent.

MMF is the most commonly used agent to treat steroid-refractory chronic GvHD. Responses of 90% and 75% in first- and second-line settings are seen when MMF is added to standard regimens that contain combinations of tacrolimus, cyclosporine, and prednisone [4]. Psoralen and ultraviolet A radiation (PUVA) therapy play a role for patients with refractory cutaneous chronic GvHD. Extracorporeal photopheresis (ECP), a modification of PUVA treatment, has also shown benefit, with the best responses in patients with skin, liver, eye, and oral mucosa involvement [7, 10]. The anti-CD20 monoclonal antibody rituximab has also been used effectively for musculoskeletal and cutaneous chronic GvHD, with durable responses 1 year after initiation, and it provided a 75% reduction in steroid doses. Alternatively, intravenous pentostatin given every 2 weeks for 6 months has been shown to produce 50% response rates among patients with chronic GvHD who failed two prior immunosuppressive regimens [9, 15]. Another alterna-

tive agent is imatinib because patients with refractory GvHD with fibrotic features have been noted to have antibodies that activate the platelet-derived growth factor receptor pathway which is blocked by imatinib [4].

Drug levels of cyclosporine and tacrolimus need to be monitored at regular intervals and dosing adjusted to maintain levels within the therapeutic range. The target range varies depending upon the underlying indication for which the allogeneic HSCT was performed. In general, levels in the higher range are maintained in patients with nonmalignant conditions because the patient gains no benefit from having GvHD (a priori graft-versus-malignancy effect). Conversely, patients treated with an allogeneic HSCT for malignancies tend to require lower drug trough levels in order to maximize GVM effect.

Because many drug-drug interactions are associated with cyclosporine and tacrolimus, it is important to regularly review the patient's medication profile to identify potentially deleterious interactions. For example, −azole class of drugs can significantly increase calcineurin inhibitor drug levels; some recommend decreasing calcineurin drug dosing to 10–50% of the current dose in order to minimize toxicity from a supratherapeutic level.

Patients need to take their immunosuppressive medications exactly as instructed; they need to contact their transplant provider before starting or discontinuing any new medications.

Because sun exposure may activate or exacerbate GvHD of the skin, patients should avoid direct sun exposure, always apply sunscreen liberally, and wear protective clothing whenever possible.

Chronic GvHD Supportive Measures

Chronic GvHD is the major cause of significant morbidity and mortality after allogeneic HSCT. The two most important factors that contribute to the morbidity and mortality associated with chronic GvHD are (1) a dysfunctional immune system that is not only inherent to GHVD but also exacerbated by its treatment with immunosuppressant and (2) poor nutritional status associated with chronic GvHD. In fact, GvHD was first referred to as "wasting syndrome" due to the patient's inability to gain adequate weight, loss of muscle mass, changes in hair and nails, and poor wound healing capacity. Supportive care measures such as infection prophylaxis, nutritional management, and coordinated multidisciplinary care are essential to improve longevity and quality of life for patients with chronic GvHD [1, 2, 4, 5, 7, 9, 11, 16].

Infection prophylaxis: Patients with chronic GvHD are very susceptible to encapsulated bacteria, and the institution of prophylaxis with oral penicillin has dramatically reduced the risk of life-threatening infections from these organisms. Antiviral prophylaxis against HSV, VZV, and CMV can prevent oropharyngeal infection and interstitial pneumonia in patients with active GvHD who require long-term immunosuppression. Antifungal agents that cover fungi and mold species are also needed to help prevent fungal infections in patients with chronic GvHD on active treatment due to their profound immunosuppressive state.

Nutritional supportive care: Mouth sores and other changes in the oral mucosa may cause the patient to be exquisitely sensitive to spicy, acidic, and mint-containing foods, contributing to poor nutrition due to decreased oral intake. Pain control with analgesics for patients with mouth sores results in increased oral intake. Oral beclomethasone can improve oral intake, nausea, and diarrhea without causing systemic or local toxicity. Often, patients will require supplemental enteral feeds in order to maintain adequate daily caloric intake which is increased due to their increased metabolic needs from chronic GvHD. Pilocarpine (Salagen) is used for oral sicca manifestations.

Ocular supportive care: Patients with chronic GvHD often have ocular involvement with eye pain, irritation, and/or dryness and blurry vision. Treatment with artificial tears and cyclosporine eye drops (Restasis) are effective in reducing ocular symptoms. Retinoic acid is used for ocular sicca syndrome.

Musculoskeletal supportive care: Clonazepam may be used to treat musculoskeletal manifestations (e.g., muscular aches, cramping, carpal spasm). Patients receiving chronic corticosteroid therapy

are at risk for osteoporosis and fractures and thus need to have vitamin D levels monitored. Often, patients will require calcium and vitamin D supplementation to improve bone health.

Endocrine-related supportive care: For patients on long-term steroid therapy or for female patients, estrogen replacement, calcium supplements, and anti-osteoporosis agents (e.g., alendronate (Fosamax), calcitonin) should be considered.

Supportive skin care: A skin care specialist may be needed for moderate to severe cutaneous chronic GvHD [2, 11, 16]. Because of poor wound healing, any skin injury needs to be evaluated and monitored closely, particularly for signs or symptoms of infection. Also, patients with chronic skin GvHD need to avoid direct sun exposure and use sunscreen liberally.

Specialists in dentistry or oral medicine, dermatology, endocrinology, gynecology, ophthalmology, pulmonology, nutrition, physical therapy, and occupational therapy are essential in caring for patients with acute or chronic GvHD.

Chronic GvHD is a primary factor in late transplantation-related morbidity and mortality (TRM). In addition, patients with chronic GvHD have psychosocial issues due to abnormalities of growth and development in children, and a decrease in functional performance status. These factors along with other manifestations of chronic GvHD lead patients to suffer from depression and anxiety, present with somatic symptoms, lack sexual satisfaction, and have difficulty in maintaining stable employment as an adult. These all contribute to a decreased quality of life. Support groups, individual and family psychotherapy, physical therapy, occupational therapy, and preventive and preemptive rehabilitation may help to prevent functional decline and emotional distress, thereby improving quality of life [2, 8].

Complicating care is the fact that by the time chronic GvHD develops, many patients have returned to their local community and are at a distance from healthcare providers with expertise in the identification and management of the diverse manifestations of chronic GvHD. Thus, it is imperative that all involved healthcare providers be aware of the signs and symptoms of chronic GvHD as well as being proactive in assessing these patients for more subtle signs/symptoms of depression and anxiety.

Alternative approaches to the prevention and treatment of GvHD (both acute and chronic) are being investigated in the preclinical and clinical setting. They include the application of cytokine shields to decrease the inflammatory responses thought to promote acute GvHD. Another area of active research is the identification of GvHD biomarkers and more selective methods of T-cell depletion of the HSC product as well as other graft engineering strategies. Gene transfer technologies are promising tools for manipulating donor T-cell immunity to preserve GVM or graft-versus-infection effects while preventing or blunting acute GvHD and are being actively explored [7, 17].

Ruxolitinib, a selective inhibitor of Janus kinase 1 (JAK1) and JAK2, is an approved agent for the treatment of myelofibrosis. JAK1 and JAK2 mediate receptor-mediated signaling for a variety of pro-inflammatory cytokines, including interferon-γ and IL-6, and inhibition of this pathway has been shown to suppress activation and differentiation of dendritic cells as well as T-cells. Pro-inflammatory cytokines are characteristically elevated in both acute and chronic GvHD, and inhibition of JAK1/JAK2 signaling with ruxolitinib has been shown to be effect in the treatment of chronic GvHD [18]. It is currently under investigation in the pediatric HSCT population.

Key Points

- Chronic GvHD remains a common and potentially life-threatening complication of allogeneic HSCT.
- It stems from immune dysfunction that mimics autoimmune disease.
- Chronic GvHD can affect every organ system, but the skin, mouth, and eyes are the most commonly involved sites.
- Chronic GvHD has been reported from 33% to 64% of all patients who received an allogeneic HSCT. The incidence depends upon the degree of HLA histoincompatibility and donor HSC source, with G-CSF-mobilized peripheral blood stem cells affording the highest risk.

- Treatment of these patients requires a multidisciplinary approach that includes management of immunosuppressive therapy, physical therapy, occupational therapy, oral care specialists, and skin care specialists.
- Keys to successful collaborative management include early recognition of chronic GvHD, comprehensive evaluation at the onset and periodically during the course of the disease, prompt institution of systemic and topical treatment, appropriate monitoring of the response, calibration of treatment intensity over time in order to avoid overtreatment or under treatment, and the use of supportive care to prevent complications and disability.
- Infection is the major cause of death in patients with chronic GvHD.
- While chronic GvHD may offer a benefit to patients transplanted to treat an underlying malignancy, i.e., a graft-versus-malignancy effect, there is no advantage of having chronic GvHD in the context of a nonmalignant condition.

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20 Hematologic Complications Associated with HSCT

Mala K. Talekar and Timothy Olson

Abstract

Allogeneic hematopoietic stem cell transplantation (HSCT) is an increasingly used curative treatment modality, not only for hematologic malignancies but also for a variety of primary immunodeficiencies and nonmalignant hematologic disorders. The expanded use of HSCT is due in large part to improvements in supportive care, T cell depletion approaches, immune suppression, and disease-specific conditioning strategies that have led to improved survival for patients receiving HSCT using alternative donors such as unrelated cord blood, matched or mismatched unrelated adult donors, and partially matched or haploidentical-related donors. While significant hematologic complications associated with well-established approaches for myeloablative matched sibling donor (MSD)-HSCT are relatively uncommon, the increasing use of reduced-intensity conditioning and alternative donor HSCT has been associated with a significant rise in hematologic abnormalities following HSCT. These include autoimmune hemolytic anemia, alloimmune hemolysis due to donor/recipient ABO incompatibility, thrombocytopenia, transplant-associated microangiopathy (TAM), and thromboembolism. Thus, pediatric hematology/oncology specialists who care for pediatric patients who have received HSCT need to be aware of the common presentations and treatment

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options for hematologic disorders that occur post-HSCT. These HSCT-associated hematologic complications along with their diagnosis and management are discussed in this chapter.

Hemolytic Anemia Post-HSCT

Introduction

Hemolytic anemia (HA) is defined as anemia resulting from premature destruction of circulating red blood cells (RBCs). Immune-mediated hemolytic anemia (IHA) occurs following the development of red cell antigen-specific antibodies, which in the context of patients who have received hematopoietic stem cell transplantation (HSCT), can be either host-derived (alloimmune) or donor-derived (autoimmune). IHA may be intravascular or extravascular depending upon the class of antibody involved, the ability of bound antibody to fix complement on the RBC surface, and splenic function. Hemolysis can range from mild and asymptomatic to severe, therapy-resistant, and life-threatening. Transfusion requirements resulting from IHA have an equally wide variability.

IHA following a HSCT is a well-recognized complication [1], and ironically both primary and secondary HSCT can be a salvage treatment approach for therapy-resistant IHA [2]. Posttransplant IHA occurs at an incidence of 3–9% [3–6] in contrast to the incidence of IHA in the general population of one to three per 10^5 patients per year [7, 8], and an estimated overall prevalence of 1:80,000 in pediatric patients [9]. Alloimmune hemolytic anemia post-HSCT usually results from major or minor incompatibility between donor and recipient red cell antigens, mainly of the ABO systems, giving rise to reactive alloantibodies (see section on alloimmune hemolysis below). In contrast, autoimmune hemolytic anemia (AIHA) post-HSCT is caused by donor-derived autoantibodies directed against donor RBC antigens. AIHA post-HSCT can occur in children of any age, may develop as early as a few weeks to as late as many months after HSCT, and results in significant morbidity and mortality [4, 10, 11].

Differential Diagnosis

The differential diagnosis of AIHA post-HSCT is quite broad, as it includes many other causes of transfusion-dependent anemia, including relapse of hematologic malignancy, graft failure, infection, treatment-related toxicity, and graft-versus-host disease (GvHD) (Table 20.1). The diagnosis of AIHA is made based on the presence of hemolytic anemia and the serologic evidence of autoantibodies to RBC antigens (Table 20.2). AIHA is sub-classified into three forms based on thermal reactivity of the autoantibodies: (1) warm AIHA (~70% of cases), wherein the direct antiglobulin test (DAT) is positive for immunoglobulin G (IgG) only or IgG plus C3d, (2) cold agglutinin disease (~20% of cases) in which the DAT is positive for C3d with cold agglutinin of I specificity, and (3) mixed form (DAT+ for IgG and C3d, with coexistence of warm autoantibodies and high titer cold agglutinins) [10]. The IgG autoantibodies in warm AIHA react at 37 °C and primarily lead to

Table 20.1 Differential diagnosis of prolonged anemia post-HSCT

(a) Inadequate RBC production

• Graft rejection/failure, partial engraftment
• Relapse of malignant disease
• Infection
  – Viral (EBV, HHV6, parvovirus, adenovirus)
  – Others (atypical mycobacterium, disseminated fungal infection)
• Chronic GvHD/anemia of chronic disease
• Drug-mediated (sulfa drugs, mycophenolate, ganciclovir)

(b) Decreased circulating RBC lifespan

• Chronic blood loss, due to:
  – Thrombocytopenia/coagulopathy
  – GI losses (gastritis, gut GvHD)
• Immune-mediated hemolytic anemia
  – Alloimmune (donor/recipient ABO incompatibility)
  – Autoimmune hemolytic anemia
• Transplant-associated microangiopathy
• Organ sequestration
  – Veno-occlusive disease
  – Other causes of hepatosplenomegaly

Table 20.2 Diagnostic criteria for AIHA

(a) Presence of hemolytic anemia
• Evidence of anemia: complete blood count (CBC) with low hemoglobin, elevated reticulocyte percentage, absolute reticulocyte count • Evidence of hemolysis: high indirect bilirubin, high LDH, low haptoglobin, hemoglobinuria
(b) Positive direct antiglobulin (Coombs) test
• Positive for presence of IgG and/or C3d, IgM • Thermal autoreactivity at 4⁰C or 37⁰C • Autoantibody specificity (anti-Rh for IgG, anti-I in case of IgM)
(c) Presence of spherocytic red blood cells on peripheral blood smear

extravascular hemolysis. In contrast, cold forms of AIHA are mainly due to IgM autoantibodies with optimal thermal reactivity at 4 °C, are able to fix complement and, in most cases, lead to intravascular hemolysis.

Etiology

The pathophysiologic mechanisms that drive AIHA post-HSCT are poorly understood, though it is generally thought to be caused by dysregulated immune responses due to slow maturation of adaptive immunity post-HSCT. Multiple risk factors have been identified for the development of AIHA post-HSCT (Table 20.3), though the strength of risk association for many of these factors varies among the few published series available. Reported HSCT-specific risk factors for development of AIHA include nonmalignant primary disease, use of reduced intensity conditioning including alemtuzumab or antithymocyte globulin serotherapy [12, 13], ex vivo T cell depletion of donor grafts [14, 15], umbilical cord blood transplantation [16], transplantation from a mismatched unrelated or haploidentical-related donor, and GvHD [3]. The common denominator of each of these risk factors is exogenous suppression or graft-intrinsic depletion of T cells, resulting in slowed recovery of functional T cell responses. Autoreactive lymphocytes escaping immature immune tolerance mechanisms have also been implicated [2]. Wen et al. demonstrated in a mouse model that dysregulated immune reconstitution of B and T cells post-HSCT could allow for polyclonal proliferation of hyperactive B lymphocytes that produce antibodies reactive against self-antigens such as those on red cells [17].

Table 20.3 Risk factors for developing AIHA post-HSCT

HSCT factor	High-risk group
• HSCT indication	• Nonmalignant disease
• Conditioning intensity	• Reduced intensity conditioning
• Conditioning agents	• Alemtuzumab, antithymocyte globulin
• Donor type	• Cord blood, mismatched unrelated, haploidentical
• Graft manipulation	• Ex vivo T cell depletion
• Post-HSCT complication	• Chronic GvHD

Diagnosis and Management of AIHA

Although routine surveillance with DAT is not recommended, any post-HSCT patient who develops an unexplained need for red cell transfusions after having achieved transfusion independence, or in whom baseline red cell transfusion needs acutely increase, should undergo an evaluation for AIHA [4]. As described in Table 20.2, this evaluation should include a CBC with reticulocyte count, peripheral blood smear examination, LDH, haptoglobin, urinalysis, liver function studies, and assessment of electrolytes/renal function (to assess for end-organ damage from hemolysis).

AIHA can present with acute, rapid hemolysis leading to a drastic fall in hemoglobin levels which, in life-threatening cases, necessitates timely transfusions using least incompatible red blood cell units. However, due to risks of transfusion-associated hemolysis and subsequent worsening of end-organ damage, packed red blood cell (PRBC) transfusion is generally only indicated for very severe anemia and/or hemodynamic instability. Until the kinetics of RBC destruction and hemoglobin decline for an individual patient presenting with AIHA are known, patients with an initial AIHA presentation post-HSCT require inpatient admission to a hematology or HSCT inpatient service for close

monitoring of hemodynamic status and serial complete blood count (CBC) surveillance.

Due to the wide clinical variability in the presentation of AIHA post-HSCT, including differences in pretransplant disease and immune/organ dysfunction, posttransplant complications including concurrent infections, the presence of GvHD, and the status of ongoing immunosuppressive therapy, treatment recommendations are very difficult to standardize. Available treatment alternatives (Table 20.4) are mostly based on data from case reports or small case series; however, several

Table 20.4 Treatment of autoimmune hemolytic anemia (AIHA)

	Treatment	Dose	Response rate	Risks/complications
First-line therapy	Corticosteroids	• Methylprednisolone 0.5–1 mg/kg q6–q12h • Once Hgb stable, convert to oral prednisone • Once transfusion-free with Hgb >10, slow taper over 4–6 months	*Response rate*: ~70–85% *Cure rate*: ~20–30%	• Hypertension • Diabetes • Osteoporosis • Viral and fungal infections
Second-line therapies	Splenectomy	–	*Response rate*: ~70% *Cure rate*: ~20%	• Surgical complications • Risk of sepsis with encapsulated bacteria • Poor response to revaccination post-HSCT • Increased risk of thromboembolism and pulmonary hypertension
	Rituximab	• Standard: 375 mg/m^2/dose × 4 weeks • Low dose: 100 mg/dose × 4 weeks	*Response rate*: 70–80%, *DFS*: ~70% at 1 year	• Infusion-related events • Risk of infections • Requirement for long-term Ig replacement
	Immunosuppressive drugs (azathioprine, sirolimus, vincristine cyclosporine)	–	*Response rate*: 40–60%	• Myelosuppression • HSCT-associated microangiopathy • Posterior Reversible Encephalopathy syndrome • Impaired wound healing
	Mycophenolate	–	*Response rate*: ~90% in combination with rituximab	• Myelosuppression • GI side effects
	Intravenous immunoglobulin (IVIG)	–	*Response rate*: 40–50%	• Infusion-related reactions Aseptic meningitis
Refractory to previous therapies	Erythropoietin (EPO)	• Therapy refractory AIHA esp. associated with reticulocytopenia	–	–
	High dose cyclophosphamide	• 50 mg/kg/day × 4 days		• Severe risk of fungal/viral infection
	Alemtuzumab	–	*Initial response*: ~70%, *Sustained response*: ~30%	• Infusion-related events • High risk of viral infection
	Second HSCT	–	–	–

[a]Response rates derived from multiple sources [10, 18–21, 24, 25, 27–31, 106–108]

common themes have emerged. In the absence of a concurrent life-threatening infection, initial therapy should include corticosteroids in aggressive dosing regimens, which result in high initial response rates but also significantly high rates of relapse when used as monotherapy.

Recurrent AIHA has been treated with splenectomy; however, this approach entails the risk of limited response to revaccination in post-HSCT patients and lifelong impaired immunity toward encapsulated bacteria. Rituximab is a relatively new agent being used in AIHA post-HSCT, and appears to have efficacy subacutely (over a few weeks) by depleting autoreactive B lymphocytes through CD20 engagement. Its major role may be as a steroid-sparing agent, particularly for patients with significant infection concerns and delayed immune reconstitution such as those receiving T cell depleted grafts [15, 18, 19]. Recent reports have also documented synergistic efficacy of mycophenolate mofetil (MMF) used in combination with rituximab [20]. A number of immunomodulatory agents (azathioprine, cyclosporine, sirolimus, vincristine) have been tried in refractory cases with variable response rates [1, 21, 22]. Intravenous immune globulin (IVIg) may also provide some therapeutic response, but more commonly is used for IgG replacement in any patient with AIHA who receives rituximab. Evidence supporting the use of plasmapheresis, commonly used in certain forms of AIHA occurring in the general population, specifically in post-HSCT AIHA is limited to isolated case reports [23]. Additional salvage approaches have been attempted in cases refractory to other modalities, including erythropoietin [24], cyclophosphamide [25], bortezomib [26], alemtuzumab [27, 28], total lymphoid irradiation [11], and even second HSCT [24, 25, 27–31].

All patients who develop AIHA post-HSCT should be treated in direct consultation with the HSCT center who performed the HSCT. Additionally, it is recommended that all patients with AIHA who are refractory to or relapse after corticosteroid therapy should be transferred to a center with experience treating AIHA in the context of HSCT, as the therapies and associated toxicities for patients with refractory AIHA require intensive management and considerable expertise in order to prevent deleterious outcomes. Even with optimal therapy in expert centers, refractory AIHA carries significant mortality risk of about 35% in pediatric patients [22]. In summary, AIHA occurring post-HSCT is associated with a number of specific risk factors, may have a variable course, is often therapy-resistant, and results in significant morbidity and mortality. Outcomes can be improved by early recognition, early initiation of appropriate therapy, judicious use of transfusion support, and achieving a delicate balance in terms of intensity and duration of immune suppression therapy in order to prevent recurrence of AIHA, but also to avoid undue infectious risk.

Alloimmune Hemolysis: Donor/Recipient ABO Incompatibility

Hemolysis due to donor/recipient ABO incompatibility is a risk unique to allogeneic HSCT patients. Although a requisite for solid organ transplants donor-recipient ABO compatibility is not necessary for successful HSCT [2], though incompatibility does require special considerations in terms of whether RBC depletion of the donor stem cell product is required and the selection of blood product units for transfusion support. ABO blood groups are inherited independently from HLA antigens, and thus HSCT donor/recipient ABO incompatibility occurs frequently in up to 30–50% of cases [32, 33]. Although major ABO incompatibility can lead to delayed RBC recovery, it has not been shown to impact engraftment (neutrophil or platelet recovery) regardless of the conditioning regiment, donor type, or the stem cell source [34, 35]. Though some groups have reported increased incidence of GvHD and modestly lower survival in subsets of patients receiving HSCT, most studies have shown no differences in these or other standard HSCT outcome assessments, suggesting that ABO mismatch does not significantly impact overall efficacy and safety of HSCT [32–34].

In major ABO-incompatible HSCT (Table 20.5), the donor/recipient ABO mismatch is such that the

Table 20.5 Hemolytic complications due to ABO incompatibility in HSCT

Type	Complications	Preventive measures	Treatment
Major			
Hemolysis with graft infusion (high risk)		RBC depletion of donor graft Recipient isohemagglutinin reduction	Hydration Transfusions
Delayed RBC engraftment/pure red cell aplasia (high risk) • Reticulocytopenia • BM: lack of RBC precursors • Exclusion of viral causes		Immunoadsorption Plasma exchange	Transfusions Immunosuppression adjustment Rituximab
Minor			
Hemolysis with graft infusion (low risk)		Plasma reduction of donor graft	Hydration Transfusions
Delayed hemolysis/passenger lymphocyte syndrome (low risk)		Serial CBC monitoring	Transfusions RBC exchange

recipient has preformed isohemagglutinins against donor RBC (A and/or B) antigens. For example, an HSCT in which a type O recipient receives hematopoietic stem cells (HSCs) from a type A donor would be considered to be a major ABO-incompatible HSCT, due to the preexisting presence of anti-A isohemagglutinins in the recipient. Major ABO incompatibility can result in acute hemolysis during or immediately after graft infusion, the risk of which can be reduced through graft manipulation strategies (such as apheresis or sedimentation) that reduce the RBC content at the cost of lowering the CD34+ stem/progenitor dose of the graft due to processing loss [33, 36]. Another strategy has been to reduce the recipient's isohemagglutinin titers via immunoadsorption or therapeutic plasma exchange (TPE) [37], although this process is labor intensive, difficult to standardize across centers, and requires close assessment of patient hydration and renal function [2].

In addition to graft infusion-related hemolysis, major ABO-incompatible HSCT may result in delayed red cell engraftment and/or pure red cell aplasia (PRCA) in up to 30% of patients due to the hindrance of normal erythroid maturation by residual plasma anti-donor isohemagglutinins derived from the recipient [2, 38]. The resulting anemia, which can persist anywhere from a few weeks to several months post-HSCT, may mimic other causes of anemia post-HSCT discussed above and thus poses a diagnostic dilemma. In this clinical scenario, blood product selection has to be performed with consideration to minimizing the potential for hemolysis of the transfused units (Table 20.6). Delayed red cell engraftment and PRCA occur more frequently in major ABO-incompatible HSCT performed with reduced intensity conditioning (RIC) regimens due to the persistence of recipient-derived isohemagglutinin-producing plasma cells due

Table 20.6 Risk of post-HSCT hemolysis due to donor/recipient ABO incompatibility and selection of blood products based on ABO status

ABO status HSCT recipient	HSCT donor	Risk of hemolysis	Preferred RBC[a]	Preferred PLT[a] (first choice[b])
O	A	High	O	A
O	B	High	O	B
O	AB	High	O	AB
A	O	Low	O	A
A	B	High	O	AB
A	AB	High	A	AB
B	O	Low	O	B
B	A	High	O	AB
B	AB	High	B	AB
AB	Any	Low	Per donor ABO	AB

[a]Preferred ABO type of RBC and PLT units until the following criteria are met: (1) engraftment occurs, (2) patient has not received RBC transfusions for 3 months and forward and reverse typing is fully donor with no mixed field, and (3) no clinical suspicion of ongoing related hemolysis
[b]If first choice of platelet unit not available, consult blood bank from specialized HSCT center for further recommendations

to the less intensive regimens. Additionally, reduced intensity transplant strategies for HSCT in nonmalignant diseases in which mixed donor/recipient chimerism is sufficient for HSCT success may be associated with particularly long delays in red cell engraftment or PRCA in the setting of major ABO incompatibility, due to the ongoing coexistence of donor and recipient hematopoiesis [2].

In contrast, minor ABO-incompatible HSCT (Table 20.5), in which donor isohemagglutinins are directed against recipient RBC antigens, infrequently results in clinically significant hemolysis, though the risk of graft infusion-associated hemolysis can be prevented through plasma volume reduction of the donor product leading to reduction in burden of donor isohemagglutinins [39]. Passenger lymphocyte syndrome (PLS) is an infrequent, related complication in the setting of minor ABO-incompatible HSCT [40] in which transplanted donor lymphocytes produce new isohemagglutinins starting 1–3 weeks post-HSCT, resulting in potentially severe hemolysis that persists until recipient RBCs are no longer produced. The majority of reported patients who have developed PLS have been of blood group A with donor blood group O [33]. Reduced intensity regimens and larger graft lymphocyte content may also increase the risk of clinically relevant PLS. Careful selection of blood products for donor compatibility is critical in preventing worsening hemolysis in the setting of minor ABO incompatibility and PLS (Table 20.6), with immune suppression reserved for severe cases [33].

Thrombocytopenia Post-HSCT: Pathophysiologic Causes

Introduction

Thrombocytopenia is an anticipated consequence of HSCT during the post-conditioning and pre-engraftment phase and in most cases is easily managed by platelet transfusion support at appropriate threshold triggers. The time to platelet engraftment in pediatric patients following HSCT, defined as a platelet count over $50 \times 10 \times 9\ L^{-1}$ without platelet transfusion support for the preceding 7 days, is highly dependent upon the hematopoietic stem cell (HSC) source. Most patients receiving bone marrow or peripheral blood stem cell grafts achieve engraftment within 3–4 weeks, whereas patients who receive umbilical cord blood transplants may not achieve full platelet transfusion independence until 2–3 months post-HSCT [41]. Thus, HSC source must be considered when determining whether duration of initial thrombocytopenia in individual patients is abnormal. Additionally, other factors, including presence of organomegaly and ongoing bleeding complications, may also make it difficult in an individual patient to determine whether ongoing thrombocytopenia is truly an aberrant hematopoietic or immunologic condition that warrants further evaluation.

However, because persistent or recurrent thrombocytopenia often leads to increased morbidity and is associated with an inferior survival post-HSCT [42], prompt recognition of the existence of pathologic thrombocytopenia (Table 20.7) and identification of its causes (Table 20.8) are critical in initiating corrective action to minimize associated morbidity. Generally in HSCT recipients, the etiology of refractory thrombocytopenia is multifactorial [43, 44]. As in the case for anemia discussed above, pathologic thrombocytopenia post-HSCT can result from inadequate platelet production,

Table 20.7 Signs of ongoing thrombocytopenia post-HSCT that require further evaluation

• Persistent transfusion dependence beyond median time to platelet engraftment
– >3–4 weeks for auto HSCT or AlloSCT with PSC or BM source
– >6–8 weeks for UCBT
• Increased platelet transfusion requirements after initial signs of engraftment
• Recurrence of thrombocytopenia/transfusion dependence after prior platelet normalization

AutoSCT, autologous stem cell transplant; AlloSCT, allogeneic stem cell transplant; PSC, peripheral blood stem cells; BM, bone marrow; UCBT, umbilical cord blood transplantation

Table 20.8 Causes of prolonged or recurrent thrombocytopenia post-HSCT

(a) Decreased platelet production

• Graft rejection/failure, inadequate engraftment
• Relapse of malignant disease
• Infection
  – Viral (EBV, HHV6, CMV, adenovirus)
  – Sepsis
• Drug-mediated (sulfa drugs, mycophenolate, ganciclovir, calcineurin inhibitors)

(b) Decreased circulating platelet lifespan "platelet refractoriness"

• Coagulopathy/chronic blood loss
• Immune-mediated thrombocytopenia
  – Alloimmune (early)
  – Autoimmune (late)
• Transplant-associated microangiopathy
• Veno-occlusive disease/hepatomegaly/hypersplenism

(c) Both decreased production and increased destruction
• Acute GvHD • Chronic GvHD

decreased lifespan of circulating and/or transfused platelets, or conditions associated with both decreased production and increased destruction (e.g., chronic GvHD). One useful approach to determining the cause of thrombocytopenia in a post-HSCT patient is to determine if the patient has developed platelet transfusion refractoriness (PTR), indicative of significantly reduced circulating platelet lifespan. Several formulas have been used to calculate a PTR state, including 1-, 20-, and 24-h corrected count increments (CCI; see Table 20.9 for formula), along with a related parameter determined from regression analyses of posttransfusion platelet increments known as the corrected platelet increment (CPI) [45–48]. A 1-h CCI of $<5\text{–}10 \times 10^9$ L^{-1} or a 24-h CPI of $<10 \times 10^9$ L^{-1} has been defined as threshold indicators for a PTR state.

Once discovered, PTR states can be further classified according to immune versus nonimmune causes. Failure to correct a platelet count (as determined by a post-platelet count collected within 1 h of transfusion) has been suggested to indicate an immune mechanism of platelet clearance, whereas normal correction at 1 hour, but recurrent thrombocytopenia within 24 h has been suggested to represent other sequestration/destruction causes. However, exceptions to this suggested rule do occur [45].

Etiology

In autologous stem cell transplantation, prolonged thrombocytopenia post-HSCT usually reflects either poor engraftment, often related to low-$CD34^+$ doses of the infused autologous stem cell product, or regimen-related toxicity including mucositis, veno-occlusive disease (VOD) (also known as sinusoidal obstructive syndrome, SOS), and/or coagulopathy. However, immune-mediated thrombocytopenia has also been described in the autologous transplant setting [49]. While aberrant thrombocytopenia following allogeneic HSCT may also be due to similar problems related to poor engraftment, primary disease relapse, or organ toxicity, the ongoing interplay between recipient and donor immune system components and the use of exogenous immune suppression in the allogeneic setting leads to increased frequency of thrombocytopenia occurring as the result of dysregulated immunity, a state which may persist for many months post-HSCT.

Immune-mediated thrombocytopenia in the post-HSCT setting typically results from platelet clearance through binding of either recipient (alloimmune) or donor-derived (autoimmune) antiplatelet antibodies. Risks for developing alloimmune thrombocytopenia include pre-HSCT transfusion exposure, pregnancy, or previous transplantation, resulting in alloimmunization to human leukocyte antigens (HLA) and/or platelet-specific antigens [45]. HLA alloimmunization is thought to be primarily responsible for immune PTR [50, 51]. Leukoreduction of blood products prior to transfusion appears to reduce the incidence of alloimmunization [52, 53] and is recommended for all patients who may ultimately require HSCT. Transplant-associated microangiopathy (TAM) is a distinct form of immune-mediated thrombocytopenia that may be associated with a PTR state and is discussed separately below (and in Chap. 22).

Table 20.9 Formula to determine corrected count increment (CCI)

$$\text{CCI} = \frac{(\text{Platelet count post-transfusion} - \text{Platelet count pre-transfusion}) \times \text{Body surface area}\ (\text{m}^2)}{\text{platelets transfused}\ (10^{11})}$$

Nonimmune causes of a platelet refractory state include severe infections, bleeding, VOD/SOS, and hypersplenism [45, 54, 55]. Platelet sequestration, consumption due to disseminated intravascular coagulation, hemophagocytosis, and nonspecific immune-mediated destruction are all mechanisms that drive PTR associated with sepsis [56–58]. In fact, fever alone has been associated with decreased platelet transfusion-associated CCI and is a common cause of transient platelet refractoriness [59, 60]. VOD is associated with early onset PTR [61]. Patients with splenomegaly from VOD or other causes also have considerable risk of developing a PTR state, and in contrast, patients who are asplenic at the time of HSCT have improved platelet recovery and response to transfusions.

GvHD is the most common and most proven cause of recurrent thrombocytopenia following initial platelet engraftment. In fact, the association of thrombocytopenia with onset of chronic GvHD has been shown to have a striking negative impact on overall survival and non-relapse mortality post-HSCT in a number of retrospective and prospective studies, including a prospective study of 178 adult patients with hematologic malignancies in which patients with chronic GvHD and platelet count <100 or >100 G/L had long-term OS estimates of 35% versus 86%, respectively [62]. Thrombocytopenia resulting from chronic GvHD is likely multifactorial in origin [63]. Dysfunction of the bone marrow microenvironment caused by GvHD (including increased TGFβ production [64], and decreased thrombopoietin production [65]) likely results in decreased megakaryopoiesis and platelet production in many GvHD patients. In addition, many immune suppression drugs used to treat GvHD (e.g., MMF) and antimicrobials used to treat infections in patients on immune suppression for GvHD (e.g., Ganciclovir) may also trigger decreased platelet production. However, chronic GvHD may also be associated with PTR states, as patients with GvHD are at increased risk for developing functionally significant platelet autoantibodies [62].

Management of Thrombocytopenia Post-HSCT

The approach to management and prevention of bleeding complications in patients with persistent or recurrent thrombocytopenia post-HSCT is dependent upon whether the cause is primarily due to poor platelet production or due to the development of PTR. For patients with poor platelet production, regular transfusions to achieve an individualized platelet threshold (typically a platelet count >10–20 × 10^9 L^{-1}) can decrease bleeding symptoms and risk of severe hemorrhage in the short term, while longer-term approaches (including medication adjustment, treatment of infections, and perhaps the utility of a second HSCT or CD34+ stem cell boost) can be considered. Leukocyte depletion of transfused platelet products, and other methods that may reduce the risk of subsequently developing refractoriness to transfused platelets, should be employed in these patients. Isolated reports suggest that thrombopoietin receptor agonists such as eltrombopag or romiplostim may also represent promising transfusion-sparing therapies in patients with hypoproductive thrombocytopenia post-HSCT [44].

Nonimmune causes of PTR need to be managed by treatment of the underlying disorder, including defibrotide therapy for SOS/VOD, and consideration of splenectomy in patients with severe hypersplenism due to other causes. The frequency and utility of platelet transfusions in this patient population, despite the lack of quantifiable effect in increasing platelet counts, remains controversial.

In cases of alloimmunization, transfusion with HLA-matched platelets can help improve platelet counts [45]. Although valid therapeutic options for autoimmune thrombocytopenic purpura (e.g., splenectomy, intravenous anti-Rh(D), and intravenous gamma globulin) have been shown to confer limited benefit in alloimmunization, they are not better than the transfusion of HLA-matched platelets [45, 51]. For patients with chronic GvHD or others with immune thrombocytopenia caused by donor-derived antibodies post-HSCT, immune suppression strategies have been employed that are similar to those described for AIHA above (Table 20.4). In particular, several reports have demonstrated the utility of rituximab in refractory immune thrombocytopenia following HSCT [66, 67].

Antifibrinolytics such as epsilon aminocaproic acid can be used for adjunctive support to control non-life-threatening mucocutaneous bleeding in all patients with thrombocytopenia post-HSCT, except those with aberrant coagulation states such as VOD/SOS. In patients with ongoing severe hemorrhage who are platelet refractory, continuous 24-h slow platelet infusion has been effective in improving bleeding [68]. Anecdotal success has also been reported using recombinant activated factor VII [69], though this approach should be used with caution in patients with high risk of thrombosis including patients with chronic GvHD. In summary, given the complexity of issues surrounding refractory thrombocytopenia in the post-HSCT setting, a stepwise approach to diagnosis and management is required for these patients, and management is best directed by experience transplant centers.

Transplant-Associated Microangiopathy

The syndrome of thrombocytopenia, microangiopathic hemolytic anemia, and renal insufficiency known as transplant-associated microangiopathy (TAM) is a well-described complication after HSCT [70–72]. Although similar to disorders that rarely affect the general population including hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), TAM appears to have a distinct pathophysiologic basis and is unresponsive to therapies such as plasmapheresis that are effective in TTP. The pathophysiology of TAM is thought to be initiated by two interrelated processes of microvascular endothelial injury and complement dysregulation resulting in fibrin and C4d deposition in microvascular beds, particularly in the kidney, and consequent platelet consumption, microangiopathic hemolysis, and inflammatory organ damage [73–77]. Factors postulated to increase the risk of TAM post-HSCT (Table 20.10) include busulfan-based conditioning regimens, fludarabine-based reduced intensity conditioning regimens, calcineurin inhibitors used as GvHD prophylaxis or treatment, acute GvHD itself, and certain viral infections, particularly adenovirus, HHV-6, and BK virus [78–84]. The presence of increased numbers of these risk factors as well as the onset of TAM before Day 120 post-HSCT has been associated with increased mortality [85].

Past incidence estimates of TAM have varied widely, but since the development and refinement of sensitive and specific diagnostic criteria (Table 20.11) by several working groups, true TAM is now thought to affect as many as ~30–35% of HSCT recipients at a median onset of 44 days, with about half of the cases progressing to severe disease [70, 71, 76, 84, 86]. A decade ago, TAM was associated with a severely high mortality rate (as high as 75%) [87], though recent advances in supportive care as well as targeted therapy options have resulted in improved outcomes [83].

Disease subclassification and evaluation/management of the nephrologic complications of

Table 20.10 Risk factors for transplant-associated microangiopathy (TAM)

Risk factor	Comment(s)
• Allogeneic transplantation	• Use of unrelated donors
• Conditioning agents	• Busulfan, fludarabine
• Acute GvHD	• CMV, HHV6, parvovirus B19, adenovirus, BK virus
• Use of calcineurin inhibitors, rapamycin	
• Hepatic veno-occlusive disease	
• Viral infections	

Table 20.11 Diagnostic criteria for transplant-associated microangiopathy [71, 83, 84]

A: Tissue biopsy demonstrating microangiopathic changes or B: Laboratory/clinical criteria
1. Sudden and persistent increase in serum LDH
2. ≥2 schistocytes per high-power field on peripheral smear
3. Decrease in hemoglobin concentration or increase in red blood cell transfusion requirement
4. Prolonged or progressive thrombocytopenia (platelet count ≤50×10^9 L^{-1})
5. Decrease in serum haptoglobin
6. Hypertension >95%ile for age
7. Proteinuria, additional markers of renal dysfunction
8. Negative coombs (direct and indirect) testing, normal coagulation studies
9. Terminal complement activation (elevated sC5b-9)

TAM are discussed in Chap. 22 [88, 89]. It is important, however, to point out the wide variability in symptom presentation and severity of affectation in patients with TAM. From a hematologic perspective, mildly affected patients may have only mild anemia and thrombocytopenia, with an elevated LDH and reticulocyte count, along with the presence of schistocytes and renal dysfunction/hypertension being the primary indicators of underlying TAM. In contrast, severely affected patients may exhibit severe hemolysis and a platelet refractory thrombocytopenia, in addition to advance stage kidney disease, and additional manifestations such as polyserositis, neurologic manifestations, and severe GI manifestations including mucosal hemorrhage [83].

Until recently, there was no definitive treatment for TAM. Discontinuation of calcineurin inhibitor therapy may help halt the progression of TAM, but generally does not reverse the existing clinical symptoms in the acute setting, and in some cases might lead to onset/exacerbation of acute GvHD, resulting in a deterioration of patient status. Switching cyclosporine with tacrolimus or vice versa may lead to improvement in symptoms [90–92]. Agents such as defibrotide, a polyribonucleotide agent with antithrombotic and thrombolytic activity used frequently for the treatment of SOS/VOD, may have some therapeutic benefit [93, 94]. Most recently, pharmacologic inhibition of complement has shown promise as an effective approach to break the cycle of complement activation and tissue injury in TAM. Eculizumab, a humanized monoclonal antibody against complement component C5 which is currently approved for use in atypical HUS and paroxysmal nocturnal hemoglobinuria in pediatrics, has resulted in marked improvement in TAM symptoms in multiple reports [95–98].

Thromboembolism

The incidence and prevalence of thromboembolic episodes in patients with malignancy is increasing and is an increasingly recognized complication post-HSCT. However, there is limited data regarding the incidence and treatment approaches for HSCT recipients who develop venous thromboembolism (VTE) [99].

Despite the induction of severe thrombocytopenia, HSCT is associated with multiple risk factors (Table 20.12) that may promote the development of VTE, including, but not limited to, primary malignant disease, chemotherapeutic and immunomodulatory drugs, indwelling vascular catheters, GvHD, and infections. Endothelial injury, either as a direct consequence of conditioning therapy or due to the proinflammatory state induced by GvHD, creates a microenvironment of an acquired hypercoagulable state that can lead to further inflammation and activation of endothelium-dependent prothrombotic coagulation factors [100–103]. Patients with chronic GvHD are at particularly high risk for developing recurrent

Table 20.12 Risk factors for venous thromboembolism (VTE) in HSCT (adapted from [99])

• Underlying primary malignant disease (leukemia, lymphoma)
• Indwelling vascular catheters
• Myeloablative conditioning
• GVHD
• Infections
• Prior history of thromboembolism
• Known inherited or acquired thrombophilia
• Prolonged immobilization

VTE [104] due to severe chronic inflammation and associated increases in circulating procoagulant factors such as Factor VIII.

Specific symptoms depend on the site of thrombosis, and include painful swelling and erythema in the setting of extremity deep venous thrombosis. The combination of chest pain, dyspnea, or unexplained tachycardia warrants evaluation for pulmonary embolism. Headache and acute neurologic changes warrant evaluation for cerebral sinus venous thrombosis [99]. Worsening splenomegaly and abdominal pain, though often associated with VOD/SOS, may alternatively signify portal venous or related thrombosis. Venous duplex ultrasonography can provide definitive evidence of VTE in extremity, and high-resolution CT scan of the chest can help detect pulmonary VTE events. Assessment of cerebral and abdominal thrombosis requires dedicated angiographic studies such as an MR angiogram.

Prior to initiation of anticoagulant therapy, obtaining a complete blood count with platelet count and a coagulation profile is imperative to assess bleeding risk in the post-HSCT patient. In general, patients with platelet count $>50 \times 10^9\ L^{-1}$ and no ongoing coagulopathy or bleeding concerns can receive anticoagulation according to standard pediatric hematology guidelines. Initial therapy with low-molecular-weight heparin (LMWH) is recommended over warfarin for VTE in patients with cancer based on result of two large clinical trials [105] and is also preferred in the post-HSCT setting due to challenges related to drug interactions and inconsistent dietary intake that would affect the ability to maintain consistent warfarin levels. Duration of therapy depends on presence/absence of additional risk factors. Catheter-associated VTE typically requires LMWH therapy for 3 months and/or until the venous catheter is removed. For patients with severe thrombocytopenia, discontinuation of anticoagulation may be considered in cases of resolved or minimal residual thrombus burden for the duration of the thrombocytopenic period, particularly in patients with ongoing bleeding concerns. However, in cases where the thrombotic burden is significant, involves cerebral, portal, hepatic, or pulmonary vessels or has an intracardiac component, continuation of LMWH along with an increased platelet threshold is typically warranted. In this setting, LMWH frequently needs dose modification (reduction) as a consequence of other post-HSCT complications/comorbidities such as GI bleeding, thrombocytopenia, renal impairment, GvHD, and thrombotic microangiopathy [104]. Although VTE is a significant complication of HSCT recipients, the complex pathophysiology of HSCT also predisposes to severe risk of bleeding, and thus more aggressive methods of thrombosis therapy such as catheter directed or systemic thrombolysis should be undertaken only in extreme circumstances and only in the context of a specialized, experienced thrombosis center.

Key Points

- Hematologic complications occur at increased incidence with newer approaches for HSCT, including reduced intensity conditioning, T cell depletion, and the use of alternative donors.
- Immune hemolytic anemia (IHA) is a well-recognized compilation of HSCT. IHA can be associated with a number of specific risk factors, may have a variable course, is often therapy-resistant, and results in significant morbidity and mortality. Outcomes can be improved by early recognition and initiation of appropriate therapy.
- Severe thrombocytopenia that occurs after initial engraftment post-HSCT may result from a variety of immunologic, inflammatory, and graft-specific etiologies and requires a stepwise approach for diagnosis so that appropriate management may be initiated.
- Transplant-associated microangiopathy (TAM) is defined by the triad of thrombocytopenia, renal insufficiency, and microangiopathic hemolytic anemia and has a highly variable disease course, ranging from mild anemia and thrombocytopenia to life-threatening cytopenias, hemostatic dysregulation, and renal dysfunction requiring aggressive therapy in order to prevent severe morbidity and mortality.

- Venous thromboembolism post-HSCT, though often treated with conventional methods, requires the need to strike a delicate balance between coexisting bleeding and thrombotic risks.

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21 Pulmonary Complications Associated with HSCT

Malika Kapadia and Terry Wikle Shapiro

Abstract

While outcomes for patients who undergo allogeneic hematopoietic stem cell transplantation (HSCT) have improved over the past 10–20 years, pulmonary complications after allogeneic HSCT remain a leading cause of morbidity and mortality. Overall, 25–50% of pediatric HSCT patients will develop pulmonary complications. Thus, prevention, early detection, and intervention are key to minimizing the sequelae from HSCT-associated pulmonary complications. HSCT-associated pulmonary complications can be classified as infectious or noninfectious, and they often follow a predictable timeline, occurring during discrete phases of HSCT (pre-engraftment, early post-engraftment, late post-engraftment). However, certain post-HSCT pulmonary complications span the entire post-HSCT course. The most common causes of noninfectious pulmonary complications are related to the conditioning regimen used which can result in varying degrees of acute or delayed lung injury, the degree of recipient–donor HLA histoincompatibility, the hematopoietic stem cell (HSC) source, the degree of graft manipulation, and the development of graft-versus-host disease (GvHD), both acute and chronic. Infectious etiologies can be caused by any class of pathogen including bacterial, viral, fungal, and protozoan. They usually occur during periods of profound and/or prolonged

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neutropenia and/or impaired or delayed cellular and humoral immune recovery. Immunosuppression used to prevent or treat GvHD also places a HSCT recipient at high risk for developing pulmonary infections that can be life-threatening. This chapter discusses the most common pulmonary complications associated with HSCT by time period post-HSCT.

Pulmonary Complications Associated with HSCT

Research suggests that pulmonary complications are one of the leading causes of post-hematopoietic stem cell transplantation (HSCT) morbidity and death and occur in 25–50% of HSCT patients [1–3]. The incidence of significant pulmonary complications is lower in autologous HSCT recipients than in allogeneic HSCT recipients because of the absence of graft-versus-host disease (GvHD) and no need for post-HSCT immunosuppression. However, autologous HSCT patients who receive conditioning regimens that include total body irradiation (TBI) are at a higher risk for developing post-HSCT pulmonary complications because TBI is a significant contributor to the development of pulmonary complications post-HSCT. Post-HSCT pulmonary complications can be classified as *infectious or noninfectious* and follow a predictable timeline after transplantation [2]. Table 21.1 summarizes the most common causes of pulmonary complications based upon the phases of HSCT when they are most prevalent. This table also distinguishes between infectious and noninfectious etiologies.

Some pulmonary complications can arise any time during the post-HSCT period, whereas others develop more commonly at discrete time periods. Typically, the post-HSCT course is divided into three phases: (1) pre-engraftment which spans days 0–30 post-HSCT, (2) early post-engraftment (days 31–100 post-HSCT), and (3) late post-engraftment (>day 100 post-HSCT). Common pulmonary complications seen in the first 30 days after HSCT (pre-engraftment period) can be of infectious or noninfectious etiologies. The noninfectious etiologies, which include pulmonary edema, pulmonary hemorrhage, diffuse alveolar hemorrhage (DAH), engraftment syndrome, pleural effusion, radiation, and chemotherapy-induced lung injury, are caused by the specific agents used in the conditioning/preparative regimen or due to increased inflammation that occurs around the time of engraftment. Infectious causes are due to the profound neutropenic state of the patient and the risk of opportunistic, invasive life-threatening infections. These include bacterial or fungal pneumonia, acute respiratory distress syndrome (ARDS) associated with septic shock, and respiratory viral infections. In contrast, the majority of causes of pulmonary complications in the late post-engraftment phase (>100 days) are related to delayed T- and B-cell immune reconstitution and to active chronic GvHD. Figure 21.1 depicts the time frame in which the above complications most commonly arise.

Patients after allogeneic HSCT, especially those with chronic GvHD who are being treated actively with immunosuppression, are particularly at risk for the development of encapsulated bacterial pneumonia, invasive mold fungal infections, viral pneumonia, *Pneumocystis jiroveci* pneumonia, and idiopathic interstitial pneumonitis.

Table 21.1 Timeline of typical onset of pulmonary complications after hematopoietic stem cell transplantation (HSCT)

Days from HSCT infusion	Cause	Pulmonary complications
Pre-engraftment: (Days 0–30)	• Conditioning/ preparative regimen • Neutropenia	Noninfectious: • Pulmonary edema • Pleural effusion • Engraftment syndrome • Chemotherapy-induced lung injury • Radiation-induced lung injury • Diffuse alveolar hemorrhage • Respiratory compromise and hypoxia due to VOD/SOS • Transfusion-related lung injury • Idiopathic pneumonia syndrome Infectious: • Bacterial infections (both gram-negative and gram-positive spp. • Aspergillosis • Candidemia or candidal infection • Respiratory viruses (e.g., RSV, parainfluenza, influenza, metapneumovirus, rhinovirus) • Herpes simplex virus • ARDS due to sepsis
Early post-engraftment (Days 31–100)	• Impaired cellular and humoral immunity • Delayed lung injury from the conditioning/ preparative regimen	Infectious: • Cytomegalovirus • Adenovirus • Herpes simplex virus • Aspergillosis • Respiratory viruses (e.g., RSV, parainfluenza, influenza, metapneumovirus, rhinovirus) • *Pneumocystis jiroveci* pneumonia • Toxoplasma Gondii • Gram-positive bacterial infections ARDS due to infection Noninfectious: • Idiopathic pneumonia syndrome • Radiation-induced lung injury • Chemotherapy-induced lung injury • Diffuse alveolar hemorrhage
Late post-engraftment (>100 days)	• Delayed immune recovery • On immunosuppression • Chronic GvHD	Infectious: • Cytomegalovirus • Adenovirus • Varicella–zoster reactivation • Aspergillosis • Respiratory viruses (e.g., RSV, parainfluenza, influenza, metapneumovirus, rhinovirus) • *Pneumocystis jiroveci* pneumonia • Encapsulated bacteria (chronic GvHD) • ARDS due to infection • EBV–post-transplantation lymphoproliferative disorder Noninfectious: • Bronchiolitis obliterans syndrome due to chronic GvHD • Bronchiolitis obliterans organizing pneumonia • Chemotherapy-induced chronic lung injury • Radiation-induced chronic lung injury

Data from [1, 28, 29]. *ARDS* acute respiratory distress syndrome, *GvHD* graft-versus-host disease, *IPS* idiopathic pneumonia syndrome, *RSV* respiratory syncytial virus, *VOD/SOS* veno-occlusive disease/sinusoidal obstructive syndrome

	Phase I Pre-engraftment (0-30 days)	Phase II Post-engraftment (30-100 days)	Phase III Late Phase > 100 days)
Host immune system defect	Neutropenia, mucositis, catheters and lines, acute GVHD	Impaired cellular immunity Acute GVHD	Impaired humoral and cellular immunity chronic GVHD
Infectious	gram - bacteria Gram + bacteria (Staph, Strep) Candida Aspergillus HSV CRV (RSV, influenza, adenovirus)	Aspergillus Pneumocystis CMV	Encapsulated bacteria Nocardia HZV
Non-infectious	CHF VOD ES DAH IPS	COP PTLPD	BO

Fig. 21.1 Common pulmonary complications post-HSCT by time. Post-HSCT complications usually develop at specific time periods during and/or after HSCT. This figure depicts a summary of such complications over discrete time periods

Pre-engraftment Period (0–30 Days Post-engraftment)

Introduction

In the pre-engraftment period (0–30 days post-HSCT), the differential diagnosis of pulmonary complications includes noninfectious etiologies, such as pulmonary edema, aspiration, engraftment syndrome, sinusoidal obstructive syndrome/veno-occlusive disease (SOS/VOD), DAH, as well as infectious causes (e.g., bacterial, fungal, and viral infections) that can lead to pneumonia and ARDS due to sepsis. In general, the signs and symptoms of the pulmonary complications seen in the pre-engraftment phase are nonspecific. They include fever, dyspnea, cough, and hypoxemia. However, the timing of the presenting signs and symptoms can be helpful to narrow down the likely etiology. Table 21.2 summarizes the most common pulmonary complications found in allogeneic HSCT patients in the pre-engraftment period.

Pulmonary Edema

Introduction: Pulmonary edema of cardiogenic or noncardiogenic origin can occur in the first 30 days after HSCT, sometimes complicating other concurrent disease states, such as pneumonia, sepsis, engraftment syndrome, and hyperacute GvHD. Noncardiogenic pulmonary edema can be induced by sepsis, aspiration pneumonia, viral infection (e.g., influenza), toxic effects of the conditioning regimen, or hyperacute GvHD. Fluid overload can also contribute to the development of pulmonary edema. Patients with severe hepatic SOS/VOD (another onerous complication of

Table 21.2 Respiratory complications after HSCT in the pre-engraftment period

Disease	Risk factors	Other manifestations	Radiographs	Diagnostics	Invasive testing
Diffuse alveolar hemorrhage	–	–	• Air bronchograms with diffuse opacities	• BAL	• Not needed
Bacterial pneumonia	• Immunosuppression • Mucosal compromise	• High fever • Hypoxia • Respiratory symptoms	• Focal consolidation	• Improvement with antimicrobials	• Not needed
Fungal pneumonia	• Immunosuppression • Exposure • Prior infection	• Persistent fevers despite antimicrobials	• "Halo sign" or "reverse halo sign" • Focal consolidation	• BAL • β-D-Glucan • *Aspergillus* galactomannan	• Not usually
Aspiration pneumonia	• Altered mental status • Dysphasia	• Increase work of breathing • Fever • Cough • Hypoxia	• Ground-glass opacities • Focal right upper lobe consolidation	–	• Not needed
Hyperacute GvHD	• HLA mismatch	• Rash • Diarrhea • Dyspnea • Abdominal pain	• Diffuse ground-glass appearance	• BAL • Skin biopsy	• May help exclude other etiologies
Engraftment syndrome	–	• Fevers • Rash • Fluid overload • Respiratory distress	• Hilar consolidation • Diffuse ground-glass appearance • Interstitial thickening	• BAL • Skin biopsy (to exclude other etiologies)	• May help exclude other etiologies
Inflammatory pulmonary edema	• Sepsis • Engraftment syndrome • Injury to the lung	• Fever • Hypoxia • Dyspnea • Increased work of breathing	• Diffuse ground-glass appearance	–	• Not needed
Cardiogenic pulmonary edema	• Cardiotoxic agents	• Fluid over load • Increased work of breathing • Pitting edema	• Cardiomegaly • Perihilar opacities in butterfly distribution	• Elevated BNP • Abnormal EKG • ECHO with decreased left ventricular function	• Not needed

HSCT) can present with either cardiogenic or noncardiogenic pulmonary edema with pleural effusions.

Risk factors: Risk factors for pulmonary edema in the pre-engraftment period include high-dose cyclophosphamide as part of the conditioning/preparative regimen, previous chest irradiation, total body irradiation (TBI) as part of the conditioning/preparative regimen, and history of cardiac dysfunction as a result of previous therapy for the primary disease. These known cardiotoxic modalities include cyclophosphamide, anthracyclines (e.g., doxorubicin, daunorubicin, and idarubicin), and external beam chest irradiation. In addition, patients who develop capillary leak syndrome, engraftment syndrome, or hyperacute GvHD are at an increased risk for developing pulmonary edema.

Differential diagnosis: The differential diagnosis of pulmonary edema includes interstitial pneumonitis, cardiac failure, radiation pneumonitis, infection, and diffuse alveolar hemorrhage (DAH).

Clinical and radiographic features: Clinical features of pulmonary edema are tachypnea, orthopnea, rales, and diminished breath sounds on physical examination, as well as lethargy, restlessness, hypoxemia, and weight gain. The radiographic manifestations of cardiogenic pulmonary edema include interlobular septal thickening, cephalad vascular distribution, ground-glass opacification (sometimes in a perihilar "butterfly" distribution), pleural effusions, and sometimes cardiomegaly.

Diagnostic studies: The diagnosis of pulmonary edema is made primarily based upon clinical findings. Radiographic evidence does not need to be present to confirm the diagnosis.

Management and outcome: The management of pulmonary edema centers on treating the underlying cause of pulmonary edema and providing supportive care. Aggressive diuresis is frequently employed with the use of loop diuretics such as furosemide. Thiazide may be added 30 min prior to administration of a loop diuretic to improve diuresis. When feasible, diuretics should be administered following the completion of a blood product transfusion or colloid infusion to enhance diuresis. In addition, patients post-HSCT should be weighed twice daily to monitor their fluid shift. Judicious fluid management should be employed with strict monitoring of all intake and output ("strict Is and Os"). One should volume restrict the patient and concentrate all IV fluids and medications when feasible. Patients should also have supplemental oxygen to maintain oxygen saturation >95%. Any suspected underlying infectious etiology (such as sepsis) that may be contributing to pulmonary edema should be treated.

Engraftment Syndrome

Introduction and incidence: Engraftment syndrome is a noninfectious complication that is reported in 7–10% of autologous HSCT patients and is rarely seen following allogeneic HSCT [4]. (See Chap. 12 for detailed discussion of engraftment syndrome.)

Risk factors: The most common risk factors for engraftment syndrome include autologous HSCT, infusion of a large hematopoietic stem cell dose (HSC), and the presence of an underlying infection.

Differential diagnosis: Initially, infectious etiology of respiratory distress needs to be ruled out. Hyperacute GvHD is included in the differential diagnosis of engraftment syndrome. Some clinicians consider the pulmonary manifestations of engraftment syndrome and hyperacute GvHD as the same clinical entity.

Clinical and radiographic features: Engraftment syndrome typically develops around 7–11 days following HSCT during the time of post-HSCT neutrophil recovery [5]. Its clinical features include dyspnea, high fever, an erythematous maculopapular rash (not attributable to a drug), weight gain, hypoxemia, and diffuse pulmonary opacities seen on chest radiograph (CXR) that are consistent with noncardiogenic pulmonary edema [4, 6]. The pulmonary manifestations of engraftment syndrome are thought to be due to diffuse capillary leakage from endothelial damage [6]. Findings on chest computed topographic (CT) scan include bilateral ground-glass opacification, hilar or peribronchiolar consolidation, and thickening of interlobular septa. Pleural effusions are also common.

Diagnostic studies: The diagnosis is determined primarily based upon clinical assessment, although CXR may help to confirm the diagnosis. Bronchoalveolar lavage (BAL) may show neutrophilia and diffuse inflammation [4],

but this procedure is rarely performed to confirm the diagnosis of engraftment syndrome unless there is a suspicion of infection as the etiology of the patient's symptoms.

Management and outcome: The treatment of engraftment syndrome is a short course of high-dose IV corticosteroids at a minimum of 2 mg/kg/day for 3–5 days and then quickly tapered off. (See Chap. 28 for specific prescribing considerations.)

Hyperacute and Acute Graft-Versus-Host Disease (GvHD)

Introduction and incidence: Hyperacute and acute GvHD are the consequence of HLA mismatch between the donor and recipient. With accurate HLA typing using molecular methods, hyperacute GvHD is very rare nowadays [1]. Hyperacute GvHD occurs in the first 14 days post-HSCT and is frequently (88%) associated with both skin involvement and noncardiogenic pulmonary edema [3]. Acute graft-versus-host disease (GvHD) can develop anytime within the first 100 days following allogeneic HSCT, although it is recognized that signs and symptoms can occur beyond 100 days post-HSCT. While acute GvHD rarely affects the lungs directly, it can be a risk factor for noncardiogenic pulmonary edema, diffuse alveolar hemorrhage, and later development of airflow obstruction (in chronic GvHD).

Risk factors: Risk factors include increasing HLA disparity, particularly if the donor HSC source is from peripheral blood because of the presence of mature T-cells in the HSC product as well as inadequate immunosuppression during the first 30 days post-HSCT.

Differential diagnosis: The differential diagnosis includes idiopathic interstitial pneumonia, diffuse alveolar hemorrhage, and pulmonary edema.

Clinical and radiographic features: Radiographic findings include extensive interstitial and alveolar injury, defined as multi-lobular involvement on CXR or CT scan as well as signs and symptoms consistent with pneumonia.

Diagnostic studies: Imaging studies performed are CXR and CT scan of the chest. BAL is often performed in order to exclude infection as the etiology. Once infectious etiologies and other pathologies such as DAH are excluded, a diagnosis of hyperacute GvHD is made. In cases in which an open lung biopsy is performed, histopathology of lung tissue is characterized by disorganized, epithelial cell damage, interstitial fibroplasia, and interstitial T-cell infiltration [7].

Treatment and outcome: Hyperacute pulmonary GvHD is treated with high-dose systemic corticosteroids. The effective rate of treatment of acute GvHD-induced lung injury positively correlates with the treatment of the underlying acute GvHD. In a series of 47 cases, approximately 75% of patients survived acute GvHD-induced lung injury when the acute GvHD was effectively treated [7].

Diffuse Alveolar Hemorrhage (DAH)

Introduction and incidence: DAH is a life-threatening pulmonary complication following HSCT. It is defined as bleeding into the intra-alveolar space that is most likely secondary to pulmonary endothelial injury from the conditioning regimen. The incidence of DAH is approximately 2% of all HSCT patients and is associated with both infectious and noninfectious causes (e.g., engraftment syndrome) [2, 5, 8]. It is associated with a high mortality rate of approximately 80% [8].

Risk factors: While the pathogenesis of DAH remains unclear, severe mucositis, renal insufficiency, and neutrophil recovery are highly associated with DAH. Autologous HSCT, TBI-containing conditioning/preparative regimens, the presence of a coagulopathy, and a history of previous chest irradiation are also associated with DAH.

Differential diagnosis: The differential diagnosis includes infectious interstitial pneumonitis, drug- or radiation-induced pneumonitis, and pulmonary edema.

Clinical and radiographic features: Patients with DAH often present with rapidly progressing dyspnea, cough, and hypoxemia without hemoptysis. CXR typically reveals areas of diffuse, bilateral consolidation. Chest CT scan, which is

more sensitive than a CXR, typically shows diffuse ground-glass or consolidative opacities, mainly in the middle lung fields (see Fig. 21.2).

Diagnostic studies: Imaging studies performed are CXR and chest CT scan. BAL is usually necessary in order to confirm the diagnosis of DAH once fungal and other infectious etiologies have been excluded. The classic diagnostic finding of DAH from BAL is progressively bloodier aliquots of lavaged fluid and/or staining of the BAL specimens showing ≥20% iron-laden macrophages.

Management and outcome: DAH is treated with high-dose systemic corticosteroids (0.5–1 g initially for 3 days followed by rapid taper over 2 weeks) [8]. Patients with DAH frequently require mechanical ventilation and blood product support. Coagulopathies should be corrected.

Pulmonary Infections

Distinguishing clinical, radiographic, and other diagnostic features of these infections and other common pulmonary complications in the pre-engraftment phase are compiled in Table 21.2. Because of the period of prolonged neutropenia and delayed donor adaptive immune reconstitution, allogeneic HSCT recipients during the pre-engraftment period are at a much higher risk for developing infections, including pneumonia. In one study of 427 consecutive allogeneic HSCT recipients, pneumonia developed in 19% of HSCT patients within the first 30 days post-HSCT, with 9% fungal, 4% bacterial, 2% viral, and 4% had suspected pneumonia without a specific organism being identified [9]. Among the cases of bacterial pneumonia, the most common causes were found to be *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*.

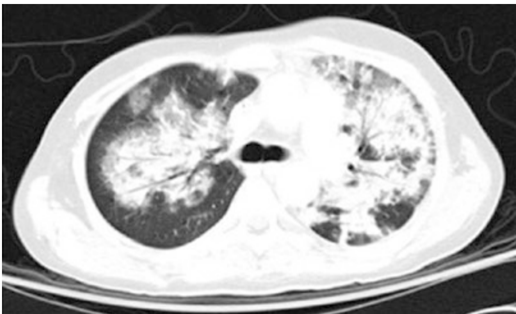

Fig. 21.2 Diffuse Alveolar hemorrhage (DAH) post-HSCT. Chest CT image showing bilateral areas of consolidation in a patient with DAH (From Amy K. Chi, Ayman O. Soubani, Alexander C. White, Kenneth B. Miller, An Update on Pulmonary Complications of Hematopoietic Stem Cell Transplantation, Chest, Volume 144, Issue 6, 2013, 1913–1922, https://doi.org/10.1378/chest.12-1708)

Bacterial Pneumonia

Introduction and incidence: HSCT recipients in the pre-engraftment phase are most at high risk for aerobic gram-positive and gram-negative bacterial infections, including pneumonia, due to prolonged, profound neutropenia [9]. The most common gram-positive bacterial organisms are *Staphylococcus epidermidis and Streptococcus* spp., whereas the most common gram-negative organisms are *Pseudomonas aeruginosa and Klebsiella.* In addition, the atypical bacteria (*Legionella* and *Mycoplasma* spp.) can be the cause of bacterial pneumonia in the HSCT recipient in the pre-engraftment phase.

Risk factors: The risk factors for developing bacterial pneumonia are neutropenia, hypogammaglobulinemia, severe mucositis, swallowing difficulties, aspiration, and possibly impaired mucociliary clearance.

Differential diagnosis: The differential diagnosis of bacterial pneumonia includes interstitial pneumonitis, atypical pneumonia, pulmonary edema, *Pneumocystis jiroveci* pneumonia (PJP), and DAH.

Clinical and radiographic features: The clinical findings are relatively nonspecific, and they include fever, hypoxemia, increased work of breathing, and dry or productive cough. *Legionella* pneumonia may start as a unilateral process that rapidly progresses to a bilateral process. CXR often shows consolidation of alveolar sacs and an isolated area of consolidation. Similar to immunocompetent patients with pneumonia, the radiographic findings lag behind by the clinical findings. Unlike immunocompetent patients, post-HSCT patients in the

pre-engraftment phase do not have leukocytosis with predominant "left shift" because of their profound neutropenia.

Diagnostic studies: A CXR should be obtained with onset of fever; CT scan of the chest should be performed in patients with persistent fevers (typically defined as 3–5 days of persistent fever). When possible, sputum cultures (or tracheal cultures if patient is intubated) should be obtained. When warranted, BAL with transbronchial biopsy and/or CT-guided needle biopsy may be performed. Blood cultures should also be obtained with new onset fever to determine if the patient also has bacteremia and/or sepsis.

Management and outcome: The management of bacterial pneumonia includes the initiation of broad-spectrum, empiric antibiotics with onset of fever. The selection of antibiotic(s) is based on the causative or suspected organism(s) and its antibiotics sensitivity profile. While specific choice of antibiotics is based on each institution's antibiogram, in general, cefepime and meropenem are used if the causative organism is *S. pneumoniae*, *Enterobacter*, *Chlamydia*, or *S. aureus*. If *Mycoplasma*, *S. pneumoniae*, *Legionella*, or *H. influenza* is suspected or identified, then azithromycin, clindamycin, or erythromycin is used. Aspiration pneumonia (due to *S. pneumoniae* and other oral flora as a result of severe mucositis) is typically treated with metronidazole or clindamycin.

Fungal Pneumonia

Post-HSCT recipients are at high risk for developing invasive fungal infections (IFI) including fungal pneumonia. Both endemic (e.g., *Histoplasma* spp., *Coccidioides* spp., and *Cryptococcus* spp.) and opportunistic (e.g., *Candida* spp., *Aspergillus* spp., and *Mucor* spp.) fungal organisms are known to cause pneumonia in the immunocompromised patient. The route by which fungal pneumonia arises is subdivided into three mechanisms: (1) fungi that invade the lung directly via inhalation of spores (e.g., *Aspergillus, Cryptococcus)*, (2) organisms that reach the lung from another site *(Aspergillus, Candida* spp.), and (3) systemic mycoses that lie dormant and reactivate in an immunocompromised patient (*Coccidioides, Mucor*, and *Histoplasma* spp.) [2, 10, 11]. In immunocompromised patients, fungal pneumonia often progresses to disseminated disease quickly and is much more difficult to treat successfully once it has disseminated.

Aspergillus Pneumonia

Introduction and incidence: Aspergillosis is the leading cause of IFI of the lung in immunocompromised patients, including those who have undergone HSCT and are awaiting immune recovery or are on immunosuppression due to GvHD. Because it often becomes disseminated, aspergillosis is associated with poor outcomes in this patient population. Invasive fungal infection mortality has been reported to be greater than 50% [11]. Overall survival is significantly less in patients with invasive fungal infections, as compared to their counter parts [11].

Risk factors: Risk factors for aspergillosis of the lungs include allogeneic HSCT, prolonged use of immunosuppression (particularly corticosteroids), GvHD, HLA disparity, TBI-containing conditioning/preparative regimens, history of prior fungal infection, and increased age of HSCT recipient.

Differential diagnosis: The differential diagnosis of aspergillosis of the lungs includes bacterial pneumonia, interstitial pneumonitis, and atypical pneumonia.

Clinical and radiographic features: Neutropenic patients may present with the classic triad of fever, pleuritic chest pain, and hemoptysis, although this triad is frequently not present. Hypoxemia may also present. The radiographic appearance is varied and includes single or multiple nodules with or without cavitation, patchy or segmental consolidation, or peribronchiolar opacities. Figure 21.3 shows an example of invasive aspergillosis of the lungs seen on CT scan of the chest in a post-HSCT recipient. This image shows a characteristic feature of a nodule surrounded by ground-glass opacity ("halo sign") that reflects angioinvasion and hemorrhage into the surrounding tissue. However, the halo sign is not specific to *Aspergillus* and can be seen with other fungi and molds including *Fusarium*, *Mucor*, and

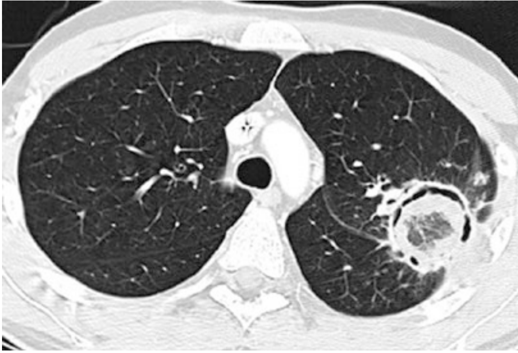

Fig. 21.3 Aspergillosis of the lung post-HSCT. Chest CT image showing aspergillosis, which typically involved segmental and subsegmental bronchi usually in the upper lobes. The lesion(s) can have a mass-like appearance and have a "halo sign" as depicted here

Scedosporium species. Note: patients who are profoundly neutropenic and/or immunocompromised may not have radiographic evidence of disease; however, lack of the radiographic findings should not delay the initiation of empiric treatment if the patient is at very high risk for developing fungal pneumonia.

Diagnostic studies: Early treatment intervention is very important in order to maximize successful outcome. Radiographic findings develop late in the course of aspergillosis. Thus, empiric treatment of suspected IFI, including aspergillosis of the lungs, is essential. Screening methods to detect *Aspergillus* include serum *Aspergillus* galactomannan, serum β-D-glucan, or serum *Aspergillus* PCR testing. Serum *Aspergillus* galactomannan assay is used most commonly, although false-positive serum galactomannan has been reported in patients receiving β-lactam antibiotics, particularly piperacillin–tazobactam, and the effects may last up to 5 days after discontinuing these antibiotics [12, 13]. β-D-Glucan screening, especially in pediatric HSCT recipients, has a low positive predictive value and is, therefore, of limited usefulness in screening for pulmonary aspergillosis [13]. Other diagnostic tools include CXR, CT scan of the chest, BAL with or without transbronchial biopsy, and CT-guided needle biopsy. Whenever possible, a biopsy of a suspected lesion should be obtained because the biopsy tissue results may help to confirm the diagnosis and guide appropriate therapy. Galactomannan testing from BAL specimen is available and has been shown to have a higher sensitivity for invasive pulmonary aspergillosis than *Aspergillus* galactomannan testing from the serum [10].

Management and outcome: Because the outcomes of IFI in immunocompromised patients are poor, prophylaxis with antifungal agents in high-risk patients (i.e., patients undergoing alternative donor allogeneic HSCT and patients receiving substantial immunosuppression) is essential. For aspergillosis prophylaxis, an echinocandin (e.g., caspofungin and micafungin) is used. Alternatively, voriconazole or posaconazole is used in very-high-risk patients.

For treatment, whether empiric or documented, voriconazole is the first-line antifungal agent of choice. However, voriconazole has no activity against mucormycosis, and outbreaks of mucormycosis in patients receiving voriconazole prophylaxis have been reported [2]. For patients who are intolerant of voriconazole or for whom the diagnosis of invasive aspergillosis is not confirmed, liposomal amphotericin B should be considered. For patients who fail treatment with voriconazole, an echinocandin alone or in combination with voriconazole or posaconazole can be used as salvage therapy [10, 14]. In selected cases, surgical intervention has been successful either as treatment or prevention of relapse in patients requiring further chemotherapy or HSCT [11, 14]. Prophylaxis with newer azole derivatives is under investigation for reducing relapse rates [15].

Candida Pneumonia

Introduction and incidence: Pneumonia due to *Candida* species in the HSCT patient population is rare due to the frequent use of prophylaxis with antifungal-azole derivatives (e.g., fluconazole).

Risk factors: Risk factors for *Candida* pneumonia include neutropenia, use of corticosteroids, oral candidiasis, and mucositis.

Differential diagnosis: The differential diagnosis includes bacterial pneumonia, interstitial pneumonitis, and atypical pneumonia.

Clinical and radiographic features: Similar to pneumonia due to other fungi, *Candida* pneumo-

nia in HSCT recipients typically presents with persistent fever that is unresponsive to broad-spectrum antibiotics. Chest CT scan findings include multiple nodules with airspace consolidation. In patients with acute lung injury due to *Candida* pneumonia, the chest CT scan may show extensive ground-glass opacities in addition to a focal area of consolidation.

Diagnostic studies: Similar to other fungal pneumonias, early treatment intervention is key to providing a successful outcome. The only screening method to detect *Candida* infection is serum β-D-glucan. β-D-Glucan screening is of limited value, especially in pediatric HSCT recipients, because it has a low positive predictive [13]. Other diagnostic tools include CXR, CT scan of the chest, BAL with or without transbronchial biopsy, and CT-guided needle biopsy. Whenever possible, biopsy of suspected lesions should be obtained because it will help make a definitive diagnosis and thus help guide the appropriate therapy.

Management and outcome: Antifungal therapy with an azole derivative (e.g., fluconazole, voriconazole, and posaconazole) is used as first-line treatment. Depending upon the species of *Candida* (such as *C. glabrata* and *C. krusei* which are resistant to fluconazole), caspofungin may be indicated although *C. glabrata* isolates that are resistant to echinocandins are on the rise.

Zygomycetes Lung Infections

Introduction and incidence: *Zygomycetes*, including *Mucor* and *Rhizopus* spp., have a reported prevalence of 1.9% in the allogeneic HSCT patient population. Data suggest that the incidence is rising with more frequent use of voriconazole prophylaxis [4].

Differential diagnosis: The differential diagnosis includes bacterial pneumonia, interstitial pneumonitis, and atypical pneumonia.

Clinical and radiographic features: The clinical presentation of pneumonia due to *Zygomycetes* in HSCT recipients has a similar, nonspecific presentation as seen with other fungal pneumonias, including persistent fever that is unresponsive to broad-spectrum antibiotics. There are no biomarkers to identify *Zygomycetes*. β-D-Glucan and galactomannan tests do not detect antigen components of the *mucorales* cell wall. *Zygomycetes*, clinically and radiologically, resembles *Aspergillus*, and, as a result, clinical distinction between the two entities is difficult. Thus, biopsy and culture are critical to distinguish *Zygomycetes* from *Aspergillus* and other more common mold species [16]. Chest CT scan findings include multiple nodules with airspace consolidation. In patients with acute lung injury due to *Zygomycetes*, the chest CT scan may show extensive ground-glass opacities in addition to a focal area of consolidation.

Diagnostic studies: Because *Zygomycetes*-associated infections are so aggressive, an emergent biopsy and/or BAL is strongly recommended in order to accurately identify the causative pathogen and thus provide the most appropriate therapy.

Management and outcome: Because *Zygomycetes*-associated infections, including those of the lung, have an extremely poor prognosis in immunocompromised patients, treatment of *Zygomycetes* infection should be initiated as soon as possible in order to improve outcome. Aggressive treatment is required and includes systemic therapy with amphotericin B and, whenever possible, wide surgical debridement and/or excision of the involved tissue.

Other Fungi

Fusarium and *Scedosporium* species can also cause pulmonary infections but are extremely rare. For example, the incidence of *Fusarium* among patients who underwent allogeneic HSCT ranges from 0.5 to 2% [9].

Early Post-engraftment (31–100 Days Post-HSCT)

Many of the most common etiologies of pulmonary complications that are seen during the early post-engraftment period (days 31–100 post-HSCT) overlap with the pre-engraftment and/or late post-engraftment periods. These include

DAH (which is discussed under "pre-engraftment") and the risk for infections, particularly community-acquired viruses, such as RSV, influenza, adenovirus, rhinovirus, and human metapneumovirus as well as CMV (which are discussed under "late post-engraftment"). While the risk for bacterial pneumonia is present during pre- and early post-engraftment periods, gram-positive organisms are far more common than gram-negative organisms. In addition to the early post-engraftment period, PJP is more prevalent and remains so through the late post-engraftment period. Both idiopathic interstitial pneumonitis (IPS) and bronchiolitis obliterans organizing pneumonia (BOOP) reach median peak incidence during the early post-engraftment period and are discussed in this section.

Idiopathic Interstitial Pneumonia Syndrome (IPS)

Introduction and incidence: Idiopathic interstitial pneumonia syndrome (IPS) is a noninfectious inflammatory process involving the intra-alveolar lining of the lung without a clear causative etiology, in which all infectious, cardiac, and renal causes have been excluded [17]. It results in widespread pulmonary damage shortly after allogeneic HSCT. Clinically, it behaves similar to an infectious pneumonia; however, IPS tends not to respond to antimicrobial therapy. IPS usually occurs within 120 days post-HSCT with the median time of onset between 42 and 58 days [18–21]. The incidence of IPS is 5–25% within the first 120 days of HSCT [17, 19]. It has a high mortality rate of 50%–70% despite improvements in diagnostic tools and in supportive care measures [17, 19, 20].

Pathogenesis: The exact pathogenesis of IPS has not been completely elucidated. However, it is thought that agents used in the conditioning regimen cause damage to pulmonary epithelium which triggers recruitment of macrophages and T-cells to the sites of injury, causing a significant inflammatory response (see Fig. 21.4 for a pictorial representation of the presumed process of IPS) [1–3, 10, 22, 23]. The underlying primary disease is also thought to contribute to the predisposition of IPS. The presence of acute GvHD and immunosuppression appears to exacerbate IPS.

Risk factors: Risk factors for IPS include previous diagnosis of leukemia or myelodysplastic syndrome (MDS), prior allogeneic HSCT, myeloablative conditioning (TBI and/or high-dose cyclophosphamide), chest irradiation, immune-mediated lung injury (acute GvHD), pulmonary infections (e.g., CMV), and increased age of the recipient at time of HSCT [19, 21]. Other factors such as previous exposure to bleomycin, carmustine, methotrexate, melphalan, and cytarabine chemotherapy appear to contribute to an increased risk but have not been statistically significant.

Differential diagnosis: The differential diagnosis for IPS includes but is not limited to infectious pneumonia (bacterial or viral), acute GvHD, drug reaction, inhalation exposure, chronic hypersensitivity pneumonia, collagen vascular disease, and asbestosis [17, 19].

Clinical and radiographic features: IPS typically presents with a nonproductive cough, fever, dyspnea, rales, hypoxemia, and worsening respiratory status. It can be categorized in three different patterns depending upon the site of injury; these are (1) pulmonary parenchyma, (2) vascular endothelium, and (3) airway epithelium [17]. The typical clinical course evolves quickly from mild respiratory symptoms to respiratory failure leading to demise within a few weeks. The radiographic findings are nonspecific with diffuse ground-glass appearance bilaterally, airspace consolidation, and pulmonary edema noted on CXR and CT scan of the chest [17]. The diagnostic criteria of IPS include the presence of diffuse radiographic infiltrates, clinical symptoms of pneumonia (hypoxia, cough, and dyspnea), and evidence of abnormal pulmonary physiology (i.e., an increased A-a gradient and/or restrictive pattern on PFTs) as well as exclusion of active lower respiratory tract infection.

Diagnostic studies: Diagnostic studies include CXR (two views if possible), pulmonary function tests (PFTs), oxygen saturation, ABG, high-resolution chest CT, as well as BAL with or without transbronchial biopsy. CT-guided needle

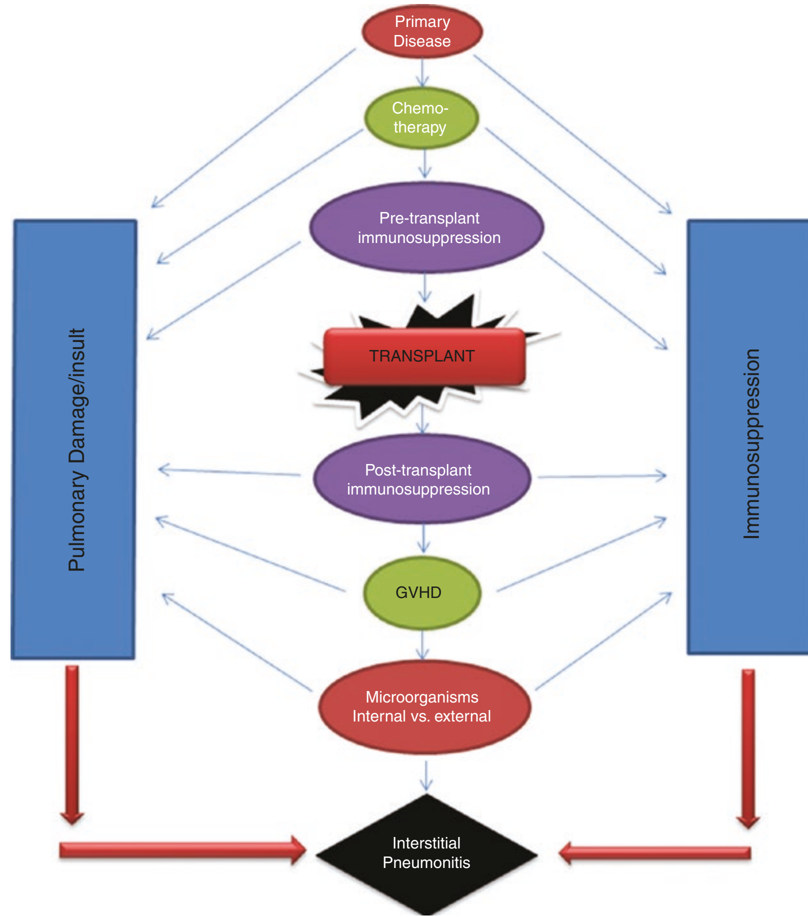

Fig. 21.4 Evolution of idiopathic pneumonia syndrome (IPS). This illustration represents the evolution of the development of IPS in the post-HSCT recipient. IPS is a multifactorial disease process. The underlying primary malignancy predisposes the post-HSCT recipient to an initial pulmonary insult that is exacerbated by the chemotherapy used in the conditioning regimen. The presence of acute GvHD and the use of immunosuppression further contribute to the development of IPS

biopsy or open lung biopsy may be performed to exclude an infectious etiology.

Management and outcome: Preventative measures for infections such as antibacterial, antifungal, antiparasitic, and antiviral therapies are often instituted while awaiting the results of the tests performed to determine the etiology of the respiratory failure. Once an infectious etiology has been excluded, treatment of IPS with corticosteroids should be instituted promptly. In addition, supportive care including oxygenation and respiratory support (i.e., mechanical ventilation) should continue [19, 24]. Recently, the addition of tumor necrosis factor-alpha (TNFα) inhibitors such as etanercept and infliximab to corticosteroid treatment is being actively investigated; however, further work needs to be done in order to prove the effectiveness of the addition of these inhibitors on overall survival [19, 24].

Bronchiolitis Obliterans Organizing Pneumonia (BOOP)

Introduction and incidence: Table 21.3 presents a comparison between bronchiolitis obliterans organizing pneumonia (BOOP) and bronchiolitis obliterans syndrome (BOS) in terms of characteristic pathology, affected lung tissue, PFT measurements, radiographic findings, treatments, and outcomes. BOOP usually occurs on average 3 months post-HSCT (range, 3–14 months) and is characterized by moderate-to-severe restrictive lung disease. The cause of BOOP after HSCT is unclear, although risk factors such as chronic GvHD, matched unrelated donor (MUD) allogeneic HSCT, haploidentical HSCT, and the use of tacrolimus for GvHD prophylaxis have been identified. Various immunologic, toxic, and/or inflammatory insults to the lung may lead to the pathognomonic findings associated with

Table 21.3 Comparison of BOOP and BOS

	BOOP	BOS
Median time of onset post-HSCT	• 3 months post-HSCT (range 3–14 months)	• 12 months post-HSCT (range 6–24 months)
Cause	• Unclear	• Unclear
Pathology	• Nonspecific inflammatory injury	• Fibrotic deposition in small airways and terminal bronchioles that results in bronchiole destruction and scar tissue
Airways affected	• Small airway and alveoli	• Small airways • Alveoli are NOT involved
Pulmonary Function Test (PFT)	• Restrictive pattern with decreased FEV1/DLco	• Obstructive pattern with airflow obstruction
Radiographic findings	• Chest x-ray with fluffy airspace disease	• Not detected on standard chest x-ray or CT scan • Can be detected with high-resolution CT scan • Need bronchoscopy to exclude infection as cause of airflow obstruction
Treatment	• Short course of corticosteroids	• Indolent course • Goes undetected until severe • Treat with corticosteroids, cyclosporine, tacrolimus, and bronchodilators, although typically unresponsive to treatment
Outcome	• High mortality rate	• Severe and irreversible • High mortality • Lung transplant

BOOP. These lesions consist of exudates with plugs of granulation and connective tissue in the distal airways extending in to the alveoli; interstitial inflammation and fibrosis are also present [25, 26].

Risk factors: The risk factors of BOOP include chronic GvHD, MUD allogeneic HSCT, haploidentical HSCT, and use of tacrolimus for GvHD prophylaxis.

Differential diagnosis: The differential diagnosis of BOOP includes infectious interstitial pneumonia as well as drug- and radiation-induced pneumonitis.

Clinical and radiographic features: The clinical features of BOOP include quickly progressive dyspnea preceded by a flu-like illness. Overall, the onset is acute. Pulmonary function tests (PFTs) show restrictive changes with a decreased FEV1/DLCO ratio. Chest CT scan typically shows predominantly peripheral, patchy infiltrates distinguishable from bronchopneumonia (which has a classic mosaic pattern).

Diagnostic studies: Diagnostic studies include PFTs, CXR, and chest CT scan with contrast. A BAL with or without transbronchial biopsy, CT-guided needle biopsy, or an open lung biopsy may need to be performed in order to exclude an infectious etiology.

Management and outcome: Treatment of BOOP includes oral corticosteroids, inhaled corticosteroids, and every other day azithromycin. Additional immunosuppression has been used. BOOP is very responsive to corticosteroid therapy, with about 80% of patients responding, which is much more favorable than the outcomes of patients with BOS [27].

Late Post-engraftment (>100 Days Post-HSCT)

Many of the pulmonary complications that typically occur during the late post-engraftment period (>100 days post-HSCT) are as a consequence of chronic GvHD (both infectious and noninfectious causes). The other major factor contributing to pulmonary complications is delayed immune reconstitution, particularly adaptive immunity (i.e., T- and B-cell recovery). These late pulmonary complications include bronchiolitis obliterans

syndrome (BOS) (which differs from BOOP and is considered by many to be a clinical manifestation of chronic GvHD of the lung), posttransplantation lymphoproliferative disease (PTLD) involving the lungs, infectious pneumonias due to encapsulated bacteria (e.g., pneumococcus), and *Aspergillus* or viral pneumonia including CMV, VZV, and community-acquired viruses (e.g., RSV, parainfluenza, human metapneumovirus, adenovirus, and rhinovirus) [1, 2, 28–30].

Bronchiolitis Obliterans Syndrome (BOS)

Introduction and incidence: Table 21.3 contains a summary comparing BOOP and BOS regarding their characteristic pathology, affected lung tissues, PFT measurements, radiographic findings, treatments, and outcomes. Historically, BOS had a grave prognosis due to its ill-defined diagnostic criteria, unknown pathophysiology, and lack of effective supportive care and therapeutic options [31]. BOS arises from an immune-mediated reaction involving the small airways. This immune-mediated reaction leads to fibrotic deposition in the small airways and terminal bronchioles that eventually causes obliteration of the bronchioles. It is an insidious process that occurs within 2 years post-HSCT with a median onset of 12 months (range, 6–24 months) post-HSCT [31]. The incidence of BOS ranges from 2 to 10%. Its prevalence is 10% among long-term survivors and up to 14% among patients with evidence of chronic GvHD [32]. The mortality rate of BOS is 41% within the first 5 years post-HSCT [32].

Risk factors: BOS is a rare, late complication of HSCT. The cause of BOS post-HSCT is not fully understood. However, there are many risk factors that increase the likelihood of developing BOS. These include HLA mismatch, other manifestations of chronic GvHD, a history of acute GvHD, busulfan-containing conditioning regimens, peripheral blood HSC source, early post-HSCT pulmonary viral infections, ABO incompatibility, prior lung disease, and post-HSCT lung disease [31, 33].

Differential diagnosis: BOS is difficult to diagnose without a lung biopsy, as it may appear similar to other diagnoses on radiographic imaging and clinical presentation. The differential diagnosis of BOS includes idiopathic pneumonia syndrome, cryptogenic-organizing pneumonia (COP), pulmonary fibrosis, late effects from ionizing radiation, infection, asthma, or chronic obstructive pulmonary disease (COPD). The differential diagnosis also includes rare disorders such as tracheomegaly, tracheobronchomalacia, and α-1-antitrypsin deficiency [31].

Clinical and radiographic features: Clinical features of BOS are chronic nonproductive cough, dyspnea on exertion, decrease exercise intolerance, wheezing, or pneumomediastinum [31, 32]. Very often, BOS is accompanied by other manifestations of chronic GvHD. While BOS can mimic other entities on radiographic imaging, the most common CT scan findings on high resolution are reticulonodular disease and air trapping (see Fig. 21.5). The most useful diagnostic tool is PFTs. Early PFT findings include a diminished FEV1 which can also be used to measure treatment response and disease progression.

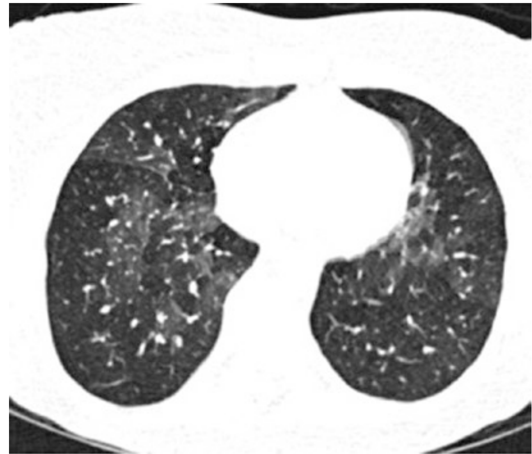

Fig. 21.5 Radiographic evidence of bronchiolitis obliterans syndrome (BOS). High-resolution CT image showing mosaic pattern in a patient with BOS with airspace and nodular opacities (From Amy K. Chi, Ayman O. Soubani, Alexander C. White, Kenneth B. Miller, An Update on Pulmonary Complications of Hematopoietic Stem Cell Transplantation, Chest, Volume 144, Issue 6, 2013, 1913–1922, https://doi.org/10.1378/chest.12-1708)

Diagnostic studies and criteria: Historically, lung biopsy was considered the gold standard to make the diagnosis of BOS; however, patients undergoing lung biopsy had significant morbidity. As a result, noninvasive diagnostic criteria have been developed for BOS. The current diagnostic criteria includes (1) FEV1 <75% predicted and an irreversible ≥10% decline in <2 years, (2) FEV1-to-vital-capacity (VC) ratio <0.7 or the lower limit of the 90% confidence interval of the ratio, (3) absence of infection, and (4) either preexisting diagnosis of chronic GvHD, air trapping by expiratory CT scan, or air trapping on PFTs measured by residual volume (RV) >120% or RV/total lung capacity (TLC) exceeding the 90% confidence interval [31, 32]. The severity of disease may be classified as mild (FEV1 measuring >60%), moderate (FEV1 measuring between 40 and 59%), and severe (FEV1 measuring <39%). BAL is a valuable tool in making the diagnosis of BOS. Alternatively, it can be useful to identify or exclude other pulmonary etiologies such as infection (which often contributes to the development of BOS) and help guide further management [31]. However, there are limitations to BAL as a diagnostic tool because the diagnostic yield may be as low as 36% [34].

Due to the limitations of BAL, if there is a high suspicion for both BOS and infection, it is common practice to proceed directly to lung biopsy despite the risks of comorbidities. *Management and outcome*: Over the last few decades, there has been significant improvement in both supportive care and treatment guidelines resulting in improved outcomes and overall survival for post-HSCT patients with BOS. Early detection improves overall survival; hence, frequent PFT monitoring in post-HSCT patients is important. It is recommended that PFTs be monitored at least every 3 months for the first year after HSCT and then annually thereafter. If a diagnosis of BOS is rendered, recommended PFT measurements should be performed more frequently in order to monitor the change in the slope of FEV1 volume over time. This measurement dictates further escalation of treatment and/or investigation of other etiologies that may be contributing to declining lung function [31, 33].

Though BOS is considered noninfectious, antimicrobial prophylaxis is recommended for patients with BOS. These include trimethoprim/sulfamethoxazole for *Pneumocystis jiroveci* pneumonia, penicillin for *Streptococcus*, and voriconazole or posaconazole for fungal coverage [31, 32].

Historically, patients with BOS have been treated with systemic corticosteroids. The downside to this approach is the increased risk of infection due to immune suppression. Recent studies have suggested that other modalities of immune suppression such as inhaled fluticasone, oral azithromycin, and montelukast (FAM) with a brief burst of prednisone (1 mg/kg/day) followed by a rapid taper has led to improved results. In a multi-institutional study of 36 patients using this approach, 94% of patients showed stabilization or improvement of their disease in 3 months and survival of 97% at 6 months [31, 35].

A high mortality rate has been associated with BOS despite aggressive interventions. However, recent estimates show a 60–70% survival rate at 2–3 years post-diagnosis and 40–50% 5-year survival as compared to 40–20% for 2–3- and 5-year survival reported previously. This improvement in survival is likely due to better supportive care and new treatment approaches [31–33].

Bacterial Pneumonia

Introduction and incidence: Lung infections that occur later than 100 days post-HSCT are generally caused by encapsulated bacteria (e.g., *S. pneumoniae* and *H. influenza)*, although their frequency is less than during the pre-engraftment phase. These types of infections continue to occur into the first year post-HSCT, largely due to defects in cellular and humoral immunity. During this period, numerous other bacteria can cause bacterial infections, including *Legionella*, *Nocardia*, and *Actinomyces* [1, 10, 36, 37].

Risk factors: Risk factors of bacterial pneumonia during the late post-engraftment phase include delayed immune reconstitution post-HSCT, hypogammaglobulinemia, and long-term immunosuppression (particularly steroids) for the treatment of chronic GvHD.

Differential diagnosis: The differential diagnosis of bacterial pneumonia includes interstitial pneumonitis, atypical pneumonia, respiratory viruses, PJP pneumonia, CMV pneumonia, *Aspergillus* pneumonia, PTLD, BOOP, and BOS.

Clinical and radiographic features: The clinical findings are relatively nonspecific, and they include fever, hypoxemia, increased work of breathing, and dry or productive cough. CXR often shows consolidation of alveolar sacs and an isolated area of consolidation. Similar to immunocompetent patients with pneumonia, the radiographic findings lag behind by the clinical findings.

Diagnostic studies: Diagnostic studies include chest radiograph (two views if possible), PFTs, oxygen saturation, ABG, and high-resolution chest CT. BAL, transbronchial biopsy, CT-guided needle biopsy, or open lung biopsy are performed if the etiology is unclear.

Management and outcome: If the post-HSCT patient has chronic GvHD, then much of their management involves the prevention of these infections including the use of prophylactic penicillin to help prevent the development of infections with encapsulated bacteria, In addition, immunoglobulin replacement is administered for patients with hypogammaglobulinemia. A thorough evaluation of fever in a post-HSCT patient who is still immunocompromised is essential.

Mycobacteria Pneumonia

Introduction and incidence: Mycobacterial and atypical mycobacterial infections are occasionally reported after HSCT [2, 38]. The overall incidence of *M. tuberculosis* infections in allogeneic HSCT recipients is 1–3% [39]. *M. haemophilum* and *M. avium* complex can be important pulmonary pathogens after HSCT as well.

Risk factors: Total body irradiation, chronic GvHD (requiring escalation of immunosuppressive therapy), and patients older than 45 years are associated with an increased risk of mycobacterial infections.

Differential diagnosis: The differential diagnosis of mycobacteria pneumonia includes other infectious etiologies such as bacterial and fungal pneumonia in addition to BOOP, BOS, and metastatic malignancy [38, 40].

Clinical and radiographic features: Radiological manifestations are variable and may include consolidation and patchy inflates, pulmonary nodules, lung cavitation, multifocal bronchiectasis, and plural effusions [38]. *M. haemophilum* and *M. avium* should be suspected in patients with skin nodules with or without pulmonary infiltrates. Other features consist of lymphadenopathy, fever, weight loss, diarrhea, nonproductive cough, chest pain, and hepatosplenomegaly.

Diagnostic studies: Because allogeneic HSCT is associated with depressed delayed-type hypersensitivity reactions, skin testing with purified protein derivative (PPD) is not likely to be useful. Sputum samples may be helpful in making a diagnosis. More useful tests consists of *Mycobacterium* PCR to confirm the diagnosis in addition to special culture that require isolated conditions. Thus, close communication with the microbiology laboratory is essential. Failure to recognize this treatable pathogen in a timely fashion can lead to a fatal outcome.

Management and outcome: Management can differ depending on tubercular versus non-tubercular etiologies. Regardless of the type of mycobacterial infection, once infection is proven, treatment should be multidrug due to increased risk of resistance. Non-tubercular *Mycobacterium* (NTM) is associated with CVC-induced bacteremia. Recovery of the immune system improves survival in patient with disseminated NTM. Treatment of NTM includes an initial and a continuation phase. Antimicrobial therapy is based on the antimicrobial susceptibility. For the initial phase, antimicrobial agents are generally prescribed for 1–2 months or until radiographic improvement is noted [38, 40]. At least three drug combinations are used in the initial phase. Typical regimens include macrolides, azithromycin, fluoroquinolones ethambutol, and rifampin. The continuation phase begins once the patient has demonstrated clinical improvement. The antimicrobial regimen for the continuation phase is usually composed of a two-drug regimen, with the total duration of therapy being 6–12 months. Non-tubercular *Mycobacterium* prognosis is 42% [38].

Tubercular infections are often sensitive to first-line drugs such as rifampin, isoniazid (INH), pyrazinamide, and ethambutol. Similar to NTM, every effort should be made to reduce the patient's immunosuppression when feasible.

Viral Pneumonia

HSCT recipients are at risk for serious lung infections due to respiratory viruses, such as influenza A and B, parainfluenza viruses (PIV) (especially PIV 3), respiratory syncytial virus (RSV), and human metapneumovirus (hMPV). Lymphopenia appears to be an important risk factor for respiratory virus infection.

Post-HSCT patients who are in the late post-engraftment phase are at great risk for reactivation as well as de novo acquisition of viral infections due to delayed reconstitution of adaptive immunity. These infections are both opportunistic and community acquired. Both types carry high mortality rates. Thus, patients need to be monitored closely for viral reactivation, and they should avoid contact with individuals with cold symptoms and avoid large crowds until immune reconstitution has occurred.

Cytomegalovirus (CMV)

Introduction: CMV pneumonia rarely occurs during the pre-engraftment period, as the major risk involves impaired cellular immunity. However, once engraftment occurs, it should be included in the differential diagnosis of cough, fever, or dyspnea, even in the absence of radiographic abnormalities. CMV is in the herpes virus family and can manifest itself in post-HSCT patients in three ways: (1) reactivation of latent CMV, (2) acquired viral pathogen from an infected HSC donor, and (3) acquired through blood transfusion [41–44]. Preemptive and prophylactic antiviral therapy has markedly reduced the incidence and severity of CMV disease and has delayed its onset, although CMV must be considered in any allogeneic HSCT recipient who is CMV seropositive or received HSCs from a seropositive donor [2, 41–44].

Risk factors: Allogeneic HSCT recipients are at high risk for CMV reactivation which may develop into CMV pneumonia. The risk is highest in seropositive recipients who receive HSCs from a seronegative donor. Another risk factor is prolonged immunosuppression, particularly the use of corticosteroids.

Differential diagnosis: The differential diagnosis of CMV pneumonia is interstitial pneumonitis, bacterial pneumonia (esp. *Legionella* spp.), radiation pneumonitis, other viral infections, cardiac failure, diffuse alveolar hemorrhage (DAH), and pulmonary edema.

Clinical and radiographic features: The clinical features associated with CMV pneumonia are nonspecific, making it difficult to diagnose early in its course. Patients may present with fever, tachypnea, rales, diminished breath sounds, lethargy, restlessness, and hypoxemia. Chest radiographs typically show bilateral, patchy areas of ground-glass or consolidation. High-resolution computed tomography (HRCT) may show ground-glass attenuation, parenchymal opacification, or innumerable small (<5 mm) nodules [2, 41–44].

Diagnostic studies: CMV by quantitative PCR from the blood should be performed one to two times a week in all allogeneic HSCT recipients, although patients can have CMV pneumonia without CMV viremia. A CXR (preferably two views) and high-resolution CT scan of the chest should also be performed if CMV pneumonia is suspected.

Management and outcome: Preemptive treatment should be started immediately in patients who show signs of viremia by PCR, i.e., prior to the manifestation of clinical symptoms of CMV pneumonia [43, 44]. Treatment of CMV infection/reactivation includes ganciclovir, foscarnet, or cidofovir. Ganciclovir is the first-line treatment in patients who have robust, sustained donor engraftment, and adequate renal function. Ganciclovir acts by inhibiting viral replication. The dosing is 5 mg/kg IV Q 12 h (see Chap. 28). Patients are transitioned to prophylaxis dosing once CMV viremia resolves because patients

who have reactivated CMV are very likely to reactivate again. Foscarnet is typically reserved for use in patients who have limited graft function. The dosing is 60 mg/kg/dose TID for a minimum of 7 days and then changed to maintenance dosing once CMV viremia resolves (see Chap. 28). Also, foscarnet may be used as prophylaxis during the pre- and early post-engraftment phases for patients who are at very high risk for CMV reactivation (i.e., recipient is seropositive and donor is seronegative). Cidofovir may be used in patients who have CMV that is refractory to ganciclovir or foscarnet. Dosage is either 5 mg/kg/dose once a week or 1 mg/kg/dose three times a week (see Chap. 28). Because of its known nephrotoxicity, cidofovir needs to be given with probenecid along with pre- and post-hydration in order to aid in preserving renal function. In addition to antiviral agents, CMV hyperimmune globulin (Cytogam) or IVIg should be considered in patients with CMV viremia, as well as those with CMV disease, e.g., pneumonia.

Community-Acquired Respiratory Viral Infections

Community-acquired respiratory viral infections (e.g., influenza, parainfluenza, respiratory syncytial virus (RSV), adenoviruses, rhinovirus, and human metapneumovirus) can occur during the post-engraftment period. Because of delayed adaptive immune reconstitution in allogeneic HSCT recipients (particularly those on immunosuppression), community-acquired respiratory viral infections can become deadly with the development of lower respiratory tract involvement. Details pertaining to specific viral lung infections in HSCT recipients' post-engraftment are provided below [36, 45].

Influenza Virus

Introduction and incidence: Influenza viruses have the potential to cause serious lung infection and respiratory failure among HSCT recipients because they tend to develop into lower tract respiratory disease. Infections with influenza tend to be seasonal, predominantly between November and April in North America. Progression to pneumonia is more likely among lymphopenic patients and, thus, is more common in the post-engraftment phase than pre-engraftment phase [36, 45]. All HSCT recipients should be immunized against influenza as soon as the vaccine is available in the early fall.

Risk factors: Risk factors for community-acquired respiratory viral infections are exposure to school-aged household contacts, hypogammaglobinemia, immunosuppressive therapy, inability to receive annual vaccines, nosocomial outbreaks, and chronic lung disease.

Differential diagnosis: The differential diagnosis of community-acquired respiratory viral infections includes bacterial pneumonia, fungal pneumonia, and PJP as well as IPS.

Clinical and radiographic features: The presence of rhinorrhea, high fevers, myalgia, malaise, cough, headache, and/or sinusitis should raise suspicion for influenza. The CT scan findings of influenza (as well as parainfluenza virus) include small peribronchiolar nodular opacities, ground-glass opacities, and/or airspace consolidation [46].

Diagnostic studies: The diagnosis of influenza can be established by rapid immunofluorescence detection of respiratory secretions (respiratory viral panel), throat swabs, or nasopharyngeal washes [46].

Management and outcome: Antiviral therapy for most community-acquired respiratory viral infections is typically supportive care and symptom management in addition to decreasing and ideally discontinuing immunosuppression if possible for HSCT recipients. Influenza can be treated with oseltamivir; however, it is time sensitive. Treatment with oseltamivir shortens the period of illness but does not rid one of the virus. Patients who present within the 48 h of onset of symptoms should have oseltamivir administered. IVIg can generally be used for patients who have had recurrent respiratory infections to provide nonspecific passive immunity.

Respiratory Syncytial Virus (RSV)

Introduction and incidence: Respiratory syncytial virus (RSV) generally begins as an upper respiratory tract infection. However, immunocompromised patients, including post-HSCT recipients, are at great risk for developing lower respiratory tract infection (LRTI) which has a high mortality rate [2]. RSV has a marked seasonal variation in incidence, with the peak between January and March in North America.

Risk factors: Risk factors for RSV pulmonary infections are the same as those for other community-acquired respiratory viral infections and include exposure to school-aged household contacts, hypogammaglobinemia, immunosuppressive therapy, nosocomial outbreaks, and chronic lung disease.

Differential diagnosis: The differential diagnosis of RSV respiratory infections includes bacterial pneumonia, fungal pneumonia, and PJP as well as IPS.

Clinical and radiographic features: For patients with RSV, presenting symptoms include fever, wheezing, increased work of breathing, and hypoxemia, and they often quickly progress. Chest radiograph shows bilateral, ground-glass opacities. Note: the chest x-ray findings often lag behind the development of lower respiratory tract infection (LRTI) symptoms; thus it should not be excluded [46]. Upper respiratory tract symptoms may precede lower tract disease by several days, although pneumonia (i.e., LRTI) can be the initial presentation.

Diagnostic studies: Similar to influenza, the diagnosis RSV can be established by rapid immunofluorescence detection of respiratory secretions (respiratory viral panel), throat swabs, or nasopharyngeal washes [46].

Management and outcome: As for most community-acquired respiratory viral infections, the management of RSV consists of supportive care, symptom management, and decrease or ideally discontinuation of immunosuppression if possible for HSCT recipients. Oral ribavirin is prescribed in patients with RSV who are at low risk for developing LRTI. Aerosolized ribavirin can be used in patients with RSV who are at high risk for developing LRTI. Patients who are at higher risk for developing LRTI include immunosuppressed patients or patients with delayed immune reconstitution. Intravenous immunoglobulin (IVIg) and palivizumab are often administered for RSV LRTI or patients who are at high risk of developing LRTI.

Parainfluenza Virus

Introduction and incidence: Parainfluenza virus is a recognized cause of both upper and lower respiratory tract infection after HSCT, and it affects 2–7% of HSCT recipients. It is seasonal, occurring most often during the fall and winter months [2, 36, 45]. There are four serotypes, with type 3 being the most common cause of lung infection after HSCT. The incubation period is 1–4 days.

Risk factors: Risk factors for parainfluenza virus pulmonary infections are the same for all community-acquired respiratory viral infections with exposure to school-aged household contacts, hypogammaglobinemia, immunosuppressive therapy, nosocomial outbreaks, and chronic lung disease being the most common.

Differential diagnosis: The differential diagnosis of parainfluenza-mediated respiratory viral infections includes bacterial pneumonia, fungal pneumonia, PJP, and IPS.

Clinical and radiographic features: The clinical features of parainfluenza are nonspecific and include cough, rhinorrhea, otitis media, fever, malaise, and/or sinusitis. The CT scan findings of parainfluenza virus include small peribronchiolar nodular opacities, ground-glass opacities, and/or airspace consolidation [46].

Diagnostic studies: The diagnosis of parainfluenza-induced respiratory viral infections can be detected by rapid immunofluorescence detection of respiratory secretions (respiratory viral panel), throat swabs, or nasopharyngeal washes [46].

Management and outcome: The management for most community-acquired respiratory viral

infections which includes parainfluenza virus centers on supportive care and symptom management. In addition, every effort should be made to decrease and, ideally, discontinue immunosuppression if possible. Aerosolized ribavirin can be used for HSCT recipients who have lower tract parainfluenza infection. IVIg can generally be used for patients who have had recurrent respiratory infections to provide nonspecific passive immunity.

Studies have demonstrated that the rate of parainfluenza respiratory viral infections is high in HSCT recipients. More than half of the HSCT recipients with parainfluenza respiratory infections will require hospitalization. These findings emphasize the importance of preventative strategies against not only parainfluenza but also all respiratory viral infections. Such strategies include droplet isolation and avoidance of sick contacts. The prevalence of respiratory viral infection, including parainfluenza, has been reported ranging from 15 to 38% and can often be fetal [46, 47].

Adenoviruses

Introduction and incidence: Respiratory adenovirus infection has been isolated in 3–5% of patients after HSCT and should be considered in the differential diagnosis of pulmonary infection [36, 48]. While adenoviruses tend to be seasonal, adenovirus infection should be suspected in all post-HSCT patients with respiratory symptoms regardless of the season.

Risk factors: The risk factors for adenovirus are GvHD, the use of T-cell-depleted donor HSC or umbilical cord blood HSCs, rabbit antithymocyte globulin as part of the conditioning regimen, and use of prolonged immunosuppression [47].

Differential diagnosis: Other viral respiratory infections should be considered part of the differential diagnosis when adenovirus is suspected.

Clinical and radiographic features: Affected patients may present with pharyngitis, tracheitis, bronchitis, pneumonitis, enteritis, hemorrhagic cystitis, or disseminated disease (viremia). Their respiratory status may progress to respiratory failure requiring mechanical ventilation and may be fatal. The specific pattern of symptoms depends at least in part on the particular serotype of adenovirus and on the age of the recipient, with younger HSCT recipients at risk for more severe infection. Asymptomatic shedding of adenovirus can often be detected in cultures from the pharynx, respiratory secretions, stool, or urine up to 2–3 months after the active infection has resolved [36, 45, 47, 48].

Diagnostic studies: Adenovirus can be rapidly detected by immunofluorescence as part of the RVP. Identification of specific serotypes can be determined, but these test results often take days to become available, whereas RVP results can be available within hours at most institutions. Adenovirus can also be detected by PCR, and this test is exquisitely sensitive. Frequent monitoring with adenovirus quantitative PCR in high-risk populations is highly recommended.

Management and outcome: Ribavirin and cidofovir are agents used in the treatment of adenovirus. More evidence for efficacy exists for cidofovir. Antiviral treatments can be used as prophylaxis, as preemptive (based on viral load cutoff values), or as therapeutic treatment in cases of frank adenoviral infection. Close monitoring with quantitative PCR followed by preemptive treatment with low dose of (1 mg/kg) cidofovir three times per week can be effective in most cases to bridge patients during their most severely immunocompromised period post-HSCT [47, 48]. Nephrotoxicity is a risk of cidofovir therapy; therefore, hyperhydration and coadministration of the drug probenecid decrease the risk of acute renal injury.

Definitive cure requires adequate immune reconstitution. Therefore, every effort should be made to decrease immunosuppression to enhance T-cell recovery. Methods to hasten this immune reconstitution after HSCT have been used with some success. These include donor lymphocyte infusion (DLI), the infusion of adenovirus-

specific cytotoxic T-cells, and adoptive immunotherapy [48].

Adenovirus can cause significant morbidly and mortality in HSCT patients. Ultimately, clearance of adenovirus requires reconstitution of immunity which is often delayed in HSCT recipients who receive T-cell-depleted or UCB donor grafts as their HSC source. Adenovirus occurs in 5–21% of allogeneic HSCT patients. Mortality rates of up to 75% have been reported for adenovirus pneumonia [47, 48].

Fungal Pulmonary Infections

Introduction and incidence: During the post-engraftment period, patients are at risk for infection with aspergillosis and other invasive fungi and molds. The median time of onset of fungal and mold infections of the lung is 100 days post-HSCT, and so patients in both the early and late phases of post-engraftment are susceptible to contracting fungal pulmonary infections [11, 14, 15].

Risk factors: Risk factors for developing fungal pneumonia during the late post-engraftment period include older age, the presence and severity of GvHD, corticosteroid therapy, and leukopenia.

Differential diagnosis, *clinical and radiographic features*, *diagnostic studies*, and *management* of aspergillosis and other fungi and molds are the same as in the early post-engraftment period and are detailed under that section.

Protozoa Lung Infections

Pneumocystis jiroveci Pneumonia (PJP)

Introduction and incidence: Prior to the universal implementation of prophylaxis with Bactrim, PJP was a leading cause of HSCT-related mortality. However, with appropriate prophylaxis, the risk for developing PJP has been greatly reduced and is now rarely seen. The incidence of reported cases is approximately 2% [49]. PJP is thought to infect most humans in early childhood and remains dormant. However, PJP may become active most often in immunocompromised HSCT patients greater than 30 days post-HSCT [1–3].

Risk factors: Risk factors for PJP include allogeneic HSCT recipients, poor T-cell function, post-HSCT corticosteroid use for the treatment of GvHD, a history of PJP prior to HSCT, and poor compliance with prophylaxis.

Differential diagnosis: The differential diagnosis includes bacterial pneumonia (especially atypical bacterial pneumonia) *Legionella* pneumonia, other protozoa respiratory infections (e.g., *Toxoplasma Gondii)*, and viral pneumonia.

Clinical and radiographic features: Clinical features include a dry, nonproductive cough, acute onset of tachypnea and dyspnea, hypoxemia, fever, and malaise. Symptoms often rapidly progress; ABG reveals decreased PO_2. CXR shows diffuse interstitial alveolar infiltrates that is bilateral and symmetric.

Diagnostic studies: BAL with immunofluorescent staining for PJP is the gold standard for making the diagnosis of PJP. Often, serum LDH is elevated but is not diagnostic.

Management and outcome: Management of PJP is three pronged: (1) support oxygenation (mechanical ventilation is often necessary), (2) administration of high-dose co-trimoxazole (see Chap. 28 for dosing guidelines), and (3) administration of corticosteroids to help dampen the associated pulmonary inflammation seen with PJP. If a patient is unable to tolerate co-trimoxazole due to myelosuppression or allergy, dapsone or pentamidine may be used. Following 21 days of therapy, patients are transitioned to prophylaxis dosing.

Toxoplasma Gondii

Introduction and incidence: *T. gondii* is a protozoan parasite that most frequently occurs in the central nervous system (brain) but can also cause pneumonia in the immunocompromised HSCT

recipient. *T. gondii* is a rare but possibly underestimated complication following allogeneic HSCT, with the incidence being reported as less than 1% [50]. This low reported incidence may be due to the limitations of diagnostic instruments used to detect toxoplasmosis.

Risk factors: Risk factors for *T. gondii* infections are *T. gondii* seropositivity, undergoing UCBT, an unrelated donor HSCT, and a T-cell-depleted donor graft. In addition, patients who received alemtuzumab as part of their conditioning regimen are more susceptible to *T. gondii* infections. Patients with chronic GvHD or patients who are unable to take Bactrim are at higher risk for contracting *T. gondii* infection.

Differential diagnosis: The differential diagnosis includes atypical bacteria, *Legionella, C. neoformans, Candida* species, aspergillosis, PJP, CMV, RSV, or parainfluenza.

Clinical and radiographic features: Clinical features include fever, cough, and increased work of breathing. A CXR typically shows bilateral pulmonary infiltrates.

Diagnostic studies: Diagnostic studies include chest radiograph and chest CT scan. However, a definitive diagnosis of *T. gondii* cannot be established without a BAL. PCR can be used to detect *T. gondii* from BAL specimens. *T. gondii* PCR of the serum can be used for routine monitoring.

Management and outcome: Prevention of *T. gondii* infection is the best management. Thus, pre-transplant serologic testing is performed to identify high-risk patients. Prophylaxis with Bactrim is used. The recommended treatment is with dual therapy using Bactrim plus pyrimethamine–sulfadoxine.

Summary

Overall, early detection and prompt investigation of pulmonary symptoms are essential for successful management of pulmonary complications in post-HSCT patients. Early diagnosis and prompt intervention can blunt disease progression and prevent poor outcomes, including death. HSCT patients who are at high risk for pulmonary complications include recipients of matched, unrelated donor HSCT, or umbilical cord blood and patients on corticosteroids. These high-risk patients need to be screened weekly with PCR tests from the blood for adenovirus, CMV, and EBV and a serum galactomannan immunoassay for early *Aspergillus* detection. A comprehensive respiratory viral panel for a broad range of viruses is performed to all potential HSCT recipients during cold and flu season. Post-HSCT patients who present with rhinorrhea, cough, shortness of breath, fever, or a change in activity tolerance need immediate evaluation. A chest radiograph should be performed with new onset of fever and/or deterioration in respiratory status in patients during the peri-HSCT period. Chest computed tomography (CT) should be performed if the patient is persistently febrile. Pulmonary function tests should be done at regular intervals post-HSCT to detect deterioration of pulmonary function before a patient becomes symptomatic. Important prophylactic measures include frequent incentive spirometry, encouraging activity, and compliance with prophylactic antimicrobials, influenza vaccination, and avoidance of sick contacts.

Key Points

- The risk of pulmonary complications post-HSCT continues to be high, and early recognition and treatment may improve outcome.
- Post-HSCT-associated pulmonary complications can be classified as infectious or noninfectious.
- The development of post-HSCT complications follows a relatively predictable timeline. However, while the risk of certain post-HSCT pulmonary complications spans the entire HSCT time course (from pre- to late post-HSCT), others develop more commonly during a discrete phase(s) of HSCT.
- While mortality post-HSCT continues to improve, respiratory failure from pulmonary complications continues to be a leading cause of post-HSCT morbidity and mortality.

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22 Renal Complications Associated with HSCT

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Abstract

Acute kidney injury and CKD remain significant complications of hematopoietic stem cell transplantation (HSCT) and are associated with morbidity and mortality. Careful assessment of kidney function, e.g., glomerular filtration rate (GFR), blood pressure, and proteinuria, is a critical first step in the detection of kidney disease and the prevention of further injury, when possible. While kidney injury can be multifactorial after HSCT, the most common causes include medications, infections, thrombotic microangiopathy, and perhaps GvHD. Close collaboration between HSCT providers, nephrologists, infectious disease experts, and, when needed, critical care teams is essential to the prevention and management of kidney injury in this high-risk population. This chapter discusses the prevalence and diagnosis of renal dysfunction post-HSCT. It also addresses common causes of kidney injury post-HSCT. Hemorrhagic cystitis is discussed in greater detail elsewhere (see Chap. 16).

Prevalence of Renal Dysfunction Post-HSCT

Patients who undergo hematopoietic cell transplantation (HSCT) are at high risk for renal complications (Table 22.1). At least one third of patients experience acute kidney injury after HSCT [1]; 5–10% of patients will require acute dialysis within the first 100 days of HSCT [2], and approximately 15% of HSCT recipients develop chronic kidney disease (CKD) within a year of transplant [3]. Depending on the type of transplant, the cumulative incidence of CKD within 5 years of HSCT is up to 44%, although the exact risk of CKD in this population is not known [4, 5].

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Table 22.1 Common causes of renal complications in HSCT

Renal complication	Prevalence	Symptoms/Signs	Associated factors	Management/treatment
Drug-induced nephrotoxicity	Common	• Rising creatinine • Electrolyte wasting	• Administration of nephrotoxic medication	• Optimize medication dosing • Management of associated electrolyte abnormalities
Thrombotic microangiopathy	10–25%	• Rising creatinine • Hypertension • Hemolytic anemia • Thrombocytopenia • Schistocytosis	• Radiation and intensive chemotherapy • Calcineurin/mTOR inhibitors • Infection	• Discontinuation of causative agent (such as calcineurin inhibitor)
Hemorrhagic cystitis/BK viral nephropathy	5–20%	• Hematuria • Dysuria • Suprapubic pain • Rising creatinine	• BK viremia	• Hyperhydration • Treatment of infection • Minimize immunosuppression
Nephrotic syndrome/ graft-versus-host disease	1–6%	• Proteinuria • Edema • Hypoalbuminemia • Hypercholesterolemia	• Minimal change disease or membranous nephropathy on biopsy Chronic GvHD	• Steroids/ immunosuppression

Detection and Diagnosis of Renal Dysfunction

Serum creatinine remains the most clinically available estimate of kidney function, although it has limitations and may overestimate glomerular filtration rate (GFR), especially in HSCT patients who have low muscle mass from poor nutritional status and inactivity. Cystatin C is a small housekeeping protein made by all nucleated cells and provides a muscle mass-independent estimate of GFR that, like creatinine, can also be obtained on a single blood sample [6]. Cystatin C may offer a more accurate estimate of GFR than creatinine, although more research is needed on the use of this endogenous marker in the HSCT population given its concentration may be affected by inflammation, steroid use, and thyroid disease [7]. Formal measures of GFR remain the gold standard, but they require injection of an exogenous tracer (nuclear isotopes such as DTPA or EDTA or contrast agents such as iothalamate or iohexol) and are therefore more expensive, invasive, and time consuming.

Proteinuria: Proteinuria as a measure of renal injury can be more sensitive than creatinine in HSCT patients. The presence of protein in the urine on urinalysis is a marker of renal inflammation and glomerular injury and is associated with an increased risk of late complications post-HSCT, including non-relapse mortality [8]. Proteinuria occurs in approximately 15% of patients by day 100 post-HSCT and is present in 4% of recipients at 1 year [8]. In cases in which persistent acute kidney injury, unexplained CKD, or significant proteinuria is present, renal biopsy can be useful in determining the underlying etiology and in making therapeutic decisions. However, in the early post-engraftment period (as defined as 30–100 days post-HSCT), renal biopsy carries significant risk of bleeding complications due to thrombocytopenia, hypertension, and high-dose chemotherapy- or radiation-induced small vessel vasculopathy.

Hypertension: Hypertension can be both an acute and chronic complication of HSCT and can occur alone or in association with acute kidney injury or CKD. Therefore, careful monitoring of blood pressure can aid in the detection of kidney disease after HSCT. The prevalence of hypertension in the post-HSCT population is triple that of the general population and occurs 25 years earlier in post-HSCT patients [9]. In addition to intrinsic kidney injury, common causes of ele-

vated blood pressures after HSCT include fluid overload, pain from mucositis, corticosteroid use, and calcineurin inhibitor therapy for graft-versus-host disease (GvHD) prophylaxis and treatment.

Drug-Induced Renal Toxicity

Prior to and over the course of HSCT, patients are exposed to many nephrotoxic medications including conditioning chemotherapy and radiation. As noted, calcineurin inhibitors, such as cyclosporine A and tacrolimus, are among the mainstays of therapy for prevention and treatment of GvHD. Because of their narrow therapeutic index, calcineurin inhibitor drug levels are usually monitored closely, and in cases in which patients have been exposed to elevated levels, calcineurin inhibitor toxicity should be considered and renal function monitored prospectively. Calcineurin inhibitors lead to renal vasoconstriction mediated via the renin-angiotensin system and the associated renal tissue hypoxia can cause reversible renal damage (Fig. 22.1). Calcineurin inhibitor toxicity can also manifest as acute tubular dysfunction preventing magnesium reabsorption, leading to urinary losses of magnesium. Finally, direct toxicity to tubular epithelial cells and irreversible necrosis of vascular smooth muscle contribute to arteriolar hyalinosis, the hallmark of calcineurin inhibitor nephrotoxicity on renal biopsy. The nephrotoxicity of calcineurin inhibitors must be balanced with the risk of GvHD by careful titration of dosing based on drug trough level monitoring.

Several antimicrobials, such as vancomycin and aminoglycosides, are frequently employed to prevent or treat the infectious complications of HSCT and are known to have significant nephrotoxicity. While lipid-based amphotericin B has decreased the associated nephrotoxicity, amphotericin B remains a major cause of kidney injury in HSCT patients. Typically, a rise in creatinine can be noted within 7 days of starting therapy, and electrolyte abnormalities are commonly associated with amphotericin B administration, particularly after prolonged use. Cidofovir is first-line therapy for the treatment of adenovirus infection and is also often employed in the treatment of refractory herpes viral infections, such as CMV. Cidofovir causes proximal tubular necrosis due to accumulation intracellularly in the renal cortex and is generally administered with aggressive hydration and probenecid, which mitigates the toxicity by competing for the intracellular transport [10]. Foscarnet is frequently used in the pre-engraftment period for CMV; the major dose-limiting toxicity is renal impairment, which in some cases has led to severe electrolyte disturbances and death from seizures or cardiac dysfunction [11].

These antimicrobial drugs are often used in combination or sequentially with each other and other nephrotoxic medications as well as in the context of other renal insults, such as dehydration. It is important to note that, in addition to causing kidney damage, many of these medications are cleared by the kidney and thus need to be dose-adjusted in the setting of a decreased GFR.

Thrombotic Microangiopathy

Thrombotic microangiopathy (TMA) is clinically characterized by kidney injury, hypertension, thrombocytopenia, and microangiopathic hemolytic anemia and occurs in 10–25% of transplant recipients. Transplant associated-TMA occurs in the setting of endothelial injury causing platelet aggregation, platelet consumption, and microvascular fragmentation of erythrocytes, which leads to fibrin deposition and thrombosis in the microcirculation of the kidney and other organs. Many factors have been implicated in the endothelial injury that ultimately leads to TMA. These include radiation and chemotherapy during conditioning, calcineurin and mTOR inhibitors, infection, cytokine release, and complement dysregulation [12].

When TMA is suspected, initial management should include consideration of dose reduction or withdrawal of the suspected causative agent, whenever possible. For example, transition from a calcineurin inhibitor to another agent for GvHD prophylaxis, such as mycophenolate mofetil (MMF), should be considered if clinically possible. Of note, plasmapheresis has not demonstrated the same utility that has been seen in classic thrombotic thrombocytopenic purpura (TTP), but ritux-

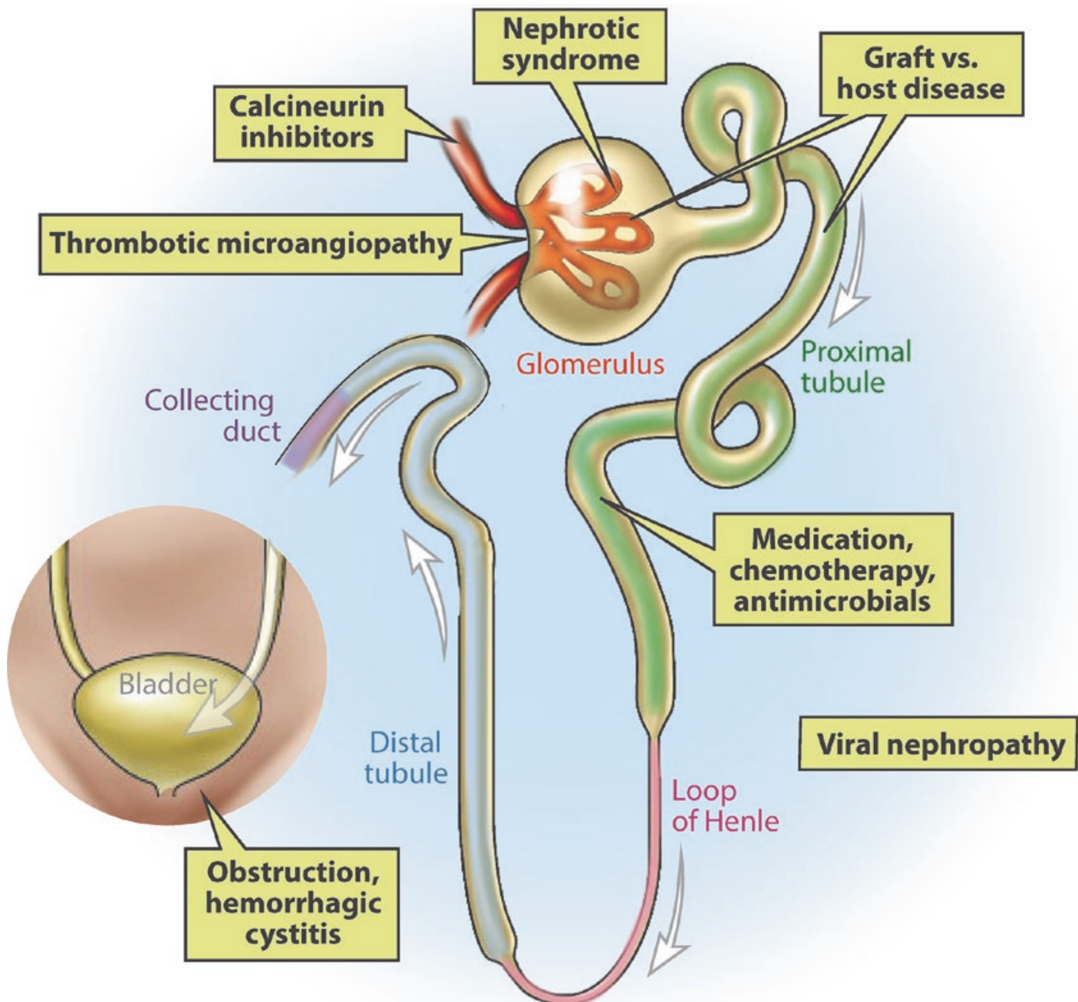

Fig. 22.1 Sites of post-HSCT renal injury by cause. The afferent renal vasculature may be constricted by calcineurin inhibitors and upregulation of the renin-angiotensin system. These agents may also be implicated in endothelial damage leading to thrombotic microangiopathy affecting the small blood vessels of the kidney (arterioles and glomerular capillaries). Graft-versus-host disease may affect the glomeruli, manifesting in nephrotic syndrome, or lead to tubular injury. Multiple drugs, including common antibiotics, can also lead to tubular injury, and viral infections may result in nephropathy due to direct cytotoxic injury or to an obstructive nephropathy, as is commonly seen with BK viral infections. Copyright Seif & Associates, Inc., 2017

imab and the complement inhibitor eculizumab have shown promise in selected cases [12–14].

Infection and Renal Dysfunction

BK virus, which is relatively ubiquitous in the general population, remains latent in the urothelial cells of the kidney and urinary tract and can reactivate in the setting of immunosuppression. Active infection most frequently manifests as hemorrhagic cystitis following HSCT (see Chap. 16). The associated clots can cause urinary tract obstruction leading to acute kidney injury. When BK virus is detected in the urine, serum PCR for BK viremia needs to be monitored. While patients with BK viral loads on the order of a million to a billion copies/mL in the urine can be asymptomatic, those with a serum BK viral load greater than 10,000 copies/mL may be

at relatively high risk for developing symptoms of cystitis or intrinsic kidney injury, although the exact PCR cutoff associated with symptomatic disease remains unknown [15, 16]. Management of hemorrhagic cystitis is largely symptomatic, with hyperhydration, bladder irrigation, pain management, and platelet transfusion (and is discussed in greater detail in Chap. 16). In addition, BK virus nephropathy can cause CKD, even in the absence of hemorrhagic cystitis. As with other viral infections that may affect the kidney, such as adenovirus and CMV, antiviral medications are often considered for symptomatic nephropathy and in some cases of hemorrhagic cystitis. Because cidofovir is the first-line antiviral agent used, the risks of nephrotoxicity must be weighed against the benefit of treating the BK virus, although treatment doses for BK virus are typically much lower than for systemic adenovirus. Intravesicular administration of cidofovir has sometimes been used in conjunction with bladder irrigation to address hemorrhagic cystitis. Leflunomide, fluoroquinolones, and IVIG have also been used as adjuncts in the treatment of BK virus [17]. It is important to recognize that no agent has been shown to be effective against BK virus in well-designed clinical trials, and patients typically require some degree of immune reconstitution to recover from this infection.

Graft-Versus-Host Disease (GvHD) and Glomerular Disease

Nephrotic syndrome, characterized by proteinuria, edema, hypoalbuminemia, and hypercholesterolemia, can occur post-HSCT but is relatively rare. Many have proposed that this may be a manifestation of GvHD in the kidney as it has a clear association with chronic GvHD. Membranous glomerulonephritis is the most common pathology noted on renal biopsy in the setting of nephrotic syndrome, followed by minimal change disease [18]. While membranous glomerulonephritis is attributed to the deposition of immune complexes, minimal change disease may be related to T-cell-mediated attack on podocytes. Whether nephrotic syndrome represents GvHD of the kidney or not, it is generally effectively managed with immunosuppression.

Key Points

- Acute kidney injury and chronic kidney disease are common after HSCT, occurring in more than a third of patients.
- Hypertension and proteinuria are important signs of potential renal dysfunction.
- Many of the medications used in HSCT are nephrotoxic, particularly chemotherapy, radiation, calcineurin inhibitors, and antimicrobials.
- Thrombotic microangiopathy, characterized by kidney injury, hypertension, thrombocytopenia, and microangiopathic hemolytic anemia, is a cause of kidney disease after HSCT and is managed with removal of the caustive agent.
- BK virus is associated with renal injury due to obstruction from hemorrhagic cystitis or from a direct cytopathic effect.

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23 Cardiac Complications Associated with HSCT

Valerie I. Brown

Abstract

This chapter focuses on cardiotoxicity that may occur during the peri- or early post-HSCT period. The long-term cardiac sequelae associated with HSCT are addressed in Chap. 26. Cardiac complications may be related to the toxic effects of the conditioning regimen, radiation, prior exposure to cardiotoxic agents, infections, and graft-versus-host disease (GvHD). HSCT-associated cardiac complications include heart failure, arrhythmias, pericarditis, myocarditis, endocarditis, pericardial effusion, and cardiac tamponade. Cardiac complications may occur acutely during the peri-HSCT period (as defined as the time during conditioning through engraftment) or may be delayed by weeks to years. With the exception of sinus tachycardia, cardiac complications are rare during the peri-HSCT period. However, when they do occur, they are usually life-threatening because heart failure, cardiac tamponade, and dysrhythmias are the most common early cardiac complications. More frequently, the manifestations of the cardiac damage *acquired* during the peri-HSCT period may be delayed for years, requiring lifelong cardiac monitoring (at least annually with ECHO and ECG) for all post-HSCT patients who are at risk.

Risk Factors

The risk factors for developing HSCT-associated cardiac complications are listed in Table 23.1. The most significant risk factors are history of receiving anthracyclines (e.g., doxorubicin, daunorubicin, and idarubicin), exposure to a total dose of cyclophosphamide of 120–150 mg/kg or more, receiving total body irradiation

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Table 23.1 Risk factors for developing HSCT-associated cardiac complications

Etiology	Comments
• Preexisting cardiac disease	–
• Prior exposure to cardiotoxic chemotherapy	• Anthracyclines • Cyclophosphamide • 5-FU
• Radiation	• Total body irradiation (TBI) • Prior mediastinal radiation
• Sepsis	–
• Mitral valve disease	–
• Ejection fraction <50% or shortening fraction <27%	• As determined by ECHO or MUGA
• Underlying diagnosis associated with cardiac disease	• Hurler syndrome • Down syndrome • Thalassemia
• History of prolonged QTc	• As determined by ECG

(TBI), and prior exposure to radiation to the chest. The patient's comorbidities, pre-HSCT physical condition, and performance score contribute to the risk of developing HSCT-associated cardiotoxicity. Patients who have underlying conditions associated with cardiac disease, such as Hurler syndrome, Down syndrome, and thalassemia, are at a greater risk for developing HSCT-related cardiac complications [1]. Other risk factors are sepsis, a history of mitral valve disease, and a history of prolonged QTc [2]. An ejection fraction as determined by echocardiography (ECHO) that is less than 50% (normal, 55–75%) is a risk factor for HSCT-related (but not necessarily life-threatening) cardiotoxicity [3].

Etiologies

Acute cardiac-related toxicities are rare in pediatric HSCT patients. However, the majority are life-threatening and often result in death if not promptly recognized and treated. The most common etiologies of HSCT-associated cardiac complications are listed in Table 23.2 and are detailed below [4].

Table 23.2 Most common etiologies of HSCT-associated cardiac complications

• Chemotherapy-induced cardiotoxicity
• Radiation-induced cardiotoxicity
• Arrhythmias
• Graft-versus-host disease-induced cardiotoxicity
• Infection
• Pericardial effusion
• Cardiac tamponade

Chemotherapy-Induced Cardiotoxicity

Acute chemotherapy-induced cardiac toxicity is most likely related to high-dose cyclophosphamide-containing conditioning regimens [5, 6]. The dosage of cyclophosphamide, the type of conditioning regimen (myeloablative versus nonmyeloablative), TBI, and coadministration of other chemotherapeutics can potentiate the risk of cardiotoxicity. The different chemotherapy-induced cardiac events range from mild, transient blood pressure changes, ECG changes, and transient sinus tachycardia to life-threatening arrhythmias, myocarditis, pericarditis, myocardial infarction, and cardiomyopathy that often result in congestive heart failure. Patients that have previously received ≥100 mg/kg of anthracyclines and then ≥120 mg/kg cyclophosphamide as part of the conditioning regimen are at increased risk for developing cardiac damage. Cyclophosphamide-induced cardiotoxicity typically occurs within 2–10 days after its first administration, but it can appear up to 3 weeks later [5]. Cardiac troponins can be helpful in the diagnosis of cardiac damage in this setting. The cardiac damage is usually irreversible due to loss of myocardial fibrils and cellular degeneration. This results in thickening of the left ventricular wall, blood-tinged pericardial effusions, and fibrinous pericarditis.

Clinical manifestations: Cyclophosphamide-induced cardiac damage is manifested by chest pain, nausea, palpitations, tachypnea, tachycardia, peripheral edema, pulmonary edema, cardiomegaly, and poor peripheral perfusion. Symptoms may progress to hemorrhagic pancarditis, cardiac tamponade, and death.

Diagnostic evaluation: An ECHO or MUGA as well as cardiac biomarkers with troponin measurement are performed in order to determine the extent of cardiac damage. An ECG usually shows decreased voltage. ECHO typically shows pericardial effusion, decreased shortening fraction, and increased end diastolic volume [6]. (See Table 23.3 for a summary of the common cardiac diagnostic tests and monitoring along with their utility.)

Table 23.3 Common cardiac diagnostic tests and monitoring

Test	Utility
• Creatinine kinase	• Enzyme found in multiple tissue types including the heart • Nonspecific measure of cardiac damage
• CK-MB	• Found mostly in the heart • Ratio of CK-MB to total CK is used to determine likelihood of heart damage
• Troponin	• Determines damage to the heart
• Brain natriuretic peptide (BNP)	• Grades severity of heart failure
• Chest X-ray	• Detect cardiomegaly • Detect pulmonary edema
• Electrocardiogram (ECG, EKG)	• Shows abnormal heart rhythms • Detects heart muscle damage
• Echocardiography (ECHO)	• Evaluates motion of heart's chambers and valves • Determines ventricular function • Detects vegetations • Can be performed transthoracic or transesophageal
• MUGA	• Nuclear scan • Determines heart wall motion at rest or after exercise
• CT scan or MRI of the chest	• Detects pericardial and pleural effusions • Determines heart size • Can diagnoses coronary artery disease
• 24-h Holter monitor	• Detects arrhythmias over a period of time

Management and outcomes: Treatment is focused on alleviating heart failure by aggressive fluid restriction, diuretics, and digitalis. If a large pericardial effusion is present, then pericardiocentesis may be indicated. While a rare complication of HSCT, cyclophosphamide-induced cardiotoxicity is associated with a high mortality rate. The shorter the time from the administration of cyclophosphamide to the first symptom of cardiac dysfunction is associated with early death. Thus, early recognition and prompt intervention may improve outcome [5, 6].

Radiation-Induced Cardiotoxicity

With the lowered dose rates and fractionation of TBI, cardiac toxicity as a result of radiation alone is rare nowadays. However, the combination of radiation with chemotherapy (such as cyclophosphamide) in the conditioning regimen is synergistic. Other risk factors for radiation-induced cardiotoxicity include prior radiation to the chest and borderline cardiac function prior to the start of conditioning [4]. While only 10% of HSCT patients die due to cardiac toxicity, therapeutic options are very limited to reverse this cardiac damage. Thus, the optimal strategy is to minimize exposure to these cardiotoxic modalities.

Pathophysiology: Cardiac damage occurs as a result of damage to the cellular structures in the connective tissue of the myocardium, leading to decreased cardiac enzyme activity [4]. When cardiac enzyme is decreased, the contractility and conductivity of cardiac cells become compromised. The degree of damage is related to the radiation dose, volume, and treatment technique. Radiation therapy can cause pericardial disease (most common), myocardial infarction, ischemic heart disease, cardiomyopathy, and coronary heart disease. Damage to the myocardium from radiation may take years before it becomes evident [7].

Clinical manifestations: The clinical manifestations of radiation-induced cardiotoxicity include angina, dysrhythmias, hypotension, jugular venous distension (JVD), peripheral edema, and the presence of third and fourth heart sounds.

Any of these symptoms warrant further investigation in a timely manner. Patients with congestive heart failure rapidly progress to hemorrhagic myocarditis, cardiac tamponade, and even death.

Diagnostic evaluation: An ECHO or multigated acquisition (MUGA) scan is performed as well as checking cardiac biomarkers and troponins to assess the degree of cardiac damage.

Management and *outcomes*: Similarly to chemotherapy-induced cardiotoxicity, the treatment of radiation-induced cardiac damage focuses on ameliorating heart failure with aggressive fluid restriction, diuretics, and digitalis. Again, pericardiocentesis may be warranted in cases of large pericardial effusions. Long-term follow-up is necessary because a significant number of these patients will go on to have a decrease in the ejection fraction over time [4].

Arrhythmias

A patient's heart rate should always be evaluated in the context of the patient's age and clinical condition. The most common etiologies that contribute to the development of an arrhythmia in the context of HSCT in pediatric patients are electrolyte imbalances, hypoxemia, acidosis, sepsis, drug toxicity (e.g., vasopressors), and multi-organ failure. Arrhythmias typically arise within the first month after transplant with a median of 6 days post-HSCT [8]. Currently, serious arrhythmias are very rare in pediatric HSCT patients unless there is an underlying cardiac condition present. The two most common arrhythmias are bradycardia and sinus tachycardia, and very often they are transient.

Diagnostic evaluation: If an arrhythmia is suspected or detected, then serum electrolytes, blood glucose, calcium, magnesium, CBC, toxicology screen, VBG or ABG, and thyroid studies should be sent. A chest X-ray, ECHO, and ECG should be performed as well. If indicated, a Holter monitor should be placed, and a cardiology consultation obtained.

Bradycardia

Bradycardia is a serious complication in HSCT patients because it compromises systemic perfusion and slows the ventricular rate. It is also associated with a fall in cardiac output. The most common cause of bradycardia in the HSCT setting is dimethyl sulfoxide (DMSO) toxicity during infusion of hematopoietic stem cells (HSCs) that have been cryopreserved in DMSO [9]. DMSO is a necessary agent used to preserve the integrity of cells during the freeze-thaw process (see Chap. 8). Thus, cardiac monitoring during HSC infusion is part of an institution's standard operating procedures. Other causes of bradycardia include hypoxia, acidosis, other drugs (e.g., fentanyl, clonidine), electrolyte imbalances (e.g., hypoglycemia, hypocalcemia, and hypokalemia), hypothermia, and vagal stimulation.

Management and outcomes: While often reversible by treating the underlying cause, the immediate treatment of bradycardia is to provide adequate oxygenation and ventilation and to provide pharmacologic support with epinephrine if the bradycardia is persistent and becomes life-threatening. Atropine may be administered if the etiology of the bradycardia is thought to be due to vagal stimulation. More importantly, it is essential to determine the underlying cause of the bradycardia and then treat it.

Tachycardia

Tachycardia may be due to sinus tachycardia or tachyarrhythmia. While tachyarrhythmias, such as supraventricular tachycardia, are rare, their presence warrants a cardiology consult [8]. In contrast, sinus tachycardia is frequently noted in patients during the peri-transplant period (from the start of the conditioning regimen through engraftment). In this scenario, the cause of tachycardia is more likely due to a non-cardiac etiology. Tachycardia is a normal physiologic response to stress, anxiety, and pain due to catecholamine release, leading to increased heart rate and contractility. Other common causes of sinus tachycardia include fever, infection, anemia, hypoxia, hypovolemia, electrolyte derangement, and acid-base imbalance (see Table 23.4). Numerous medications can cause tachycardia, namely, antihistamines, phenothiazines, antidepressants, and general anesthetics. Less common causes include anaphylaxis and hyperthyroidism.

Table 23.4 Etiologies of sinus tachycardia in the pediatric HSCT patient

Common	• Pain • Stress, anxiety • Hypovolemia • Fever, infection • Anemia • Hypoxia • Electrolyte derangement • Acid-base imbalance • Drug-induced
Less common	• Anaphylaxis • Hyperthyroidism

Management: The most important aspect of the treatment of tachycardia, particularly sinus tachycardia, is to treat the underlying, non-cardiogenic cause.

Graft-Versus-Host Disease (GvHD)-Induced Cardiac Complications

Cardiac complications due to graft-versus-host disease (GvHD) are rare. Cardiac damage in the setting of GvHD is thought to be caused by donor lymphocyte infiltration [10, 11]. This infiltrative process can appear on MRI as the presence of nodules. ECG demonstrates LV wall thickening and impaired LV filling. In contrast, ECHO typically shows a normal ejection fraction.

Clinical presentation: The clinical presentation resembles that of impaired myocardial contractile function (thus difficult to discern the etiology).

Management and outcomes: If cardiac GvHD is suspected, then a cardiology consultation is warranted. Cardiac GvHD is treated with high-dose steroids, fluid restriction, and supportive care.

Cardiac Complications Due to Infection

Cardiac infections are rare in the HSCT setting but can affect the pericardium, endocardium, and myocardium. They can be of bacterial, viral, or fungal origin.

Bacterial

In general, the most common bacterial heart infection associated with HSCT is bacterial endocarditis that can involve the heart valves and/or the endocardium and is very rare [12].

Pathogenesis: Gram-positive bacterial species more commonly cause endocarditis [12]. The bacteria adhere to the valves and produce vegetations. Only a low titer of bacteria is needed to establish an infection. The bacteria themselves are found deep within the vegetations and thus difficult to treat effectively. Over time, the bacterial vegetations will eventually erode away the valve or leave scar tissue on the valve, rendering the valve ineffective.

Clinical manifestations: The clinical manifestations of bacterial endocarditis include fever, chills, cough, malaise, headache, development of a murmur, and positive blood cultures.

Diagnostic evaluation: Valvular vegetations along with abscesses and valvular insufficiency are detected by ECHO. A transthoracic ECHO can be done in infants, children, or obese individuals in whom an ECHO cannot be performed readily. An ECG may show conduction or rhythm disturbances as well. Blood cultures should be obtained. Because it is difficult to isolate the bacteria from the blood if bacterial endocarditis is suspected, the microbiology clinical laboratory should be notified accordingly.

Treatment and outcomes: The mainstay treatment of cardiac bacterial infections is prompt initiation of antibiotic therapy and fluid management. If damage to a valve is severe, then heart valve replacement may be necessary. The use of empiric, broad-spectrum antibiotics is critical for the prevention and early treatment of bacterial infections of the heart. Otherwise, it can be fatal.

Fungal

In general, fungal infections of the heart are rare [13]. However, patients with preexisting invasive fungal infections are at a higher risk. Myocarditis and pericarditis are more common than endocarditis.

Pathogenesis: Initially, fungus spreads by direct invasion from the lung tissue to the heart or by hematogenous spread. Involvement of the endocardium is more common in the presence of disseminated fungal disease, such as aspergillosis. Thromboemboli are spread from the primary

source and may lead to myocardial infarction if they lodge in the coronary arteries. Alternatively, fungus invades the myocardium and then spreads to the pericardium [14]. Aspergillosis is the most common cause of fungal pericarditis as well as endocarditis. *Candida albicans* has also been found to cause cardiac infections [12].

Clinical manifestations: The clinical manifestations of fungal heart infections are similar to those seen with bacterial cardiac infections. However, symptoms in fungal cardiac infections may mimic those of a respiratory infection including tachypnea, tachycardia, and cough as well as refractory substernal chest pain.

Diagnostic evaluation: In general, it is difficult to diagnose a cardiac infection due to fungus. The ECG will show ischemic changes, and blood work will show elevation of cardiac isoenzymes. Blood cultures are often positive. A fungal infection should be considered if the patient has persistent fevers that are not responsive to broad-spectrum antibiotics and the patient's blood cultures are negative with no source of fever identified. Pan CT scans should be performed to investigate for sites of fungal infection, although the heart will not necessarily appear.

Management and outcomes: For the best outcome, empiric antifungal treatment should be initiated early on. The remainder of care is supportive with fluid management, digitalis, diuretics, and pain management (e.g., nitroglycerin for chest pain).

Viral

The most common cardiac infection of viral etiology is myocarditis that is predominantly caused by adenovirus and enterovirus (specifically Coxsackie A). Viral infection of the myocardium causes inflammation that leads to irreversible damage to the heart and cardiac dysfunction [15].

Clinical manifestations: A patient with viral myocarditis presents with symptoms of heart failure including tachycardia, cool extremities, pale or mottled skin, muffled or decreased heart sounds, jugular vein distension (JVD), hepatomegaly, and peripheral edema.

Diagnostic evaluation: While it is relatively difficult to diagnose viral endocarditis or myocarditis, viral cultures obtained from blood, nasal passages, and perirectal areas, viral PCR, ESR, CRP, CBC, electrolytes, blood glucose, and cardiac biomarkers should be sent. Of note, a biopsy of the myocardium is the gold standard to diagnose viral endocarditis and should be performed as early as possible [15]. ECHO typically shows general myocardial dysfunction with global hypokinesis, increased heart size, pericardial effusion, and wall movement abnormalities. An ECG demonstrates small voltage, tachycardia, and QT prolongation.

Management and outcomes: Early detection is important with the goal of treating the underlying virus. In addition, management includes supportive care: management of heart failure to maintain adequate perfusion with fluid management, digitalis, and diuretics.

Pericardial Effusion

In children, the pericardial space normally contains up to 20 mL of fluid. When the volume of fluid exceeds the pericardial space (i.e., pericardial effusion), this extra fluid produces pressure against the heart causing the heart to pump ineffectively. The incidence of pericardial effusion in the pediatric HSCT recipient is very low. However, when present, the most common causes include an infectious etiology (bacterial, fungal, tuberculosis, and viral), capillary leak syndrome, renal failure, drug toxicity (e.g., cyclophosphamide), and GvHD. In one retrospective study, the incidence of clinically significant pericardial effusion was 4.4% with a median time of development of 30 days post-HSCT (range, 19–210 days) [16]. The majority of cases were associated with GvHD, and all of the patients have had a pre-HSCT left ventricular ejection fraction >45%.

Clinical manifestations: The presentation of pericardial effusion is dependent upon the rate at which the fluid has accumulated. Rapidly accumulating pleural effusions typically are more symptomatic, whereas slowly progressing ones can remain asymptomatic for a long period of time. The most common clinical manifestations are dyspnea, orthopnea, cough, painful respira-

tions, chest pain, tachycardia, and pericardial friction rub.

Diagnostic evaluation: Chest X-ray often shows a "water bottle" sign in which the cardiac silhouette is enlarged and looks like a flask or water bottle. In addition to chest X-ray and blood work (electrolytes, blood glucose, CBC, and cardiac enzymes), an ECHO, ECG, and possibly CT scan of the chest should be performed. Cardiac consultation should be obtained to perform pericardiocentesis. Pericardiocentesis should be performed for both diagnostic and therapeutic purposes. The fluid obtained from the pericardiocentesis should be evaluated for infectious etiologies, the presence of blood, and protein and glucose levels.

Management and outcomes: In addition to pericardiocentesis, the underlying cause should be treated, i.e., early initiation of appropriate antimicrobials as indicated. Anti-inflammatory agents and steroids have been used for the treatment of pericardial effusions, but the use of steroids is controversial because of the potential of reaccumulation once the steroids are tapered off. If the pericardial effusion is compromising heart function that results in cardiac tamponade, a subxiphoid pericardial window with pericardiostomy is necessary to adequately drain the fluid and relieve the tamponade. Early recognition and treatment of a pericardial effusion (prior to the development of cardiac tamponade) can prevent death related to pericardial effusion [16].

Cardiac Tamponade

Cardiac tamponade develops after a pericardial effusion has caused reduced ventricular filling which results in cardiac compromise. Cardiac tamponade is a medical emergency because it ends in ineffective pumping of blood that often leads to death. Pericarditis or pericardial effusion often precedes cardiac tamponade, and so the causes of cardiac tamponade are the same as those of pericarditis or pericardial effusion, with infections and chemotherapeutic agents used in conditioning regimens (e.g., cyclophosphamide) as the most common etiologies.

Clinical manifestations: Clinically, patients present with the same symptoms as pericardial effusion. Classically, they present with the "Beck triad" which is hypotension, increased jugular vein distension (JVD), and diminished heart sounds [17]. Patients often present with pulsus paradoxus.

Diagnostic evaluation: The diagnosis must be made in a timely fashion because acute cardiac tamponade is a medical emergency. Diagnostic studies are the same as those done to evaluate for pericardial effusion.

Management and outcomes: If cardiac tamponade is suspected, then a cardiology consultation should be obtained emergently. Treatment is to perform emergently a pericardial window with pericardiostomy.

Pulmonary Hypertension

Overall, the development of pulmonary hypertension in pediatric HSCT recipients is very rare but often fatal [18]. Pulmonary hypertension is associated with increased pulmonary vascular resistance and elevated right ventricular pressure that can lead to permanent changes in the pulmonary vasculature. These changes often result in right ventricular failure and death. The most common classifications of pulmonary hypertension that are found in pediatric HSCT recipients (both autologous and allogeneic) are pulmonary arterial hypertension (PAH), pulmonary veno-occlusive disease (PVOD), and PVOD with pulmonary arterial involvement. PVOD is predominantly an obstruction of the pulmonary venules. In one study, Schechter et al. reported that 15% of pediatric patients treated with high-dose chemotherapy (carboplatin and thiotepa) followed by autologous peripheral blood stem cell transplantation in tandem for CNS tumors had biopsy-proven PAV with PAH [19]. In another study, Desai et al. reported that 19% (4 of 21) of pediatric patients who received busulfan and melphalan for high-dose chemotherapy followed by autologous peripheral blood stem cell transplantation for high-risk neuroblastoma developed pulmonary hypertension [20]. Two of

the four patients died as a result of the pulmonary hypertension although they had other significant comorbidities.

Pathophysiology: Both pulmonary arterioles and venules can be affected in HSCT-associated pulmonary hypertension. Histopathologically, all vascular layers of arterioles can be affected in PAH. In contrast, the histopathology of PVOD is characterized by extensive occlusion of the pulmonary venules with fibrous tissue eventually forming sclerotic occlusion without thrombosis.

Clinical manifestations: PAH and PVOD present similarly and can only be distinguished by lung biopsy to determine the vascular compartment injured. However, it is not important to make this distinction because treatment for both is very similar. Initially, pulmonary hypertension presents with shortness of breath, fatigue, weakness, dizziness, and hypoxemia. Edema, hepatomegaly, and ascites typically develop later on secondary to increased venous congestion. If left unrecognized or untreated, patients will develop progressive tachypnea and hypoxemia, eventually leading to respiratory failure. Thus, the diagnosis of pulmonary hypertension should be considered early on in any HSCT recipient who presents with unexplained hypoxemia or respiratory distress.

Diagnostic evaluation: A chest X-ray may show prominence of the pulmonary artery, enlarged hilar vessels, and decreased peripheral vessels, and these findings are found in over 90% of patients with advanced pulmonary hypertension. ECG may demonstrate evidence of right arterial enlargement or right ventricular hypertrophy with peaked P waves, R axis deviation, and a right bundle branch block pattern. High-resolution CT or MRI can detect dilated pulmonary artery. Cardiac MRI is used to evaluate right heart cardiopulmonary function and structure. ECHO is used to comprehensively evaluate right heart function. Cardia catheterization should be considered in any HSCT recipient suspected of having pulmonary hypertension, as it is the gold standard in the diagnosis of pulmonary hypertension because cardiac catheterization will measure directly cardiac output and pulmonary artery pressures and is used to calculate pulmonary resistance.

Management and outcomes: In general, management of pulmonary hypertension focuses on optimization of cardiac function, particularly if right ventricular compromise is present. Management of pulmonary hypertension includes oxygen, diuretics, afterload reducing agents, inotropes (e.g., milrinone), and phosphodiesterase-5 inhibitors (e.g., sildenafil). Because the development of pulmonary hypertension can quickly lead to right heart failure and death, symptomatic patients need to be evaluated and start treatment promptly [18, 21].

In summary, cardiac complications vary greatly from transient sinus tachycardia to life-threatening, irreversible cardiac damage and heart failure that can result in death. The underlying causes are multifactorial and include drug toxicity, prior chest irradiation, infection, GvHD, and preexisting cardiac disease/dysfunction. Pretransplant cardiac evaluation is a key component to help prevent or at least minimize cardiac complications that patients may experience post-HSCT. While the manifestations of cardiac damage may be immediate, albeit rare, they are more often delayed by months or even years. Thus, post-HSCT recipients require regular, lifelong evaluation for cardiac damage because outcomes are better when cardiac damage is detected early on.

Key Points

- HSCT-associated cardiac complications can occur acutely or delayed.
- Acute cardiac complications that are life-threatening are rare in the HSCT patient (<2%).
- Cardiac complications in HSCT patients are more often delayed by months to years and thus requiring lifelong cardiac monitoring annually at a minimum.
- The most common risk factors are preexisting cardiac disease, ejection fraction <50%, chemotherapy, radiation, sepsis, history of mitral valve disease, and underlying diagnosis associated with cardiac disease or dysfunction.
- The most common cardiac toxicities are chemotherapy- or radiation-induced cardiac damage, arrhythmias, GvHD-induced damage,

infection, pericardial effusion, and cardiac tamponade.
- Suspicion of cardiac toxicity warrants an emergent evaluation that includes blood work for cardiac biomarkers, ECG, ECHO, and possibly CT or MRI of the chest, and very often cardiology consultation.

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Neurologic and Sensory Complications Associated with HSCT

24

Valerie I. Brown

Abstract

Severe neurologic complications associated with hematopoietic stem cell transplantation (HSCT) have been reported in approximately 14–25% of all pediatric HSCT patients. The majority of severe neurologic events occur within the first 100 days after HSCT. Risk factors for developing severe neurologic events in the context of HSCT include the age of the HSCT recipient (children <3 years old), underlying disease, prior therapy, conditioning regimen, hematopoietic stem cell (HSC) source, unrelated donor, graft versus host disease (GvHD), immunosuppressive agents, metabolic disorders, and prior central nervous system (CNS) infections. The most common causes of developing a severe neurologic complication are medications (particularly calcineurin inhibitors), radiation-induced, hemorrhage-related, immune-mediated, and infection-induced. The *most common severe neurologic events* are seizures, cerebral vascular events as well as change in consciousness (mental status changes), and encephalopathy/leukoencephalopathy. Severe neurologic events account for 10–15% of all deaths in HSCT patients. Sensory complications such as radiation-induced cataracts, platinum-induced high-frequency hearing loss, and high-dose chemotherapy-induced taste dysfunction are seen quite often in pediatric HSCT recipients. Because pediatric HSCT recipients are still developing, effort needs to be made to minimize neurologic and sensory complications.

Introduction

Approximately 20% (range: 9.7–65%) of all HSCT patients will experience a severe neurologic event which includes seizures, change in mental status, stroke, cognitive decline, and posterior reversible encephalopathy syndrome (PRES), and these events are more common in

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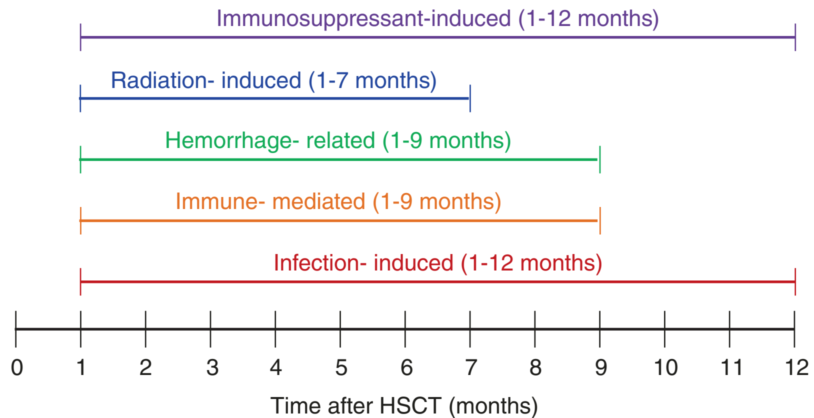

Fig. 24.1 Timing of the most common causes of HSCT-associated severe neurologic events: Each colored bar represents the typical timeframe for each category of severe neurologic events in relation to the time after HSCT

allogeneic versus autologous HSCT [1, 2]. Almost all of the HSCT-related neurologic events occur within 1 year post-HSCT with the majority occurring within the first 6 months. Figure 24.1 summarizes the timing of the most common causes of severe neurologic complications. Some of these neurologic sequelae can be lifelong and devastating, and severe neurologic complications account for 10–15% of all deaths in HSCT patients [3]. However, one study found that if neurologic complications do not persist after 6 months post-HSCT, then there was no impact on mortality [1].

Risk Factors and Etiologies

The risk factors and causes of HSCT-associated severe neurologic events are multifactorial. They are detailed below and summarized in Table 24.1. The major risk factors are the age of the recipient at the time of HSCT, use of an unrelated donor, the underlying disease being treated by HSCT, prior treatments, the conditioning regimen, the HSC source, metabolic disorders, history of a vascular disorder, and prior CNS infections. The major causes include immunosuppressive agents and other drugs as well as GvHD. Other causes include CNS relapse of the underlying disease being treated by HSCT and metabolic derangements. Generally, early neurologic complications are associated with medications, radiation used in the conditioning regimen, and infections, whereas post-HSCT late events are related to the immunodeficient state of the patient [4].

Age of recipient: The age of the recipient at the time of HSCT impacts the risk for developing neurologic complications due to HSCT. One would predict that infants and children under the age of 3 years would be more susceptible to developing neurologic complications because of incomplete brain development. However, one study showed no significant difference in incidence between younger and older children [5], and another found that life-threatening neurologic events were documented more frequently in older (median age, 11 years) versus younger (median age, 4 years) children [3].

Underlying disease: Table 24.2 summarizes the most common underlying diseases and their predisposing features that are associated with a higher risk of severe neurologic events in post-HSCT pediatric patients. Patients who have underlying neurologic abnormalities or disorders (e.g., seizure disorder or inborn error of metabolism) are at higher risk for experiencing a neurologic event while undergoing HSCT. Patients who have CNS disease at the time of diagnosis or at relapse, such as CNS involvement of leukemias, lymphomas, or solid tumors, are at higher risk for severe neurologic events as compared to those who have never had CNS involvement [6]. Also, patients with brain tumors that have residual neurologic symptoms or seizures are at higher risk for developing neurologic complications during the HSCT process, and their neurologic symptoms may be exacerbated by the condition-

Table 24.1 Risk factors for the development of HSCT-associated neurologic complications

Risk factor	Comments
• Age of recipient at time of HSCT	• While older children and adolescents have a higher incidence of CNS complications, HSCT patients <3 years old have neurocognitive sequelae after TBI due to incomplete brain development
• Underlying disease	• CNS relapse of underlying disease must be considered
• Prior treatments	• Prior use of fludarabine and intrathecal methotrexate
• Conditioning regimen	• Neurotoxicity may be acute or delayed
• Immunosuppressive agents – Calcineurin inhibitors	• Most are reversible and resolve once the agent is discontinued
• HSC source	• Most likely correlates with need of immunosuppression to prevent graft rejection and to prevent or treat GvHD
• Unrelated donor	• Most likely correlates with need of immunosuppression to prevent graft rejection and to prevent or treat GvHD
• GvHD	• Directly due to immune dysregulation associated with chronic GvHD or indirectly due to the agents used to treat GvHD (e.g., calcineurin inhibitors)
• Metabolic disorders	• Particularly if the metabolic disorder is associated with CNS sequelae
• Prior CNS infections	• HSCT patients with a history of CNS infection is at a higher risk for developing the same CNS infection post-HSCT

ing regimen [7]. Patients with sickle cell disease are at particular risk because of the very high likelihood of having pre-existing narrowed or occluded vessels in the brain [8–10]. This pathology lends to abnormal cerebral blood flow that increases the risk for stroke, and these patients are more susceptible to stroke during HSCT. In addition, a high incidence of PRES was noted in allogeneic-HSCT recipients with sickle cell disease [8]. Patients with inborn errors of metabolism such as Hurler's syndrome may have a history of encephalopathy or hydrocephalus and are thereby more vulnerable to suffer a severe neurologic event during the peri- and post-HSCT periods [11].

Prior treatment: Patients who have suffered from a neurologic event from prior therapy are at higher risk for experiencing a severe neurologic event after HSCT [5]. These include vincristine-induced peripheral neuropathy, asparaginase-related stroke, and ifosfamide-induced encephalopathy. Patients who developed syndrome of inappropriate antidiuretic hormone (SIADH) after receiving Cyclophosphamide, ifosfamide, or melphalan are at higher risk for neurologic events with HSCT [12, 13]. Patients who have had neurocognitive deficits or encephalopathy due to intrathecal methotrexate are more likely to have these worsen during HSCT.

Table 24.2 Predisposing features of underlying diseases

Underlying disease	Predisposing feature(s)
• Leukemia/lymphoma • Solid tumor	• CNS involvement at diagnosis or relapse
• Brain tumor	• Residual neurologic symptoms • Seizure disorder
• Sickle cell disease	• Vessel narrowing or occlusion → stroke
• Metabolic disorder	• Encephalopathy • Hydrocephalus

Conditioning regimen: Certain agents used as part of conditioning regimens are known to be highly associated with specific neurologic complications [3, 14]. Table 24.3 summarizes these agents and their associated neurotoxicities. However, the risks can be minimized with close monitoring, pharmacokinetic dosing, and prophylaxis. For example, the incidence of seizures from busulfan has decreased significantly with the advent of pharmacokinetic targeted dosing and the use of prophylactic anticonvulsants [15, 16]. Keppra is the most commonly used anticonvulsant agent in this case [17]. Seizures and mental status changes that occur with cyclosporine-induced SIADH can be avoided with close monitoring of serum sodium, urine output, and urine-specific gravities as well as with strict fluid management and early intervention with

Table 24.3 Commonly used HSCT agents and their associated neurotoxicities

Agent	Associated neurotoxicity, acute
• Busulfan	• Seizures
• Cyclophosphamide	• Seizures • Mental status changes due to SIADH (transient)
• Carboplatin	• Ototoxicity (hearing loss) • Peripheral neuropathy • PRES
• Fludarabine	• Confusion • Visual disturbances • Acute toxic leukoencephalopathy
• Cytarabine	• Cerebellar toxicity • Seizures
• Melphalan	• Seizures • Encephalopathy
• Etoposide	• Peripheral neuropathy • PRES • Acute dystonia
• Thiotepa	• Meningitis
• Corticosteroids	• Psychosis
• Alemtuzumab • Rituximab	• Progressive multifocal leukoencephalopathy (PML)
• Calcineurin inhibitors – Cyclosporine A – Tacrolimus – Mycophenolate mofetil – Sirolimus	• Fine tremor • Burning sensation of soles and palms • Visual disturbances • Seizures • PRES • Mutism • Pseudotumor cerebri • Hearing loss • Optic neuropathy
• Acyclovir	• Seizures • Encephalopathy
• Amphotericin B	• Confusion
• Posaconazole	• Exacerbates vincristine-induced neuropathy
• Voriconazole	• Visual disturbances • Hallucinations
• Cefepime	• Seizures • Encephalopathy • Myoclonus
• Imipenem	• Seizures
• Linezolid	• Ischemic optic neuropathy • PRES • Peripheral neuropathy
• Metronidazole	• Reversible dysarthria • Ataxia
	Associated neurotoxicity, delayed
• Radiation	• Leukoencephalopathy – Increased risk if prior intrathecal methotrexate or high-dose methotrexate • Intracranial hemorrhage
• Methotrexate	• Leukoencephalopathy – Increased in combination with radiation
• Alkylators	• Intracranial hemorrhage

Table 24.3 (continued)

Agent	Associated neurotoxicity, acute
• Carmustine	• Encephalopathy
• Cytarabine	• Encephalopathy
• Antithymocyte globulin	• Visual encephalopathy
• Alemtuzumab	• Visual encephalopathy • CNS infection • Guillain-Barre syndrome
• Calcineurin inhibitors – Cyclosporine A – Tacrolimus – Mycophenolate mofetil – Sirolimus	• Demyelination • Increased risk for PML

diuretics and mannitol. Fludarabine is associated with a dose-dependent neurotoxicity similar to PRES, known as acute toxic leukoencephalopathy characterized by cognitive impairment with visual and sensory defects [4, 18]. MRI imaging may show bilateral abnormal signaling within the deep white matter. Although now rarely used in current conditioning regimens, cytarabine causes cerebellar toxicity in approximately 10% of HSCT patients. Other acute neurotoxicities, such as confusion from fludarabine and peripheral neuropathy from carboplatin, are usually transient. Worsening of hearing loss, especially sensorineural high-frequency, is very common in patients who receive carboplatin (see below for a detailed discussion) [19, 20]. This is particularly common in patients undergoing autologous HSCT as part of the consolidation phase for the treatment of high-risk neuroblastoma who have already suffered significant high-frequency hearing loss from platinum-based prior therapy. The onset of neurologic toxicities may be acute or delayed as seen with radiation-induced leukoencephalopathy. The administration of either intrathecal or high-dose methotrexate prior to HSCT increases the risk of TBI-induced leukoencephalopathy [5]. Because TBI and high-dose alkylators can cause disturbances to the vascular endothelium along with severe thrombocytopenia secondary to myeloablative conditioning, patients are at high risk for intracranial hemorrhage [4, 5]. Patients who receive anti-thymocyte globulin (ATG) or alemtuzumab as part of their conditioning regimen are at high risk for viral infections including those known to penetrate the CNS to cause viral encephalopathy [5, 12]. Close viral blood monitoring and early initiation of preemptive therapy have contributed to circumventing the development of viral encephalopathy.

Hematopoietic stem cell (HSC) source: The cumulative risk for the development of a severe neurologic event at 3 years post-HSCT by hematopoietic stem cell (HSC) source is 2.4% for autologous, 15% for matched-related donor, and 38.6% for unrelated donor HSCT [5]. These findings correlate with the likelihood that the HSCT recipient is on concomitant therapy for the prevention or treatment of graft versus host disease (GvHD) either as a direct side effect of the drug(s) or indirectly as a result of being immunocompromised or pancytopenic (i.e., infection or hemorrhage).

Graft versus host disease (GvHD) and its treatment: Neurologic complications are much more common due to the treatment (i.e., immunosuppressive agents) rather than GvHD itself. They vary in degree from transient and mild to irreversible and life-threatening. Calcineurin inhibitors, such as cyclosporine A and tacrolimus, can cause a wide range of neurologic side effects, and these side effects are most often, but not exclusively, seen with elevated calcineurin inhibitor serum levels [12]. Most if these side effects are reversible and resolve completely once these agents are discontinued. These include fine tremor (which is the most commonly seen side effect), confusion or agitation, burning sensation of the palms or soles, somno-

lence, headache, visual disturbances (including hallucinations), aphasia, cortical blindness, and ataxia. Calcineurin inhibitor-induced seizures are exacerbated in the context of hypertension and electrolyte disturbances (particularly hypomagnesemia, hypo-, and hypernatremia). Thus, it is imperative to aggressively treat hypertension and correct any electrolyte abnormalities. PRES or white matter changes can be seen with elevated calcineurin inhibitors (with cyclosporine A more than tacrolimus) and is discussed in detail later in this chapter. While calcineurin inhibitors are frequently the cause of HSCT-associated neurotoxicity, they are associated with fewer long-term neurologic sequelae [1]. Coordination disturbances, sleep disturbances, mood disturbances, psychosis, akinetic mutism, optic neuropathy ototoxicity, and myoclonus are associated more so with tacrolimus than cyclosporine A. In chronic GvHD, neurologic complications can also be as a result of immune dysregulation. These complications include acute demyelinating encephalitis, acute inflammatory demyelinating peripheral neuropathy, (e.g., Guillain-Barre syndrome), CNS vasculitis, myasthenia gravis, relapsing-remitting disorders (e.g., multiple sclerosis), polymyositis, and polyneuropathy [4, 12]. GvHD-associated vasculitis can result in an aneurysm causing focal parenchymal hemorrhage. Peripheral neuropathy has been seen in the context of chronic GvHD as well but is very rare.

CNS infections: CNS infection is a rare but most often fatal cause of neurologic complications seen post-HSCT. In general, the infections are opportunistic. Common viral etiologies include HSV, HHV-6, VZV, BK virus, adenovirus, and CMV. Fungal (e.g., *Aspergillus*, *Candida*, and *Cryptococcus*) and protozoal (e.g., *Toxoplasma*) organisms are also found [4, 21]. HSCT patients who are at highest risk for CNS infections are those who received an allogeneic HSCT (particularly unrelated donor), graft manipulation with T cell depletion, or are on prolonged immunosuppression. The clinical presentation includes fever, headache, confusion, and focal neurologic signs. The neurologic symptoms can be divided into three general syndromes: (1) seizures, (2) focal brain disease (most commonly due to hematogenous spread from outside of the CNS), and (3) diffuse neurologic involvement. The diagnosis is determined by physical examination, by culture or PCR assay of cerebral spinal fluid (CSF) and blood samples, and by imaging, e.g., CT scan or MRI. Generally, treatment involves prolonged administration of the appropriate antimicrobials, depending upon the etiology of the infection. Of note, many of the antimicrobials used to treat CNS infections can have neurologic side effects. For example, the antifungal agent, voriconazole, frequently causes altered visual perception. Also, acyclovir can cause encephalopathy and PRES. Cefepime has been found to cause severe encephalopathy, especially in the context of renal insufficiency. Metronidazole is known to cause cerebellar dysfunction, sensorimotor peripheral neuropathy, optic neuropathy, and autonomic dysfunction. Finally, neurotoxicity associated with quinolones includes seizures, encephalopathy, myoclonus, and toxic psychosis.

Fungal etiologies: *Aspergillus* is the most common cause of post-HSCT CNS fungal infection. CNS fungal infections may result in mycotic aneurysm, vasculitis, and/or subarachnoid hemorrhage. CNS aspergillosis is usually disseminated from lungs or sinuses by vascular spread. The presentation is typically nonspecific but includes an altered level of consciousness (with or without focal neurologic signs) or meningeal irritation. It may lead to a mycotic aneurysm rupture in the subarachnoid space. Radiographic imaging typically shows lesions within the cerebral hemispheres with edema, mass effect, and areas of ischemia or hemorrhage. Treatment is with voriconazole or liposomal amphotericin B. Meningitis is rare, but *Candida* species are the most common cause followed by *Cryptococcus*. *Mucorales* (e.g. *Mucor*, *Absidia*, and *Rhizipus* species) can also give rise to CNS infections in immunocompromised patients. It occurs by spread from nasal, oral, or cranial sinuses to the orbit or cranial base and erodes through to the brain parenchyma. Early diagnosis and biopsy of suspected lesions are imperative. Surgical debridement is essential for any hope of cure, and

even then, these infections are almost always fatal. Combination treatment with liposomal amphotericin B and an echinocandin, such as caspofungin, is standard treatment.

Protozoal etiologies: Reactivated *Toxoplasma gondii* is a known cause of brain abscess in allogeneic-HSCT recipients. Patients often present with signs and symptoms of increased intracranial pressure (ICP) because of *Toxoplasma gondii's* tropism for periventricular locations causing obstructive hydrocephalus. Reactivated toxoplasmosis presents with multiple abscesses in the white or gray matter of the cerebral hemispheres as seen on MRI of the brain. CSF can be tested for *Toxoplasma gondii* DNA by PCR. Brain biopsy is required for histological diagnosis. The treatment is with trimethoprim-sulfamethoxazole and clindamycin or pyrimethamine. Toxoplasmosis is extremely rare nowadays because of the wide use of trimethoprim-sulfamethoxazole as prophylaxis in post-HSCT and immunocompromised patients.

Viral etiologies: HHV-6 is the most frequent cause of viral encephalitis [4]. Patients present with fever, headache, obtundation, and short-term memory loss. HHV-6 virus encephalitis has been associated with the use of alemtuzumab. HHV-6 virus can be detected by PCR from CSF fluid. MRI commonly shows bilateral abnormalities in the limbic system. Treatment is with ganciclovir or foscarnet. CNS infection due to CMV is rare. Typically, it occurs late post-HSCT (median > 4 months post-HSCT) and has a high mortality rate in all allogeneic HSCT patients (>80%). The CSF can be evaluated by PCR for the presence of CMV DNA. Imaging studies show ventriculoencephalitis with microglial nodules. The treatment is with ganciclovir or foscarnet. Varicella zoster virus (VZV) CNS infections occur on average 4–5 months post-HSCT. Nowadays, VZV CNS infections in post-HSCT recipients are extremely rare with widespread use of prophylaxis with acyclovir. It presents with fever, headache, somnolence, and often seizures. Symptoms may also include facial nerve palsy, hearing loss, arm weakness, and neurogenic bladder due to spinal cord involvement. Dermatomal zoster may precede CNS disease. Often, VZV CNS infection leads to significant vasculopathy, affecting both large and small vessels. While pleocytosis in the CSF is not detected in a third of patients with VZV CNS infections, detection of VZV DNA by PCR from the CSF is very sensitive and specific. CNS infection due to VZV is often fatal despite treatment with acyclovir. While Herpes Simplex Virus (HSV) 1 or 2 infections are relatively frequent in post-HSCT patients, dissemination to the CNS is rare. MRI imaging shows alterations of the mesial temporal structures. HSV-PCR from CSF is highly diagnostic. Treatment is with high-dose acyclovir. Two-thirds of patients will go on to have neurologic sequelae.

Bacterial etiologies: Bacterial CNS infections present as meningitis or brain abscess [4]. Classic signs and symptoms of meningitis may be blunted or absent in post-HSCT patients due to an impaired inflammatory response. In contrast, brain abscesses present with altered consciousness and a rapidly evolving focal neurological deficit (e.g., hemiparesis) with fever. Both gram-negative rods and gram- positive cocci as well as anaerobic organisms may cause CNS infections. Although extremely rare in the HSCT setting due to the use of trimethoprim-sulfamethoxazole as prophylaxis, the gram-positive organism, *Nocardia asteroides*, may cause a brain abscess as a consequence of hematogenous spread usually from pulmonary infection. Imaging studies with contrast typically show ring enhancing multiple or multiloculated abscesses. Treatment is with Trimethoprim-Sulfamethoxazole.

Miscellaneous infectious etiologies: Progressive multifocal leukoencephalopathy (PML) is a rare demyelinating disease associated with JCV infection. Its development is associated with the agents, fludarabine and rituximab. This latent infection develops late (>1 month post-HSCT) and can be delayed by years after HSCT. It is characterized by multifocal areas of demyelination of the brain with progressive neurological deficits. MRI shows hyperintense, multifocal, asymmetric lesions in the white matter with mass effect on T2-weighted and flair images and minimal contrast enhancement following gadolinium injection. While a brain biopsy pro-

vides the definitive diagnosis, PCR of JCV DNA of the CSF can provide the diagnosis but does not exclude the diagnosis in the PCR test is negative. Currently, there is no effective treatment, but reduction of immune suppression should be attempted.

HSCT-Associated Neurologic Complications

Serious neurologic complications are relatively rare in pediatric HSCT patients, but they do account for 10–20% HSCT-related deaths [3, 6]. These include seizures, altered states of consciousness (including encephalopathy, leukoencephalopathy, and PRES), and cerebral vascular events. They may be diffuse or focal and transient or more long-lasting.

Seizures: Seizures occur in approximately 5–11% of post-HSCT patients [22]. In one study, seizures accounted for half of the neurologic complications in pediatric post-HSCT patients [1]. Risk factors include medications (particularly busulfan, fludarabine, cyclosporine A, imipenem, DMSO, and acyclovir), electrolyte disturbances, hypertension, stroke, cerebrovascular bleeding, infections, and a prior history of seizures [22]. While the presentation is highly variable, generally, these seizures are most often tonic-clonic but can be focal. There is also prodromal encephalopathy. In one study, the most common etiologies of HSCT-associated seizures are PRES and CNS infections [22]. The ideal treatment of seizures in the peri-HSCT period is prevention. Thus, medication levels, particularly those of busulfan and cyclosporine A, should be monitored closely and appropriately dose-adjusted, blood pressure tightly controlled, and electrolyte abnormalities (particularly of magnesium and sodium) corrected promptly. In addition, treatment of any suspected or proven etiology should commence immediately, and anticonvulsants, such as keppra, phenytoin, and lorazepam, should be started. Typically, patients who develop HSCT-related seizures do not go on to develop epilepsy, and chronic anticonvulsant therapy is not necessary [22]. However, HSCT recipients who develop seizures have a reduced 5-year overall survival of 32.3% versus 45.8% in those who do not [22]. Relapse of the underlying disease, such as lymphoma or leukemia, needs to be considered, as onset of seizures may be a symptom of relapse.

Cerebral vascular events: Cerebral vascular events typically occur during hematologic recovery and include intracranial hemorrhage (ICH), hemorrhagic stroke, subdural hematoma, and ischemic stroke (both infectious and noninfectious). Risk factors include severe thrombocytopenia, neutrophil recovery, use of immunosuppressive agents, vascular injury, vessel occlusion, hypercoagulable state, and fungal infections. These events are mostly due to the severe thrombocytopenia that HSCT recipients experience while awaiting full hematologic recovery and thus most often occur during the first month after HSCT. In addition to severe thrombocytopenia, rapid engraftment of white blood cells can potentiate a cerebral vascular event. Side effects of immunosuppression, including increased risk for infection and hypertension, can potentiate a cerebral vascular event post-HSCT. Conditions and infections, such as veno-occlusive disease of the liver and fungal infections, are associated with platelet consumption and thus place HSCT recipients at a higher risk for cerebral vascular events. Patients who are prone to vessel narrowing or occlusion, such as those with sickle cell disease, are at an increased risk for stroke during the peri-HSCT period. The diagnostic workup includes obtaining a coagulation profile and performing a CT scan of the brain to evaluate for acute hemorrhage or infarct. An MRI should be performed if the CT scan is nondiagnostic. The treatment of a cerebral vascular event includes correcting any coagulopathy, platelet transfusion support, treatment of underlying hypertension, treatment of any underlying infection, administration of seizure prophylaxis and management of increasing intracranial pressure with intubation, hyperventilation, and mannitol. A neurosurgical consultation may be necessary to fully manage a patient with a cerebral vascular event.

Posterior reversible encephalopathy syndrome (PRES): PRES is a clinicoradiographic syndrome first described by Hinchey et al. [23] in 1996 that

presents with acute mental status changes (including altered consciousness, confusion, hallucinations, and lethargy), visual disturbances (including blurred vision, hemianopsia, and cortical blindness), headaches, seizures, nausea and vomiting, as well as paralysis [22, 24, 25]. The symptoms peak within 12–48 h and typically improve within 1–2 weeks. An MRI of the brain, which is the preferred imaging modality, typically shows extensive, multifocal areas of T2-signal hyperintensity in the posterior regions of the cerebral hemispheres of the white matter that are suggestive of edema and represent areas of decreased perfusion resulting in cortical ischemia. Most often, the occipital lobes are involved (>2/3 of cases) and less so in the cerebellum, brain stem, and basal ganglia [~ref Masetti et al. Pediatrics]. PRES is seen in 6–9% of HSCT recipients and usually develops in the first 100 days post-HSCT. PRES is most commonly induced by elevated serum levels of calcineurin inhibitors (cyclosporine A more so than tacrolimus) [25]. PRES has also been associated with sirolimus, gemcitabine, cytarabine, methotrexate, fludarabine, G-CSF, linezolid, ciprofloxacin, carbamazepine, rituximab, infliximab, alemtuzumab, and epinephrine [26–29]. One report identified age >2 years at the time of HSCT, a diagnosis of hemoglobinopathy, fludarabine-based conditioning regimen, a mismatched unrelated donor, cord blood HSCT, and use of G-CSF [25]. The pathophysiology of PRES is unclear, but it most likely involves endothelial damage that leads to blood-brain barrier dysfunction and then cerebral vasogenic edema. The differential diagnosis includes CNS infection, post-HSCT lymphoproliferative disease (PTLD), stroke, PML, metabolic disturbances, and thrombotic thrombocytopenia purpura (TTP). In addition to an MRI of the brain, a lumbar puncture should be performed. While analysis of the cerebral spinal fluid is not helpful in making the diagnosis of PRES, it is helpful in excluding CNS infection in the differential diagnosis. Blood chemistries and liver function tests should be sent to eliminate metabolic disturbances and TTP in the differential diagnosis. If the patient is taking a calcineurin inhibitor, then a drug level of this agent should be sent. An electroencephalogram (EEG) may also be helpful in making the diagnosis of PRES: In the acute phase, unilateral or bilateral focal slowing and/or periodic lateralized epileptiform discharges in the parieto-occipital or temporo-occipital regions of the brain. The mainstay of treatment for PRES is to decrease, and if possible, discontinue the calcineurin inhibitor and change to an alternative immunosuppressant. Because hypertension is often associated with the onset of calcineurin inhibitor-induced PRES, hypertension should be aggressively treated. In addition, any metabolic disturbances should be corrected. Typically, neurologic symptoms begin to improve a few days after presentation; resolution of abnormalities seen on MRI generally correlates with clinical improvement. The majority of patients have a full neurologic recovery. However, permanent neurologic damage is more likely to occur if PRES is not promptly recognized and treated. Furthermore, pediatric patients who develop PRES after allogeneic HSCT tend to have a higher mortality rate than patients who do not. However, one study found that the 8-year overall survival rates were not statistically significantly different at 35.1% versus 56.1% of HSCT recipients who had developed PRES versus those who did not [25].

Metabolic encephalopathy and *leukoencephalopathy*: Encephalopathy is global brain dysfunction that is associated with drug toxicity, electrolyte disturbances, organ failure, infection, nonconvulsive seizures, and GvHD; it is reportedly seen in approximately 6% of HSCT recipients. Metabolic leukoencephalopathy is caused by irritation of the white matter of the brain. Risk factors include older age of the recipient, use of busulfan in the conditioning regimen, severe electrolyte abnormalities (particularly hyper- and hyponatremia), drugs (such as cyclosporine A and antibiotics), renal insufficiency, uremia, liver failure, viral infections (such as HHV-6 and BK virus), toxoplasmosis, and use of mind-altering drugs. Encephalopathy and leukoencephalopathy usually occur 2–3 months post-HSCT and present with an altered sensorium, lethargy or stupor, focal neurologic signs, and hemiplegia. The diagnostic workup includes clinical examination with

a detailed neurologic examination, serum chemistries (electrolytes, BUN, creatinine, and liver function tests), a lumbar puncture for cerebral spinal fluid (protein, glucose, and infection studies), and drug screening, if indicated. The radiographic imaging of choice is an MRI which will show hyperintensity on T2/FLAIR. An EEG will show diffuse slowing. In some cases, a brain biopsy is warranted. A neurology consult should be done as well. The management of encephalopathy and leukoencephalopathy focuses on correcting or eliminating the underlying cause. For example, electrolyte and other metabolic disturbances should be corrected; uremia and hepatic failure should be treated if possible; seizures should be treated with anticonvulsants; and infections should be treated with the appropriate antimicrobials. Metabolic encephalopathy may not always be reversed, and only approximately 15% of HSCT patients fully recover neurologically. Approximately 38% have a partial recovery but with sequelae including seizures and learning disabilities. Death is not uncommon.

Acute irreversible and *chronic leukoencephalopathy*: Non-metabolic leukoencephalopathy is often progressive and irreversible and may occur at any time during the peri- or post-HSCT period. It is characterized by progressive mental deterioration of greater than 2 weeks duration. Symptoms include confusion, altered mental status, abnormal movements, and seizures. Known risk factors are cranial radiation, prior intrathecal or systemic methotrexate, cyclosporine A, or JCV infection. The diagnosis is made clinically and radiographically. The radiographic findings include periventricular or subcortical white matter decay or cerebral atrophy. The management is supportive with no known curative intervention. The outcome is generally poor [30].

Immune-Mediated Neurologic Complications

The main causes of immune-mediated neurologic complications are transplantation-associated thrombotic microangiopathy (TA-TMA) as well as myositis, diffuse demyelinating leukoencephalopathy, and Guillain-Barre-like demyelinating polyneuropathy which are quite rare [5]. TA-TMA is a distinct entity of endothelial damage and arterial thrombosis that can involve renal, GI, hepatic, pulmonary, and CNS systems. Neurologic deficits have been reported in 30% of patients with TA-TMA [31]. Only a small number of patients with TA-TMA have imaging findings suggestive of PRES. There is no standard treatment for TA-TMA, but use of inhibitors of the antibody-mediated complement cascade, such as eculizumab, is being investigated. Defibrotide and rituximab may be efficacious. Myositis is associated with chronic GvHD with an incidence of 2–3% of all HSCT patients. Hallmark of chronic GvHD-mediated myositis is moderate to severe proximal muscle weakness. Elevated creatinine-phospho kinase levels are associated with clinical course. The diagnosis is confirmed by a muscle biopsy which shows segmental muscle fiber necrosis and regeneration, mononuclear cell inflammation, and lymphocytic donor cell infiltration. Treatment is with corticosteroids. Acute immune-mediated neuropathies, such as Guillain-Barre and Guillain-Barre-like syndrome (GBS), are very rare. It usually develops within the first 3 months post-HSCT. It is characterized by progressive, symmetrical ascending motor weakness, hyporeflexia, and numbness. GBS may affect the diaphragm, resulting in respiratory insufficiency and need for mechanical ventilation. Electrophysiological testing shows slowed or blocked nerve conduction. Treatment with plasma exchange, IVIg, or rituximab has been used as well.

Neurocognitive Consequences of HSCT

There is a paucity of studies evaluating the impact on cognitive function in pediatric patients who have undergone HSCT. However, there are a number of small studies and two large studies that address this issue. Based upon the preponderance of data that showed the deleterious effects of CNS-directed therapy on cognitive ability in survivors of childhood brain tumors and

leukemia, the assumption had been that pediatric patients who undergo HSCT would also be at high risk for neurocognitive decline over time. However, the results of the majority of studies of cognitive performance in pediatric HSCT recipients demonstrated normal neurocognitive development with minimal to no decline in global intelligence (IQ) or in academic achievement over time [32, 33]. Phipps et al. [32] set out to evaluate the cognitive and academic outcomes of 268 pediatric HSCT patients prior to and at 1, 3, and 5 years post-HSCT. At 1 year post-HSCT, 158 patients were alive and comprised the cohort of this study's data. While the investigators found no significant changes in IQ and in academic achievement, they noted a difference (albeit small) in certain subsets of patients. By age, patients <3 years of age at the time of HSCT had the sharpest decline as compared to other age groups but was not a significantly large decline. In terms of underlying disease, HSCT recipients with leukemia showed a decline in IQ whereas those with non-malignancies showed a significant increase in IQ over time. As a function of the type of HSCT, patients who received an unrelated HSCT demonstrated a significant decline in VIQ9 (which measures verbal intellectual abilities, e.g., acquired knowledge, verbal reasoning, and attention to verbal materials), whereas those who had a matched sibling donor or autologous HSCT had a nonsignificant improvement. Patients who received TBI as part of the conditioning regimen had a significant decline of VIQ and no change in PIQ (which measures fluid reasoning, spatial processing, attentiveness to details, and visual-motor integration). In contrast, patients who did not receive TBI had a significant improvement in both VIQ and PIQ. Patients who had acute GvHD showed a statistically significant (but relatively small) decline in all neurocognitive outcomes over time. In the 17 patients who had received both intrathecal chemotherapy and high-dose methotrexate for treatment of ALL prior to HSCT (six of which had received cranial radiation as well), full-scale IQ, PIQ, and reading achievement all declined. Despite these declining slopes, the change is only a two IQ point loss or less over a 5-year period. More importantly, the results of this study showed that socioeconomic status of the HSCT recipient had the greatest impact on all neurocognitive outcomes measured.

A follow-up study that analyzed an expanded pediatric HSCT population focused on the impact of age and use of TBI on neurocognitive outcomes 5 years post-HSCT [33]. Patients were divided into four age groups: 0–2.99 years, 3–5.99 years, 6–15.99 years, and ≥16 years. Patients <3 years old who received TBI were the only group that had a statistically significant decline in IQ by 5 years post-HSCT. While patients of all ages regardless of receiving TBI or not had a decline in IQ, the decline was more substantial in those who had received TBI regardless of age. In patients <3 years old at the time of HSCT had the greatest decline in IQ whether they received TBI or not. However, the youngest patients who received TBI had IQs that remained flat over time, whereas patients <3 years old who did not receive TBI had an improvement in their IQ after 1 year post-HSCT and were approaching their baseline IQ pre-HSCT. Thus, while the impact of HST does not generally impact long-term IQ and academic achievement, patients <3 years old at the time of HSCT who receive TBI are at the greatest risk for a permanent negative impact on neurocognitive function of significance. Therefore, non-TBI conditioning regimens should be avoided in patients <3 years old as long as overall survival is not compromised.

Sensory Complications Related to HSCT

Ototoxicity

Introduction

Ototoxicity has been reported that 2.6% of all survivors of HSCT in childhood or adolescence with hearing loss in one or both ears [34]. Hearing loss is a much more common complication in specific subsets of pediatric HSCT recipients. The sequelae of ototoxicity (both complete and partial hearing loss) have a particularly negative impact on patients whose language development

is not complete (usually ≤3 years old). Hearing loss can lead to impaired speech and language development, communication difficulties, as well as delayed emotional or social development. Children with moderate high-frequency hearing loss may not be able to understand speech even in a quiet room. Hearing loss is generally classified into three groups according to the site of damage, Conductive hearing loss occurs as a result of damage to the outer or middle ear by preventing sound waves from reaching the inner ear. Conductive hearing loss is typically temporary because its most common causes are fluid in the middle ear or otitis media. The second classification is sensorineural hearing loss due to damage to the inner ear or auditory nerve. Sensorineural hearing loss can cause difficulty in speech perception. The third group is mixed hearing loss in which both conductive and sensorineural hearing loss are present.

Risk Factors and Etiologies of HSCT-Associated Hearing Loss

Risk factors for ototoxicity in pediatric HSCT patients include prior exposure to ototoxic agents causing hearing loss, diminished renal function (specifically increased serum creatinine), pre- and post-HSCT use of ototoxic agents, radiation therapy, and age of 3 years or younger at the time of administration of ototoxic agents. In contrast, the type of HSCT (allogeneic versus autologous), use of TBI, and delivery of Amphotericin B and vancomycin are not associated with worsening hearing loss. Furthermore, baseline hearing loss at the time of HSCT does not necessarily mean that the patients will suffer worse hearing loss post-HSCT [20].

Ototoxic agents: Table 24.4 summarizes the common ototoxic agents used in the HSCT setting along with its pathophysiologic mechanism of action and end result. The most common ototoxic agents used in HSCT are platinum-based chemotherapeutics (e.g., carboplatin and cisplatin) and loop diuretics (e.g., furosemide). Aminoglycoside antibiotics are also notorious for being ototoxic but are rarely used in pediatric HSCT patients nowadays. Often, platinum-based chemotherapy agents initially cause high-frequency hearing loss but often progresses to speech frequency hearing loss with cumulative exposure [19]. In one study by Punnett et al., HSCT patients who had neuroblastoma or Hurler's syndrome and those who received carboplatin-based conditioning regimens had worse hearing post-HSCT with a 16-fold increased risk in neuroblastoma patients and a 7.7-fold increased risk in patients receiving carboplatin [20]. Of note, all nine of the neuroblastoma patients in this study received carboplatin, and, conversely, nine of ten patients who were

Table 24.4 Ototoxic effects of agents commonly used in HSCT

Agent	Mechanism of pathophysiology	End result
• Platinum-based chemotherapy – Carboplatin Cumulative dose >360 mg/m^2 – Cisplatin Cumulative dose >1000 mg/m^2	• Destruction of outer sensory hair cells followed by inner sensory hair cells in the cochlea	• Irreversible sensorineural hearing loss in high-frequency ranges initially that progresses to speech frequency range
• Loop diuretics – Furosemide	• Changes in electrolytes and/or enzymes in the inner ear • Nerve transmission is affected by fluid changes within the inner year	• Hearing loss can develop quickly • Transient, severe tinnitus, deafness and high-frequency hearing loss • Usually reversible after discontinuation of agent
• Aminoglycoside antibiotics – Gentamicin – Tobramycin	• Destruction of outer sensory hair cells in the cochlea • Most commonly occurs with prolonged elevated trough drug levels	• Usually high-frequency hearing loss

conditioned with a carboplatin-based conditioning regimen had neuroblastoma.

Radiation: Radiation of the head and neck can cause ototoxicity. In addition, to hearing loss, radiation can cause tinnitus. Radiation-induced ototoxicity can be self-limiting to irreversible, and the loss of can be acute or delayed by 3–10 years. Radiation-induced hearing loss can be conductive (due to fibrosis of the tympanic membrane and ossicles) or sensorineural if the organ of Corti or the auditory nerve is within the radiation field.

Patient's age at the time of exposure: Because the auditory system and language is still developing early in life, children 3 years and younger are most vulnerable to damage after exposure to ototoxic agents. This damage may be exacerbated due to the slower clearance of platinum-based chemotherapeutics in younger children [19].

Diminished renal function: Many HSCT patients have some degree of renal dysfunction at the time of HSCT. One study showed that a rise in serum creatinine was associated with worse hearing post-HSCT with an adjusted odds ratio of 2.2 with every increase of 5 μmol/L of serum creatinine [20]. Many agents that are ototoxic are also nephrotoxic. The increased serum creatinine may be acting as a surrogate marker of prior exposure to agents that are both nephron and ototoxic. Alternatively, mild renal insufficiency may lead to a change in the metabolism of ototoxic agents, causing an exacerbation of ototoxicity.

Incidence

Baseline hearing loss at the time of HSCT in pediatric patients has been reported to be 43% [20]. The subset of HSCT patients who are most likely to suffer from hearing loss are those with high-risk neuroblastoma who are receiving high-dose carboplatin chemotherapy followed by autologous HSCT as the consolidation phase of therapy (odds ratio = 16). These patients are particularly vulnerable for worsening hearing loss because they have received cisplatin as part of their induction phase of therapy and then high-dose carboplatin in the myeloablative consolidation phase. After HSCT, hearing loss has been reported to be worse in 44% of patients with 38% having a moderate hearing loss (as defined as a threshold >40 dB) and 11% with severe loss (as defined as a threshold of >70 dB) [20]. Landier et al. reported that 67% of high-risk neuroblastoma patients had severe hearing loss post autologous HSCT [19], and the risk of severe hearing loss in high-risk neuroblastoma patients was increased >3-fold after consolidation with high-dose carboplatin. Furthermore, >50% of patients required hearing aids [20].

Management and Outcomes

The best management of hearing loss is its prevention. However, because of the proven efficacy of platinum-based chemotherapies for the treatment of certain malignancies, such as neuroblastoma, it is inevitable that these patients will receive these agents in order to achieve a cure. Thus, the use of these agents should be avoided altogether, kept to a minimum or substituted with a non- or less ototoxic agent without sacrificing outcome. For example, the use of busulfan or melphalan may be used for high-dose myeloablative consolidation therapy in place of carboplatin-based therapy in high-risk neuroblastoma patients with significant hearing loss at the end of the induction phase of therapy. Judicious use of loop diuretics and avoidance of aminoglycoside antibiotics are also done to ameliorate the risk of worsening ototoxicity during the peri-HSCT period. Because increased serum creatinine is associated with worsening of hearing loss, serum creatinine should be monitored closely. If the serum creatinine increases, then drugs need to be renally dosed in order to avoid exacerbation of ototoxicity and nephrotoxicity. Also, patients with hearing loss should avoid excessively noisy situations for up to 6 months after the ototoxic insult. Amifostine and sodium thiosulfate are being investigated as otoprotectants [35]. Monitoring for hearing loss is key. Patients who have received platinum-based chemotherapy should undergo auditory tests prior to the first exposure and then at least 3 weeks after the completion of each course of platinum-based chemotherapy. All patients must undergo auditory testing prior to HSCT as part of the pre-HSCT evaluation. It is recommended for patients to undergo annual audiometric testing thereafter.

Table 24.5 Comparison of audiographic tests

Test	Age range	Measurements obtained	Interpretation	Comments
• BAER	• Birth to 9 months	• Electrophysiologic measurement of function of auditory nerve pathway	• Hearing assessed by reviewing the size of the evoked peaks and the time to form them	• Child must be asleep or sedated • 15-min test
• VRA	• 9 months to 2.5 years	• Child turns head toward a lighted toy when sound is introduced at a specified frequency	• Assess hearing of better ear if headphones worn • Evaluates frequency range of 500–4000 Hz	• Child sits on parent's lap in a soundproof room • 30-min test
• Play audiometry	• 2.5 to 5 years	• Auditory threshold in response to speech or specific tones	• Assesses patient's auditory perception	• Child performs repetitive task each time a sound is heard • ~30-min test
• Conventional audiometry	• ≥5 years	• Auditory threshold in response to brief clicks	• Assesses patient's auditory perception	• Child raises hand when hears a sound • Wears soundproof headphones • 30-min test
• OAE	• All ages	• Cochlear hair cell response to auditory stimuli	• Determines whether hearing loss is present	• Cannot determine degree of hearing loss • May be normal in patients who have received carboplatin • 10-min test

BAER brain stem auditory evoked response, *VRA* visual reinforcement audiometry, *OAE* oto-acoustic emissions

Table 24.5 summarizes audiometric testing by age. Results of audiometric testing are plotted on an audiogram with pitch frequency (measured in Hertz) on the horizontal axis (lowest to highest) and sound intensity (measured in decibel) is plotted on the vertical axis from softest to loudest. The threshold is the frequency at which a patient can hear the softest sounds. The lower the point plotted, the worse the hearing. Conversation occurs at approximately 60 dB whereas a whisper is heard at approximately 30 dB. Normal speech frequencies range from 250 to 2000 Hz. Normal hearing is considered to be −10 to 15 dB hearing loss, mild hearing loss at 26 to 40 dB, moderate hearing loss from 41 to 55 dB, moderate severe hearing loss from 55 to 70 dB, severe hearing loss from 71 to 90 dB, and profound hearing loss ≥91 dB.

Children with any degree of hearing loss, including in the high-frequency range, need to be identified and receive intervention as early as possible. There are multiple technical devices, such as an auditory trainer.FM trainer, telephone amplifiers, text telephones, and hearing aids. Of note, hearing aids need to be refitted approximately every 6 months in younger children and have the batteries replaced very 1–2 weeks.

Visual- and Ocular Complications

Ocular complications after HSCT can affect all parts of the eye and can be divided as ocular surface disease, cataracts, and posterior segment disease. Table 24.6 summarizes the most common HSCT-associated visual disturbances and ocular complications with their potential etiologies. Ocular complications associated with HSCT have been recognized since the early 1980s. Factors that contribute to the development of

Table 24.6 Most common HSCT-associated visual disturbances and ocular complications with their potential etiologies

Visual disturbances	Potential etiologies
• Change in visual acuity	• Medication-induced (cyclosporine A) • Chronic GvHD
• Blurred vision	• Medication-induced (cyclosporine A; fludarabine) • Chronic GvHD
• Diplopia	• Medication-induced (cyclosporine A) • Chronic GvHD
• Blindness (occipital)	• Medication-induced (cyclosporine A; fludarabine)
Ocular complications	*Potential etiologies*
• ***Premature cataract formation*** – Posterior subcapsular	• TBI/radiation to the head/neck • Chemotherapy – Busulfan – Melphalan – Fludarabine • Prolonged steroid use • Chronic GvHD (not necessarily ocular)
• ***Ocular surface disease***	–
– Dry eye syndrome (DES)	– Ocular chronic GvHD
– Keratoconjunctivitis sicca	– Ocular chronic GvHD
– Viral conjunctivitis	– CMV, adenovirus
– Subconjunctival hemorrhage	– Profound thrombocytopenia
– Corneal pannus formation	– Chronic GvHD, steroids
– Corneal ulcers	– Chronic GvHD, steroids, infections (CMV)
– Excessive tearing	– Chronic GvHD
• ***Posterior segment changes***	–
– Occlusive microvascular retinopathy/ retinitis	– Infections (CMV; bacterial infections)
– Retinal vasculitis	– Medication-induced (fludarabine)
– Optic disc or nerve edema	– Medication-induced (fludarabine; cyclosporine A)
– Optic nerve atrophy	– Medication-induced (fludarabine; cyclosporine A)

post-HSCT ocular complications and visual disturbances include the use of total body irradiation (TBI), radiotherapy to the head or neck prior to HSCT. This damage may also be due to the conditioning regimen used, chronic GvHD of the eye, immunosuppression, opportunistic infections, and adverse effects of other agents frequently used in the HSCT setting.

The incidence and etiology of ocular complications and visual disturbances post-HSCT varies over time. In a retrospective study from 1999, 104 pediatric HSCT patients (less than 18 years old) that had ophthalmologic examinations 16, 21, and 36 months post-HSCT were studied for ocular and visual complications [36]. Among those found to have an ocular complication post-HSCT, the most common finding was posterior subcapsular cataract. Cataracts were found in 23% of patients with a mean time to detection of 19 months (range, 12–36 months). Among these patients, 67% had mild cataract(s) (as defined by visual acuity ≤20/40 in room light), 12.5% had moderate cataract(s) (as defined by visual acuity between 20/40 and 20/60 in light room), and 21% had severe cataract(s) (as defined by visual acuity >20/60 and requiring cataract surgery). Severe cataracts were associated with the presence of GvHD, most likely due to the use of corticosteroids to treat GvHD. Suh et al., also found that 22% of post-HSCT patients had ocular surface disease with dry eye syndrome (DES) noted in 13 of 104 patients. All patients with DES had undergone an allogeneic HCT, and 5 of the 13 had acute GvHD. Other ocular surface diseases noted in this patient cohort were blepharitis, bacterial infection, HSV keratitis, viral conjunctivitis, sub-

conjunctival hemorrhage, corneal pannus formation, and corneal ulcerations. Posterior segment changes, including occlusive microvascular retinopathy, optic nerve edema and optic nerve atrophy, were noted in 13.5% of patients ($N = 14$). Three had CMV retinal infections and one had a submacular abscess due to *Nocardia* bacterial infection. In this study, 95.7% had 20/40 or better visual acuity over time after appropriate treatment despite >50% of patients having an ocular complication post-HSCT. In another study, 27% pediatric HSCT recipients were found to have ophthalmologic abnormalities in the first year post-HSCT and that the most common findings changed over time [37]. Overall, 14% of the HSCT recipients had DES, 12% had retinal hemorrhages, 6% had optic disc edema, 2% had chorioretinal lesions, 2% had vitritis, and 2% had increased ocular pressure. At 5 months post-HSCT, over 50% of the patients with an ocular complication had DES, whereas retinal hemorrhages, optic disc edema, chorioretinal lesions, vitritis, and increased ocular pressure were more common within 3 months post-HSCT. Overall, the patients' symptoms were mild and self-limiting. Subretinal/retinal hemorrhages were associated with profound thrombocytopenia during the early post-HSCT period. In this particular study, older patients versus younger patients (13 years versus 3.4 years) were found to develop ocular complications more often. Also, opportunistic infections of the eye were rarely seen. However, systemic viral reactivation early on post-HSCT does place the HSCT recipient at a higher risk for developing eye involvement.

In comparison, Gurney et al. [34] found that approximately 40% of survivors who underwent HSCT during childhood and adolescence suffer from an ocular complication or visual disturbance. Among these, cataract formation, legal blindness in at least one eye, and double vision were the most common findings. The cumulative incidence of these individual ocular complications may vary depending upon the circumstances (e.g., TBI- versus non-TBI conditioning regimens, autologous versus allogeneic HSCT, age at the time of HSCT, the presence of chronic GvHD, the immune status of the patient, and other agents used in post-HSCT patients). Ocular chronic GvHD is involved in 40–60% of patients with chronic GvHD with symptoms that include dry, gritty or painful eyes, foreign body sensation, eyelid edema and erythema, blurred vision, excessive tearing, and photophobia. Ocular chronic GvHD is addressed further in Chap. 19.

Special consideration of HSCT-associated cataracts: It is well established that premature cataract formation is associated with exposure to ionizing radiation. Thus, HSCT patients who receive TBI as part of their conditioning regimen and/or pre-HSCT cranial irradiation are at higher risk for developing cataracts. This risk is dependent upon the total dose, fractionation, and dose-rate, with single-dose TBI being associated with the highest incidence as compared to those who received the same dose split up over six or more fractions. In addition, the cumulative incidence of cataracts is higher in patients who received a higher dose rate of radiation. Thus, older studies typically report higher incidences of cataract formation than more recent ones because of the changes in the delivery of TBI. Furthermore, the use of TBI as part of the conditioning regimen is now avoided whenever possible in very young patients or in patients with non-malignant conditions. However, the incidence of cataract formation remains significantly high in HSCT patients if they received TBI as part of their conditioning regimen [34, 37]. Patients who have received prolonged corticosteroid therapy prior to or after HSCT are at high risk of premature cataract formation. Thus, the presence of chronic GvHD (which usually requires a prolonged course of corticosteroid treatment) has been associated with premature cataract formation, and the development of cataracts can be accelerated in patients with chronic GvHD who also have received TBI [38]. Typically, cataract formation is a relatively late complication post-HSCT with a reported cumulative incidence of 36% at 15 years post-HSCT [34]. Thus, a funduscopic examination and visual acuity assessment should be performed annually in patients at high risk for premature cataract formation and other HSCT-associated ocular complications. For example, patients with evidence of cataract formation should be referred to ophthalmology for further evaluation and possible intervention, i.e.,

cataract extraction or photocoagulation, in order to preserve a patient's eyesight.

HSCT-Associated Taste Disturbances and Dysfunction

Anecdotally, altered taste (dysgeusia) and reduced taste (hypogeusia) have been noted in HSCT recipients. However, there are very few formal studies, particularly those focused on pediatric patients, investigating this issue. The chemical sense of taste is detected through taste buds which are clusters of cells containing receptor sites that specifically detect sweet, sour, salty, or bitter sensations. Taste buds are located on the papillae of the tongue. Cranial nerves transmit taste stimuli from the taste buds to the brain. Taste receptors regenerate approximately every 10 days, but their lifespan can last for a month or longer. Because taste receptors are rapidly proliferating, they are vulnerable to damage by chemotherapy including those used in various HSCT conditioning regimens. Thus, renewal of taste receptors is interrupted during the administration of chemotherapy. Over time, taste buds will begin to rapidly renew and form new connections with nerve fibers. Until then, normal taste perception is disrupted (either blunted or altered). Other agents, such as antibiotics, can alter taste perception as well.

While some may consider taste disturbances a minor issue in the context of HSCT, it can be a significant problem in the pediatric HSCT population because younger children can develop an oral food aversion due to alterations in their taste buds. It can interfere with normal oral development in very younger children. Older children and adolescents with dysgeusia and hypogeusia may not be able to resume appropriate caloric intake and achievement of a normal nutritional status. Dysgeusia can lead to serious nutritional deficiencies in all ages. Knowledge of the time course and characteristics of HSCT-associated taste disturbances is helpful in order to better counsel HSCT recipients and their families regarding what to anticipate. One such study of 51 pediatric HSCT recipients age 3–12 years old was designed to address these issues [39]. This study set out to identify threshold values and perceived stimulus intensity over time after HSCT. Each participant was given solutions of four different flavors (sucrose, sodium chloride, citric acid, and quinine hydrochloride) at four different concentrations plus deionized water as a control for a total of 15 solutions (2 mL each) to taste for `0 s and then expectorate. The sequence of the administration of the 15 solutions was randomly predetermined. Patients were tested prior to starting their conditioning regimen, twice during conditioning, and then every 3 months post-engraftment. For all four flavors, the threshold means were significantly blunted during conditioning regardless of the conditioning intensity. The threshold means reached or approached baseline values by 6 months post-HSCT. In contrast, the perceived intensity of each flavor differed over time. For example, while all four taste sensations were not detected during conditioning at the most dilute concentration tested (0.000032 M), only the sweet taste sensation was detectable at 0.0001M but at a very low level. The other three taste sensations were detected only at higher concentrations during conditioning. By 6 months post-HSCT, study participants were able to detect all four tastes at lower concentrations, with bitter and sweet at the lowest concentration.

Adult studies of taste dysfunction after allogeneic HSCT have reported longer time periods for resolution up to 1 year or longer post-HSCT, with 80% recovered by 1 year post-HSCT [40, 41]. Salt was the most profoundly affected taste sensation. Furthermore, the presence of oral GvHD was not associated with altered taste perception [42]. The difference in recovery time between pediatric and adult patients may be related to the difference in the overall rate of cell proliferation, with younger children's taste buds' regeneration occurring faster than in adults.

Key Points

- Neurologic complications associated with HSCT in children and adolescents are not uncommon and quite variable from transient and mild to irreversible and severe.
- The most common causes of severe neurologic complications are drugs, CNS infections, CNS involvement of underlying disease,

PRES, encephalopathy of unknown etiology, and metabolic disturbances.

- The etiologies vary over time and can present early on in the peri-HSCT period to delayed (>6 months post-HSCT). Early neurologic complications are most often drug-induced, whereas delayed ones are typically related to the immunodeficient state of the HSCT patient.
- It is very important to perform a detailed neurologic examination and obtain a comprehensive medication history.
- Sensory complications most commonly involve medication-induced visual disturbances, radiation-induced cataracts, medication-induced ototoxicity and taste disturbances.

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HSCT-Associated Complications of the Skin, Hair, and Nails

25

Valerie I. Brown

Abstract

HSCT-associated skin, hair, and nail changes are very common. Skin complications range from breakdown of the skin integrity due to chemotherapy or total body irradiation (TBI) during the peri-HSCT period to significant changes (sclerodermatous and lichenoid changes) due to chronic graft-versus-host disease (GvHD) that can be lifelong and life-threatening. The most common causes of skin complications are drug allergy, engraftment syndrome, chemotherapy-induced, radiation-induced, GvHD, infection, and contact dermatitis. Nail changes can occur in HSCT recipients, and they are most often caused by chemotherapy, radiation, and GvHD. Hair changes associated with HSCT include partial or total alopecia that is usually temporary but can be permanent and most often due to chemotherapy, radiation, or chronic GvHD. Change in color or texture can also occur in HSCT recipients. This chapter addresses skin, nail, and hair complications found in HSCT recipients with the exception of GvHD-related because this topic is detailed elsewhere in Chapters 17 (Acute Graft-Versus-Host Disease: Diagnosis, Prophylaxis, and Treatment) and 19 (Chronic Graft-Versus-Host Disease).

Skin-Related Complications of HSCT

Introduction

The skin plays multiple roles: it provides a major barrier against microbial infections, protects against minor trauma, prevents body fluid loss, and plays a significant role in body temperature regulation. Impairment of skin integrity can impact these important functions. The maintenance of skin integrity is particularly important in patients undergoing HSCT because they are sus-

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ceptible to skin infections if the skin has broken down. Because a patient may not be able to manifest symptoms of a skin infection or cellulitis, i.e., erythema, warmth, and/or purulent discharge, due to profound neutropenia, a thorough inspection of the skin during the peri-HSCT period is very important in order to detect any signs or symptoms of infection. Furthermore, patients treated for GvHD with steroids over a prolonged period of time are at greater risk for skin atrophy and breakdown.

Risk Factors, Etiologies, and Clinical Manifestations

The risk factors of skin complications during the peri-HSCT period include toxicity due to the conditioning regimen (chemotherapy and radiation), the presence of a central venous catheter, the presence of a surgical incision, GvHD, poor nutrition, and diarrhea. In addition, immobility, impaired oxygenation of tissues, medications, infections, immunosuppression, and cytopenias are risk factors of impaired skin integrity. Peripheral edema can cause mild erythema to significant stretching and oozing of the skin. The differential diagnosis of the causes of HSCT-related skin complications includes drug allergy, toxicity of chemotherapy and/or radiation, GvHD, engraftment syndrome, infection, folliculitis, eczematous changes due to food or formula, and contact dermatitis. Vitamin and mineral deficiencies can result in perioral scaling with a pellagroid appearance due to skin fragility [1]. In one small study [1], the most common cause of skin changes in pediatric patients post-HSCT is reaction to drug(s), with drug-induced hyperchromia (or hyperpigmentation) as the second most common finding.

The clinical manifestations of impaired skin integrity during the peri-HSCT period range from a macular or popular rash with or without pruritus and hives to blisters or bullae and complete breakdown of areas of skin. These findings can arise any time during the peri-HSCT period starting with the conditioning regimen and can last for weeks, months, or even years. Often a HSCT patient's skin becomes very dry during the first month post-HSCT, and skin desquamation is very common, particularly if the HSCT recipient perceived radiation. Other skin manifestations seen in the peri- and post-HSCT periods include hyper- and/or hypopigmentation, skin erythema, petechiae, bruising, alopecia (discussed separately in this chapter), urticarial, vesicles, bullae, nevi, fissures (particularly perianal), ulcerations, sclerodermatous and lichenoid changes, and striae due to corticosteroids.

Chemotherapy: Chemotherapeutic agents commonly used in conditioning regimens, including busulfan, etoposide, melphalan, thiotepa, and ATG, typically cause moist desquamation (i.e., dermatitis), producing an exudate or oozing of the skin. It can range from hyperpigmentation to painful desquamation. The most commonly affected areas include the neck, upper trunk, abdomen, palms, and skinfolds. Some manifestations of skin involvement are more commonly seen depending upon the specific chemotherapy. For example, the metabolites of thiotepa are excreted through sweat pores, accumulating on the skin. It particularly collects in axillary, neck, and inguinal skinfolds as well as under ventral venous catheter (CVC) dressings and under constrictive clothing, such as elastic waistbands and socks. If not washed off promptly, the excreted thiotepa metabolites will cause severe irritation (reminiscent of a skin burn), moist desquamation, and eventual hyperpigmentation [2]. Frequent showers (paying close attention to skinfold areas) with water only, minimizing skin dressings, and no application of creams, lotions, or gels to the skin will help to minimize the risk of severe skin irritation. Bathing should occur as frequently as every 6 hours with all bed linens and clothes changed at the time of each bath. This regimen should begin 6 hours after the start of the first infusion of thiotepa and continued until 24 h after the completion of the last dose. Also, only loose-fitting clothing should be worn during this time period. In very small children, use of diapers should be avoided if possible, but if not, then diapers need to be changed immediately after each void. Thiotepa-induced dermatitis is delayed, typically by 1 week or so. Silvadene applied to affected skin areas is the typical treat-

ment. While less commonly used in conditioning regimens nowadays, etoposide can cause bullae formation of the hands. However, mucous membranes, pharynx, and conjunctiva may be involved as well. Another agent commonly used during conditioning in pediatric patients is antithymocyte globulin (ATG). The administration of ATG is associated with hypersensitivity and anaphylaxis. In addition to fevers and hypotension, patients may develop urticaria (hives), erythema, and/or pruritus. The erythema and pruritus are usually transient, but the urticaria may be present for several days. Premedicating with Benadryl and corticosteroids can minimize the risk of these reactions.

Total body irradiation (TBI): The skin changes that can occur after exposure to total body irradiation range from mild skin irritation and erythema to the development of bullae. These changes can be exacerbated if ointments, lotions, gels, or creams are applied to the skin prior to and during TBI. Thus, topical agents should not be applied to the skin starting at least 24 h before the delivery of the first fraction of TBI through 24 h after the last fraction in order to avoid minor skin erythema and irritation from developing into frank skin burns, for example.

Impaired skin integrity: Intact skin provides the most important barrier to infection. If the skin integrity is compromised, then the risk for infection increases significantly. Although dressings are used at CVC sites, the skin under the dressing can be irritated, even denuded, from a combination of adhesives and tape over the dressings and from chemoradiation. The skin surrounding the CVC site also may become sensitive and irritated and occasionally become friable and denuded. If the area does become irritated or broken down, then nonadhesive dressings, nonirritating soaps, and topical antibiotics should be used.

Skin infections: A comprehensive daily skin assessment is imperative during the peri- and very early post-HSCT periods in order to identify areas of skin irritation and breakdown early on and to promptly intervene with empiric antimicrobial therapy. Common signs of infection, such as erythema, warmth, and tenderness, may not be present due to profound neutropenia. Aspergillus skin infection may present initially only as a small, gray-black dot that can rapidly progress to disseminated disease. If cutaneous aspergillus is suspected, then dermatology should be consulted to perform a skin biopsy and culture of the area. In addition, empiric antifungal coverage should be initiated or expanded if the patient is already on an antifungal agent for prophylaxis. The risk for HPV and herpes simplex virus skin infections, which can manifest as gingivostomatitis, lip lesions, and warts, correlates with the duration of immune suppression [1].

Diarrhea due to the toxicity of the conditioning regimen, GI infections, antibiotic treatment, and graft-versus-host disease (GvHD) may lead to perirectal irritation or skin breakdown which greatly increases the risk for infection. Thus, frequent inspection and application of a protective barrier cream are effective strategies to prevent perirectal skin irritation and breakdown.

Viral infections that involve the skin are seen in HSCT patients. Varicella zoster virus (VZV) infection typically occurs because of viral reactivation. Most cases of reactivation occur late post-HSCT. Initially, VZV reactivation manifests itself as a vesicular rash arising in specific dermatome(s). In some cases, the rash is preceded by pain which may persist after the rash resolves. In other cases, the classic "dew drop on a rose petal" description of VZV rash is not present, especially in immunosuppressed patients. Thus, if VZV is suspected, then one of the skin lesions needs to be tested for VZV. HHV-6 is another virus that can be accompanied by skin rash. This rash can mimic acute GvHD of the skin, i.e., a maculopapular rash. HHV-6 can be detected from the blood by PCR. If HHV-6 is detected, then antiviral therapy needs to be initiated immediately because HHV-6 has been associated with graft rejection. Thus, donor chimerism should be monitored closely in patients with HHV-6 infection [3].

Candidal rash in the diaper area is a common skin infection seen in young HSCT patients in the peri-HSCT period. Candidal overgrowth often occurs due to antibiotic use, diarrhea, and chemotherapy-induced dermatitis. Once the skin breaks down, it will not heal until the patient

engrafts because white blood cells are necessary for skin regeneration. Thus, strategies to prevent skin breakdown in the perineal area are essential in all age groups. This area needs to remain clean with frequent diaper changes in very young HSCT patients. Application of protective, moisturizing creams which provide a mechanical barrier and of antifungal powders and creams provides protection against skin breakdown. Older HSCT recipients may develop cutaneous candidal infections in skinfolds under the breasts and scrotal area.

Impaired oxygenation and immobility: Impaired oxygenation and immobility can lead to decubitus ulcers due to immobility and impaired oxygenation. When there is pressure on the skin and underlying tissues that is greater than the closing pressure of the capillaries, the capillaries become occluded, impairing tissue oxygenation. If blood flow is not restored, then the vessels with collapse, tissues will become anoxic, and ultimately cells will die, resulting in necrotic tissue. The optimal approach for the treatment of decubitus ulcers is to prevent them from occurring. Preventative measures include frequent repositioning, ambulation, and specific "alternating pressure" or foam mattresses. Treatment includes wet-to-dry dressings, but it is best to consult an institution's would team.

Diagnosis and Evaluation

History and physical examination: When assessing a change in the skin of a HSCT recipient, it is important to know key pieces of information regarding the change in skin. These include the duration of the skin change (e.g., rash), i.e., did it just appear or has it been getting progressively worse. It is important to ascertain if the character of the rash has changed, i.e., evolution from a red-violaceous to a black, necrotic-looking dot which is suspicious for aspergillus skin infection. If the rash comes and goes, one needs to ask if it is temporarily related to the administration of a medication or certain food(s). It is important to determine if the rash is painful or pruritic. One also needs to inquire about recent environmental changes or other exposures. Most important is a thorough head-to-toe inspection of the patient's skin, including the perirectal area and skinfolds, particularly during the peri-HSCT period.

While the etiology of skin complications that arise in HSCT patients is diagnosed most often on a clinical basis, dermatology consultation may be necessary to perform a biopsy or scraping of the affected area and assist in establishing a diagnosis, such as GvHD, drug-mediated, or infectious-related. If the biopsy site requires a suture(s), the site should be monitored very closely for infection and exposed to the air as much as possible.

Supportive Care, Management, and Outcomes

In general, the use of gentle cleansers without alcohol and fragrances is recommended during the peri-HSCT period and in patients with chronic GvHD. Sitz baths are often utilized to ensure that the rectal area is thoroughly cleaned. Use of chlorhexidine wipes daily is an effective method for daily skin hygiene in patients during the peri-HSCT period. Liberal use of skin barriers, topical antibiotics, and antifungal agents should be applied after each cleaning. Jewelry should be removed, clothing with elastic band or are constrictive should be avoided, and all artificial nails removed during the peri-HSCT period.

While most of the pigmentation changes associated with dry desquamation resolve eventually, it often takes months. Furthermore, skin can be permanently altered, as is often seen with chronic graft-versus-host disease (GvHD). In the early and late post-HSCT periods, patients need to limit skin exposure by wearing hats and long-sleeved cotton shirts as well as liberally using sunscreen (≥ SPF 15) while exposed to the sun. It is very important to undergo prophylactic skin protective actions even after the skin has healed because HSCT recipients are at risk for secondary skin cancers [4, 5]. In addition, sun exposure may trigger skin GvHD exacerbation.

HSCT-Associated Hair Complications

While reversible alopecia is the most often seen post-HSCT, a proportion of patients will go on to develop permanent alopecia post-HSCT, and alopecia areata and alopecia universal are associated with the calcineurin inhibitor, tacrolimus, and, less so, cyclosporine A. However, there are only a few studies investigating permanent alopecia following HSCT. One study from the Netherlands reviewed the incidence and risk factors of pediatric HSCT patients over a 23-year period [6]. They found that 15.6% of patients had permanent alopecia defined as a clinically apparent decreased hair density. All had diffuse alopecia except one who had alopecia totalis. These patients had normal eyebrows, eyelashes, and body hair distribution as they got older. In terms of risk factors, permanent alopecia developed more often in patients who received a busulfan-containing conditioning regimen as compared to those who did not (23% versus 13%). Irradiation to the head was not associated with permanent alopecia. GvHD, both acute and chronic, was associated with patients who had permanent alopecia, but the severity was not, nor was the skin as a target organ for GvHD. In another study, Choi et al. [7] found that 12% of post-HSCT pediatric patients developed permanent alopecia. In contrast, this study found that thiotepa, and not busulfan, was associated with permanent alopecia. They also found that 67.1% of patients experienced a loss of hair density. The mean age was significantly younger (mean age = 5.2 years) in patients with permanent alopecia than those that did not (mean age = 7.6 years).

In addition to alopecia, the texture and color of hair in post-HSCT recipients may be different than pre-HSCT. Furthermore, patients with chronic GvHD may suffer from premature graying, thinning, and/or brittle hair. While not life-threatening, these changes in hair post-HSCT, particularly permanent alopecia, may cause distress and other psychological issues because of its impact on self-esteem. For permanent alopecia, currently, there is no effective treatment that will reliably restore normal hair growth, and there is no effective preventative measure either.

HSCT-Associated Nail Complications

The chemotherapy agents used in conditioning regimens, including busulfan, cyclophosphamide, and melphalan, commonly cause nail changes including hypopigmentation, linear banding, and ridging. Skin chronic GvHD often involves nails. Most common features include nail dystrophy, longitudinal ridging or splitting, brittle nails, onycholysis, pterygium unguis, and nail loss [8]. In one study, 45% of pediatric HSCT patients with skin chronic GvHD had nail dystrophy, with longitudinal ridging and distal splitting being the most common findings [9]. Five of the 31 HSCT patients who had documented nail examinations had pterygium inversum unguis, and all five patients had severe chronic GvHD of the lung.

Key Points

- Changes in skin, hair, and nails are very common in post-HSCT recipients.
- Skin complications range from minor skin irritation and erythema to life-threatening skin breakdown.
- There are many causes of the skin changes found in HSCT patients. These include chemotherapy and irradiation used as part of the conditioning regimen, acute and chronic graft-versus-host disease (GvHD), drug allergy, engraftment syndrome, infection, and contact dermatitis.
- The most common HSCT-associated hair change is alopecia that is usually temporary but can be permanent. Other hair changes include a change in color or texture.
- The most common causes of HSCT-associated nail changes are chemotherapy, radiation, and GvHD.

- While not life-threatening, changes in hair and nails, particularly permanent alopecia, can elicit significant distress in post-HSCT patients, negatively impacting self-esteem.

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Part V

Life After HSCT

Immune Reconstitution After Hematopoietic Stem Cell Transplantation

26

Mala K. Talekar and Timothy Olson

Abstract

Immune reconstitution after hematopoietic stem cell transplantation (HSCT) is critical in order for HSCT recipients to recover their ability to fight all types of infections. Restoration of individual components of the immune system occurs with different timelines. Factors that influence this differential recovery include the type of HSCT, type of donor, donor histocompatibility, donor hematopoietic stem cell source, age of the donor and recipient, conditioning regimen used, the underlying disease or disorder, graft manipulation strategies, and the presence of graft-versus-host disease (GvHD). In general, innate immunity recovers much earlier post-HSCT as compared to adaptive immunity. Complete immune reconstitution may occur from several months to up to 2 years after HSCT. This chapter discusses the typical time course of immune reconstitution by cell type as well as methods to monitor immune reconstitution and an approach to revaccination post-HSCT.

Introduction

The ability of hematopoietic stem cell transplantation (HSCT) to provide definitive cure without excess transplant-related mortality (TRM) for both malignant and nonmalignant diseases of hematopoiesis and immunity is dependent upon the ability of donor hematopoietic stem cells (HSC) to replace and restore all aspects of innate and adaptive immunity (Fig. 26.1). The immunosuppressive effect of HSCT conditioning renders patients temporarily vulnerable to a wide array of bacterial, viral, and fungal infections that contribute to TRM [1]. Cytokine fluctuations,

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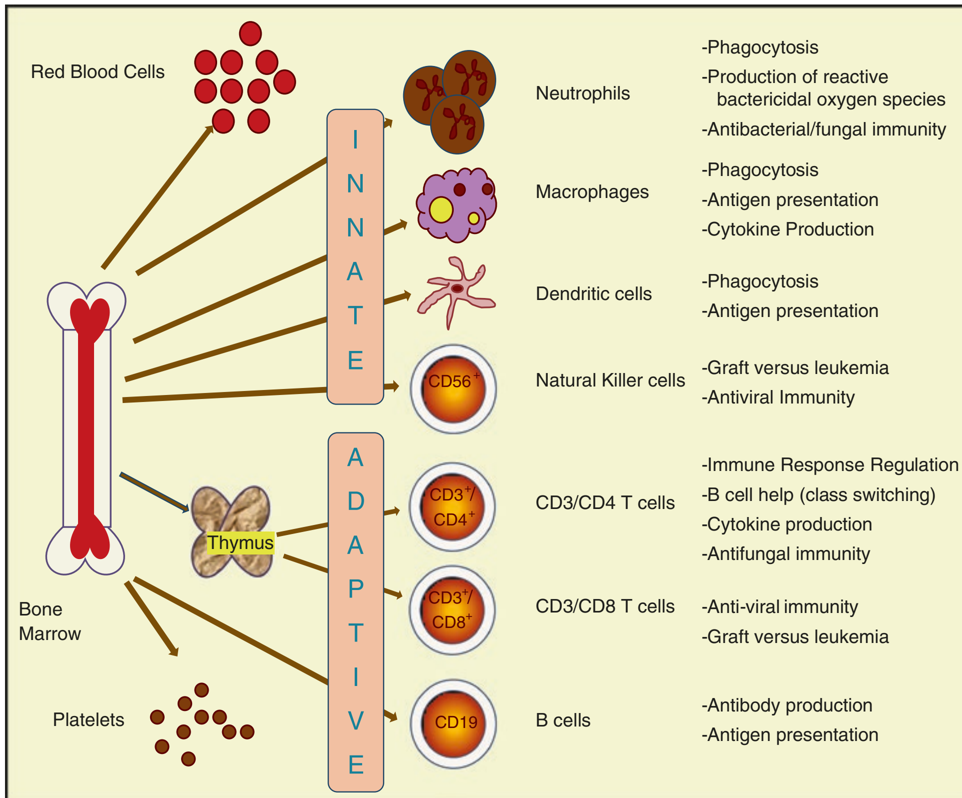

Fig. 26.1 Cells derived from hematopoietic stem cell transplant (HSCT) grafts and their roles in immune responses post-HSCT. This illustration represents the different cell types derived from the hematopoietic stem cells contained within the donor graft. On the far *right side*, the major function(s) of each cell type is noted

conditioning regimen-related immune dysregulation, and states associated with profound lymphopenia after HSCT are also the leading causes of severe, life-threatening HSCT complications [2–5]. Intact immune surveillance is additionally critical to graft-versus-malignancy (GVM) effects that lower relapse rates following HSCT for certain malignancies [6]. Furthermore, quality of donor immune reconstitution is critically important for determining the degree of correction for underlying immune deficiencies and for preventing immunologic graft rejection in HSCT for nonmalignant diseases [7].

Individual components of immunity are restored at quite distinct rates following HSCT. Many factors specific to an individual transplant strategy and the underlying indication for HSCT greatly influence these rates. The type of the transplant (autologous vs allogeneic), type of donor (matched sibling, unrelated donor, umbilical cord blood), donor histocompatibility (how closely matched the donor is to the recipient), donor hematopoietic stem cell (HSC) source (cord blood, bone marrow, mobilized peripheral blood stem cells), donor and recipient ages, conditioning regimen, GvHD prophylaxis and treatment choices, and in vivo or ex vivo graft manipulation strategies all contribute to the pace by which immunologic recovery following HSCT occurs [8, 9].

Functional immune reconstitution begins with initial engraftment and, on average, proceeds to completion anywhere from several months to up to 2 years after HSCT (Fig. 26.2). Recovery of innate immune system components (monocytes, granulocytes, and natural killer (NK) cells in that order) occurs first, followed by a slower and highly variable recovery of adaptive immunity [8, 9].

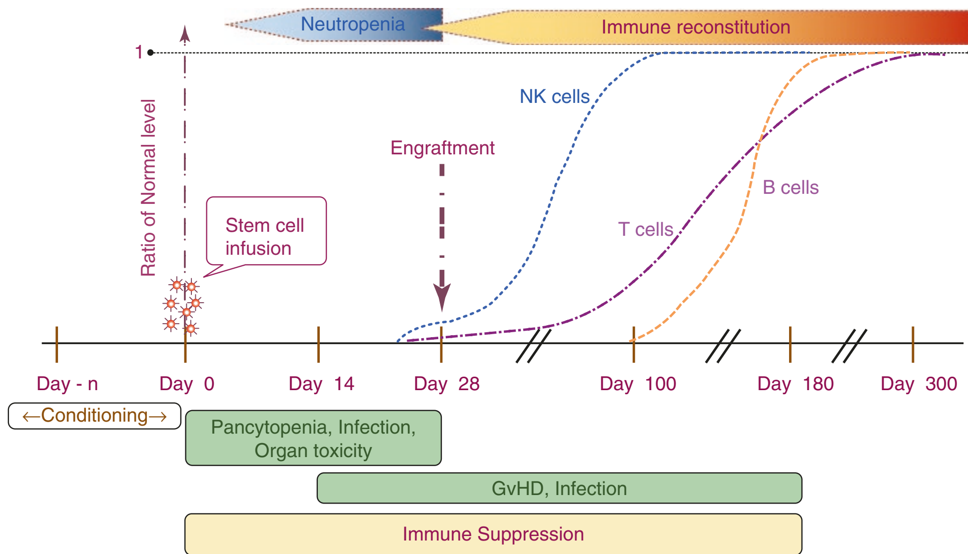

Fig. 26.2 Time course of immune reconstitution following matched sibling donor bone marrow transplantation. This schematic depicts the time course of quantitative immune reconstitution following matched sibling donor bone marrow transplantation in the absence of additional factors associated with delayed reconstitution

Reconstitution of the Innate Immune System

Neutrophils

Neutrophil recovery is considered one of the gold-standard signs of hematopoietic engraftment following HSCT. Neutrophil engraftment is defined as the first of 3 consecutive days post-HSCT with absolute neutrophil count (ANC) ≥500 cells/μL [10] and is highly dependent on HSC source (Table 26.1). In the autologous HSCT setting, patients receiving G-CSF mobilized peripheral blood stem cell transplantation (PBSCT) generally achieve neutrophil engraftment by 10–15 days, whereas neutrophil engraftment following autologous bone marrow transplant (BMT) takes longer and is more variable among studies (14–26 days) [11]. A meta-analysis of time to neutrophil engraftment in adults receiving allogeneic HSCT for hematologic malignancies revealed similar outcomes with an average time to neutrophil engraftment of 16 and 20 days for PBSCT grafts versus BMT, respectively [12]. In contrast, neutrophil engraftment following umbilical cord blood transplantation (UCBT) generally takes up to a week longer (range, 20–30 days) than following HSCT with other stem cell sources [13–17].

The impact of HSC *dose* on time to neutrophil engraftment appears to depend on the HSC *source*. Whereas the total $CD34^+$ cell dose from bone marrow grafts does not affect time to neutrophil engraftment in pediatric allogeneic BMT, G-CSF stimulation of bone marrow (BM) grafts does lead to early engraftment [18]. In contrast, cell dose significantly impacts neutrophil engraftment following UCBT, with UCB units containing $>3 \times 10^7$ nucleated cells/kg having much improved rates and durability of engraftment [19]. Following recovery of normal neutrophil numbers, studies are conflicting as to whether substantive qualitative neutrophil function defects may persist for a longer period of time [20–22].

Natural Killer Cells

Natural killer (NK) cells have gained increasing focus in the field of allogeneic HSCT over the past decade, with a number of discoveries that NK cells can play critical roles in allogeneic immune responses, both by mediating graft-

Table 26.1 Differences in speed of quantitative immune recovery based on stem cell source

Source:	Bone marrow	Umbilical cord blood	T-Cell-repleted PBSC	T-Cell-depleted PBSC
Neutrophil engraftment (days)	14–21	20–30	10–16	10–16
NK-cell recovery (days)	<30	<30	<30	Varies[a]
T-Cell recovery (months)	3–6	3–6	3–6	>6 months[a]
B-Cell recovery (months)	6–12	2–6	4–12	Varies[a]

Numbers reflect estimated time to quantitative recovery of each cell type (ANC > 500 μL^{-1}; CD3+ T cells >500 μL^{-1}; CD19+ B cells >200 μL^{-1}). Qualitative functional reconstitution takes longer than times indicated here for all subsets. *PBSC* peripheral blood stem cells

[a]Speed of NK-, T-, and B-cell recovery in T-cell-depleted PBSC grafts depends on specific method of T-cell depletion

versus-malignancy (GVM) responses and by modulating GvHD risk through a combination of interactions involving both activating receptors and inhibitory killer IG-like receptors on the NK-cell surface [23]. These studies have led to an evolving literature describing the functional reconstitution of NK cells and mechanisms to manipulate this reconstitution. NK cells are the first lymphocyte population from the donor graft to recover post-HSCT, with recovery to baseline numbers in peripheral blood by 1 month regardless of whether unmanipulated PBSCT, BMT, or UCB grafts are used [24, 25]. In the setting of T-cell-depleted unrelated donor or haploidentical related donor grafts, where GVL effects of NK cells are most important, recovery of NK cells is highly dependent on methods of T-cell depletion and on the presence or absence of post-HSCT immune suppression. Specifically, the use of positive selection for $CD34^+$ cells is associated with much slower reconstitution of functional NK-cell activity when compared to T-cell-depleted grafts obtained via either direct depletion of $CD3^+$ or $TCR\alpha\beta^+$ T cells [26, 27].

The initial NK cells that recover post-HSCT are functionally immature. Full restoration of normal immunophenotypic NK-cell populations takes between 3 and 6 months post-HSCT, and T-cell depletion of donor grafts may negatively affect the speed with which NK cells become functionally mature. Interestingly, CMV infection post-HSCT may enhance functionality and cytotoxicity of recovering NK cells [28], which may provide an explanation for the prior observation that CMV reactivation correlates with lower risk of relapse in patients receiving HSCT for acute myeloid leukemia [29].

Monocytes

Donor-derived monocytes are one of the earliest cell populations to be detected in peripheral blood following HSCT, often preceding the return of neutrophils by 1–2 days. While quantitative data defining the typical kinetics of monocyte recovery are somewhat lacking [2], adequate recovery of monocytes by Day +100, defined as an absolute monocyte count of >300 cells/μL in peripheral blood, correlates with improved overall survival in adult patients with hematologic malignancy following both myeloablative and reduced intensity conditioning [30, 31]. Adequate monocyte recovery may be critical in facilitating normal immune function post-HSCT in several ways. Directly, monocytes give rise to tissue macrophages that phagocytose pathogens and orchestrate immune responses through coordinated release of pro- and anti-inflammatory cytokines. Indirectly, monocytes give rise to specialized components of the HSC niche in bone marrow that play critical roles in maintenance and differentiation of hematopoietic stem and progenitor cells at baseline [32] and may play similar roles post-HSCT in graft function and lymphopoiesis [33]. One negative aspect of monocyte reconstitution pertaining to immune regulation is that recovery and proliferation of certain subsets of $CD16^+$ monocytes have been linked to the development of acute graft-versus-host disease (GvHD) and normalize with successful GvHD-directed therapy [34].

Reconstitution of the Adaptive Immune System

T Cells

Reconstitution of functional T-cell subsets is one of the most critical determinants of HSCT success, whether through promotion of GVM effects, resolution of T-cell deficiency in combined immune deficiency disorders or prevention of graft rejection or post-HSCT opportunistic viral and fungal infections. T-cell recovery post-HSCT occurs either by (1) peripheral expansion of infused mature donor T cells or (2) de novo thymic production of T cells from donor hematopoietic precursors. In the latter process, bone marrow-derived donor lymphoid precursors, home to the recipient thymus, proliferate and undergo "education" through positive and negative selection, leading to development of functional $CD4^+$ and $CD8^+$ T cells. This de novo production process is particularly important for $CD4^+$ T-cell recovery post-HSCT [8, 35–37]. While de novo T-cell reconstitution is ultimately required for a diverse functional T-cell repertoire post-HSCT, disruption of thymic architecture and function caused by HSCT conditioning regimens results in minimal thymic output of de novo T cells for at least 6 months post-HSCT, as measured by the technique of T-cell receptor excision circles (TRECs) [38]. Therefore, in the first few months post-HSCT, proliferative expansion of infused T cells becomes the dominant mechanism of T-cell recovery, anti-pathogen immunity, and GVM effects. Strategies incorporating ex vivo or in vivo T-cell depletion of donor grafts thus have profound impacts on immunity in the first 6 months, though as discussed in specific sections below, these strategies may also impact longer-term thymic output [2].

In conventional HSCT, using related or adult unrelated donors without ex vivo or in vivo (serotherapy) T-cell depletion, the initial T-cell populations to recover include effector cytotoxic $CD8^+$ T cells [39], which may be primarily responsible for GvHD and GVM effects early after HSCT. $CD8^+$ T cells normalize faster (2–8 months) than helper $CD4^+$ T-cell populations, which should reach >200 cells/μL by 6 months and often do not reach normal levels until >1 year post-HSCT. Anti-inflammatory regulatory CD4+CD25+FoxP3+ T cells (Treg), which may be critical in limiting pathogenic allogeneic GvHD responses, recover somewhat early in the first few weeks post-HSCT [2], and lower initial Treg recovery correlates with higher rates of GvHD. Interestingly, recovery of thymic production of Treg may take longer than 2 years post-HSCT [39], which may explain the long duration of chronic GvHD symptoms and subsequent resolution that some HSCT patients experience.

Overall, gradual $CD4^+$ T-cell recovery over time, regardless of other HSCT factors, is an independent predictor of survival. However, early rise and subsequent declines in CD4+ T cells within the first 90 days post-HSCT, likely reflecting GvHD or infection, worsen mortality risk [2, 15]. Naïve T cells recover earlier (8–12 months) than memory T cells (approximately 24 months). Importantly, quantitative recovery of T-cell numbers precedes the return of fully functional T cells as measured by mitogen response testing, which is performed by culturing peripheral blood mononuclear cells (PBMCs) in the presence of phytohemagglutinin (PHA) or pokeweed mitogen (PWM) and assessing for the ability of T cells in the PBMC pool to undergo proliferation. In the absence of GvHD therapy or T-cell depletion, mitogen testing results typically return to normal within the first year post-HSCT [40]. T-cell proliferative responses to recall antigens, including candida and tetanus toxoid, which can also be performed through lymphocyte culture testing, may take considerably longer to normalize.

The above represents the best-case scenario for T-cell recovery post-HSCT. In addition to GvHD, which globally slows the pace of diverse T-cell immune reconstitution due to intensive systemic immune suppression, several additional scenarios may impact the speed of T-cell immune reconstitution (Table 26.2).

Serotherapy: Use of anti-thymocyte globulin (ATG) as an in vivo T-cell depletion approach to prevent graft rejection/GvHD following HSCT

Table 26.2 Factors leading to delayed functional T-cell reconstitution following HSCT

• Serotherapy (alemtuzumab, ATG)
• Ex vivo T-cell depletion
• Acute/chronic GvHD and its treatment
• Systemic immune suppression for other reasons (e.g., autoimmune cytopenias)

has many potential benefits, but it may also considerably slow the rate of immune reconstitution following HSCT. While recovery of innate immunity is unaffected and $CD8^+$ T-cell and B-cell recovery is also only modestly impaired, ATG seems to significantly impair $CD4^+$ T-cell recovery, with many patients not achieving a $CD4^+$ T-cell count >200 μL^{-1} until nearly 1 year post-HSCT [41]. A recent study demonstrates that the risk of delayed $CD4^+$ T-cell reconstitution correlates directly with the area under the curve (AUC) for ATG exposure, with the timing and dosing of ATG within the conditioning regimen being major determinants of AUC [42]. Alemtuzumab, an alternative agent used as serotherapy for the purpose of in vivo T-cell depletion, causes an even greater delay than does ATG in terms of both numeric and functional T-cell reconstitution and delays B- and NK-cell reconstitution as well. Both the timing and dose of alemtuzumab correlate with severity of this delay [43–45].

Ex vivo T-cell depletion: Evolving strategies for ex vivo T-cell depletion over the past 15 years have greatly improved outcomes following mismatched unrelated (MMUD) and haploidentical-related (Haplo) HSCT. Early approaches utilized the concept of positive immunomagnetic selection of $CD34^+$ HSC cells, thereby severely reducing all immune cells present in the infused graft to as few as 2–3 × 10^4 cells/kg [46]. High doses of $CD34^+$ HSC cells selected in this manner resulted in excellent initial engraftment but virtually eliminated adaptive immune reconstitution from the proliferative expansion mechanism discussed above. With this method, full B- and T-cell recovery after haplo-HSCT may take years, often with no detectable T-cell recovery in the initial 5–6 months post-HSCT [47]. A more recent approach to haplo-HSCT, utilizing direct depletion of $CD3^+$ T and $CD19^+$ B cells (the latter depleted to prevent EBV-post-transplant lymphoproliferative disease), enables retention of lymphoid progenitors and NK cells in the infused graft that results in improved immune reconstitution. With this approach, NK-cell recovery (1000 cells/μL) occurs rapidly within the first month. CD3+ T-cell recovery also occurs within 3 months with full T-cell reconstitution (>1000 CD3+ cells/μL) occurring at about 12 months [48, 49]. Building on this concept further, several groups have recently published results from promising strategies in which only GvHD-causing TCRαβ + T cells are depleted from donor grafts along with B cells, allowing for rapid proliferative expansion of infused donor $TCR\gamma\delta^+$ T cells, which may help prevent leukemia relapse, immunologic rejection, and opportunistic viral infections [27, 50].

Cord blood transplantation: While stem cell doses in CBT are lower than with BMT or PBSCT, overall T-cell number UCB units are similar to other graft sources. However, T cells derived from UCB are immunologically distinct, with predominance of a naïve phenotype, increased regulatory T cells, and reduced allogeneic reactivity [51]. In contrast to the delayed neutrophil recovery following UCBT, T-cell recovery can occur quite early after UCBT [52] at a similar rate to BMT [13] and more rapidly than T-cell-depleted PBSCT [16, 53]. Complete T-cell reconstitution in the absence of serotherapy with ATG or alemtuzumab takes about 7–12 months for both UCBT and BMT [52]. Inclusion of ATG in the conditioning regimen is particularly detrimental for T-cell reconstitution in the UCB setting because cells with a naïve phenotype that are most susceptible ATG comprise the majority of T cells in UCB grafts [2, 54–56].

Severe combined immunodeficiency (SCID): While approaches to HSCT for patients with SCID vary dramatically from unconditioned matched sibling donor HSCT to fully T-cell-depleted haploidentical or mismatched unrelated donor (MMUD) HSCT, T-cell recovery begins rapidly in patients with SCID, regardless of approach. Analyses have demonstrated that

thymic production of TREC+ T cells occurs as early as 3–6 months post-HSCT [57], much earlier than in many other HSCT scenarios. However, T-cell reconstitution is not always complete, with as many as 50% of patients having long-term CD4+ T-cell counts <500 cells/μL. Patients with SCID who receive conditioning and/or grafts from MSD without T-cell depletion are more likely to achieve normal T-cell counts in the long term [58].

Dendritic Cells

Dendritic cells (DCs) are critical antigen-presenting cells involved in initiating adaptive immune responses. DCs present processed peptide antigens to T cells in the setting of either pro- or anti-inflammatory cytokine, thus determining whether antigen-specific T cells become anergic or regulatory or exert cytotoxic responses. Thus, DCs play critical roles in allogeneic recovery of immune function post-HSCT [2]. DC content is much higher in mobilized PBSCT grafts versus BM grafts and correlates closely with CD34+ HSC dose [59]. However, factors influencing early DC recovery post-HSCT are not well understood. Initial DC reconstitution is rapid, correlating with myeloid engraftment, then declines significantly, taking years to return to normal levels [60]. Particularly low numbers of monocytoid and plasmacytoid DC levels correlate with increased risk of severe aGvHD development. The roles that DC reconstitution plays in full reconstitution of immunity post-HSCT and in acute and chronic GvHD are just beginning to be understood [61].

B Cells

B cells are nearly undetectable in the first 2 months post allo-HSCT [9, 41, 62], though the few that are present are typically of donor origin [63]. Most of the data regarding B-cell reconstitution in pediatric HSCT literature reports on CD19+ B cells alone and defines recovery as a CD19+ cell count greater than 200 cells/μL. However, numeric B-cell recovery is quite distinct from full recovery of humoral immunity. Initial B-cell recovery is mostly composed of a repertoire of naïve B cells and very few memory B cells, in line with normal B-cell development in immune ontogeny [64]. Recovery of IgM production occurs first, followed by recovery of IgG subclass production in a highly variable time course occurring anywhere from 3 to 12 months post-HSCT. Full B-cell reconstitution includes the ability to undergo class switching and produce functional antibody responses to vaccines and, in the absence of extenuating factors, typically takes about 1–2 years following HSCT. Recovery of the ability to generate responses to polysaccharide antigens takes even longer [9]. Memory B-cell reconstitution can take as long as 2 years post-HSCT. In a cohort of 13 primary immunodeficiency patients, Scarselli et al. found that switched memory B cells (CD19+CD27+IgD−IgM−) recover earlier and better than IgM memory B cells (CD19+CD27+IgD+IgM+). They also found that recovery of memory B cells correlated with good in vivo humoral function [65].

Slow and/or low B-cell reconstitution increases the risk of infections and major complications significantly [41, 66, 67]. B-cell recovery can be delayed by a number of factors (Table 26.3), including most commonly GvHD and its treatment [68, 69]. The use of rituximab for EBV prevention/treatment or for chronic GvHD [70, 71] significantly impairs both numeric B-cell recovery and maturation of humoral immunity post-HSCT. As an alternative to rituximab, ex vivo CD19+ B-cell depletion is often performed for patients receiving ex vivo T-cell depletion to prevent severe EBV

Table 26.3 Factors leading to delayed functional B-cell reconstitution following HSCT

• Serotherapy (alemtuzumab, ATG)
• Ex vivo CD19+ B-cell depletion
• Rituximab therapy/prophylaxis for EBV or autoimmune cytopenias
• Acute/chronic GvHD and treatment with immune suppression
• Specific HSCT indications (e.g., severe combined immune deficiency)

reactivation, though the impact of how this CD19+ B-cell depletion impacts the pace and extent of B cell reconstitution is not well studied.

Conditioning regimens that include ATG are also associated with some degree of impaired B-cell reconstitution, reflecting the need for adequate T-cell help in regenerating functional B cells [41]. Alemtuzumab use can delay B-cell reconstitution, either through direct B-cell depletion or through T-cell-related effects, although the extent and consistency of this effect is not clear [72, 73]. Interestingly, B-cell reconstitution, in the absence of ATG conditioning, may be faster after UCBT (2–6 months) compared to other stem cell sources (4–12 months for PBSCT; greater than 6 months for BMT), likely reflecting the fact that UCB B-cell progenitors exhibit the same rapid B-cell development that is seen in infants [13, 16, 74, 75].

B-cell reconstitution following HSCT for SCID is particularly well studied and quite distinct from reconstitution following HSCT for other indications [76]. In general, patients with SCID who receive HLA-identical related donor grafts do not require conditioning regimens to develop fully functional B-cell reconstitution of either donor or recipient origin. However, patients with SCID who lack such donors are at high risk of requiring long-term Ig replacement therapy due to delayed or permanent B-cell dysfunction. In this setting, risk factors for poor B-cell reconstitution include SCID genotype, lack of intensive conditioning, and poor B-cell and total donor chimerism following HSCT [77].

Assessments of Immune Reconstitution and Revaccination

An example of a schedule for immune reconstitution assessments is given in Table 26.4. Schedules for assessing functional immune reconstitution

Table 26.4 Schedule of immune reconstitution assessments post-HSCT

Time from HSCT (months)	1	4	8	12	18[a]	24
Abs lymphocyte count	×	×	×	×	×	×
Flow cytometry (absolute #'s)						
$CD3^+$	×	×	×	×	×	×
$CD4^+$		×	×	×	×	×
$CD4^+/CD45RA^+$		×	×	×	×	×
$CD4^+/CD45RO^+$		×	×	×	×	×
$CD8^+$		×	×	×	×	×
$CD16^+/CD56^+$	x	×	×	×	×	×
$CD19^+$	x	×	×	×	×	×
$CD19^+/IgD^-/CD27^-$		×	×	×	×	×
T-cell mitogen testing (PHA/tetanus/Candida)				×	×	×
TRECs		×	×	×		×
Immune globulins (IgG, IgA, IgM)		×	×	×	×	×
Vaccine response						
Anti-tetanus				× (pre)[b]		× (post)[b]
Anti-pneumococcal				× (pre)[b]		× (post)[b]

[a]Eighteen months post-HSCT assessments only required if abnormal at 12 months
[b]Denotes pre- and post- re-immunization with tetanus and pneumococcal vaccines. Lymphocyte subsets are as follows: $CD3^+$, all mature T cells; $CD4^+$, helper T cells; $CD4^+/CD45RA^+$, naïve T cells; $CD4^+CD45RO^+$, memory T cells; $CD8^+$, cytotoxic effector T cells; $CD16^+/CD56^+$, NK cells; $CD19^+$, B cells; $CD19^+IgD^-CD27^-$, Ig class-switched B cells. *TRECs* T-cell receptor excision circles. *PHA* phytohemagglutinin

vary considerably among HSCT centers, but several common principles are consistent across centers. Few centers test directly for reconstitution of innate immunity outside of basic CBC monitoring, though clinical testing for quantitative NK-cell recovery is available and is often part of screening for reconstitution of T-cell immunity. Initial assessments for T-cell reconstitution typically involve monitoring absolute lymphocyte and $CD3^+$ T-cell recovery. Once CD3+ T-cell counts recover to at least the 200–500 cells/μL range, additional assessments for numeric T-cell recovery, including $CD4^+$, $CD8^+$, and naïve (CD45RA) and memory (CD45RO) populations, are also performed, as are assessments of de novo T-cell production via TREC assays. The decision to stop prophylaxis for *Pneumocystis jirovecii* and for herpes simplex virus based on T-cell reconstitution also varies by institution but at a minimum generally requires the patient to be off systemic immune suppression with a $CD3^+$ T-cell count >500 μL^{-1} and a CD4+ T-cell count >200 μL^{-1}. Prior to revaccination, T-cell functional capability is usually assessed through mitogen stimulation assays, such as proliferation in response to phytohemagglutinin, as discussed above.

Table 26.5 Recommended revaccination schedule following HSCT

	Time post-transplant (months)[a]						
Vaccine	6	12	14	16	18	24	Booster needed?
Influenza (inactivated)	×						
Diphtheria, tetanus, pertussis		×	×	×			4 years after 3rd dose; • DTaP for patients 7 years and younger; • Tdap for patients >7 years
Haemophilus influenzae type b		×	×			×	
Hepatitis B		×	×	×			
Inactivated polio vaccine		×	×			×	Only if <4 years old for all three doses
Pneumococcal Conjugate 13 Valent		×	×	×			
Hepatitis A		×			×		
Meningococcal Conjugated		×			×		Cannot be given at the same time as PVC13, so administer 1 month after PVC13
Measles, mumps, rubella						×	4 weeks after 1st dose
Varicella						×	3 months after 1st dose
Human papillomavirus						×	Repeat at 2 and 6 months after 1st dose; administer for ages 9–26 years

[a]Inactivated influenza vaccination may be given regardless of immune status. For remaining vaccines, patients receiving autologous HSCT may initiate schedule at 12 months post-HSCT. Patients receiving allogeneic HSCT must meet immune criteria ($CD20^+$ B cells >50 μL^{-1}; $CD3^+CD4^+$ T Cells >200 μL^{-1}, normal T-cell response to mitogen) to begin schedule

Assessment of B-cell recovery typically begins with measuring CD19$^+$ B-cell counts. Assessments of immune globulins (IgG, IgM, and IgA) are performed to determine whether patients will require long-term Ig replacement. Assessment of B-cell class-switching capability requires flow cytometry-based analysis of B-cell subsets, including measurements of memory IgM$^+$CD27$^-$IgD$^-$ memory B cells.

Once B-cell reconstitution has been assessed, we recommend checking pre- and post-vaccine titers prior to and after initiation of re-immunizations. Re-immunization schedules also vary according to institutional practice, but consensus exists for a few general guidelines (see Table 26.5 for example schedule). Most vaccinations should not be given prior to 12 months post-HSCT, with the exception of the inactivated influenza vaccine, which may be administered as early as 100 days post-HSCT. Live virus vaccines are generally delayed until 2 years post-HSCT or for up to a year following initiation of inactivated vaccines. Finally, initiation of vaccines post-HSCT should not start until patients have been off chronic, systemic immune suppression for GvHD for a period of at least several months.

Key Points

- Achievement of efficient and complete immune reconstitution following HSCT is a critical determinant of HSCT success, enabling prevention of malignancy relapse, graft rejection, infection, and many other causes of HSCT-associated morbidity.
- Recovery of innate immunity occurs rapidly following HSCT in line with initial engraftment, though recovery, specifically of NK-cell-based immunity, may be delayed through the use of ex vivo positive CD34$^+$ cell-selection strategies and alemtuzumab administration.
- Standard kinetics of T-cell reconstitution post-HSCT are well described, though T-cell recovery may be significantly delayed due to serotherapy in the conditioning regimen, ex vivo T-cell depletion, or use of chronic immune suppression due to GvHD or other immune dysregulation complications of HSCT. Adequate T-cell recovery should be documented prior to discontinuation of antimicrobial prophylaxis post-HSCT.
- Full reconstitution of B-cell subsets and Ig production may take 1–2 years post-HSCT and need to be assessed prior to initiation of revaccination schedules.

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27 Life After HSCT: Survivorship and Long-Term Issues

Smita Dandekar

Abstract

Hematopoietic stem cell transplant (HSCT) is a curative therapeutic option for not just hematologic malignancies but also many nonmalignant conditions such as beta thalassemia, sickle cell disease, metabolic disorders, and certain primary immunodeficiencies. Continued advances in the HSCT techniques, expansion of indications for HSCT, and tremendous progress in supportive care strategies and management of HSCT-related complications have collectively resulted in an expanding population of survivors of HSCT. Progress and expansion of alternative donor hematopoietic stem cell (HSC) sources (e.g., umbilical cord blood, haploidentical donors) are resulting in a continually increasing amount of HSCTs performed in children per year. These survivors are at risk of developing treatment-related late effects, and two-thirds of the HSCT survivors will develop at least one chronic health condition. The direct impact of these long-term effects on the morbidity and mortality of the HSCT survivors makes risk-based and exposure-related screening for these late effects a critical part of their care. This chapter reviews the most common secondary neoplasms seen in survivors of HSCT during childhood and adolescence. This chapter will also discuss the HSCT-associated long-term complications affecting the cardiac, pulmonary, endocrine, musculoskeletal, renal, and neurocognitive systems. Screening recommendations are also included.

Introduction

Hematopoietic stem cell transplant (HSCT) is a curative therapeutic option for patients not only with malignancies but also with many nonmalignant conditions such as beta thalassemia, sickle cell disease, metabolic disorders, and many pri-

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mary immunodeficiencies. Continued advances in the use of alternative donors, manipulation of the hematopoietic stem cell product, expansion of indications for HSCT, and tremendous progress in supportive care strategies and management of HSCT-related complications have collectively resulted in an expanding population of survivors of HSCT. Progress and expansion of alternative donor hematopoietic stem cell (HSC) sources (e.g., umbilical cord blood, haploidentical donors) are resulting in a continually increasing number of HSCTs performed in children and adolescents each year. These survivors are at risk of developing treatment-related late effects; two-thirds of the HSCT survivors will develop at least one chronic health condition.

Survivors of childhood and adolescent HSCT carry a significantly greater burden of morbidity not only compared with the non-cancer populations but also compared with the conventionally treated cancer patients, proving the need for close monitoring of this high-risk population [1]. The type of HSCT (i.e., matched sibling donor versus matched unrelated donor versus umbilical cord blood), HSC source (i.e., bone marrow, umbilical cord blood, and peripheral blood stem cells), and the conditioning regimen used determine the type and severity of long-term complications in the HSCT patients. 70–80% of those who survive at least 2 years after an allogeneic HSCT are expected to become long-term survivors [2–4].

The Bone Marrow Transplant Survivor Study (BMT-SS) which is one of the most comprehensive and largest studies of HSCT survivors to date, studied 1479 patients who were alive at least 2 years post their allogeneic HSCT and found that allogeneic HSCT patients had a 9.9-fold increased risk of early death [3]. Though relapse of primary disease and chronic graft versus host disease (GvHD) remained the leading cause of premature death, treatment-related causes such as GvHD secondary malignancies, cardiac toxicity, and pulmonary complications attributed to 25% of the deaths.

A multi-institutional study comparing long-term health outcomes in survivors of childhood cancer treated with HSCT to survivors of childhood cancer treated with conventional therapy showed that survivors of HSCT were more likely to have a severe or life-threatening condition (Relative Risk [RR] = 3.9) [1]. They were more likely to have multiple chronic conditions (RR = 2.6) and more likely to have functional impairment (RR = 3.5) and activity limitations (RR = 5.8) than the conventionally treated patients. HSCT survivors were drawn from the BMT-SS study and conventionally treated patients were drawn from the Childhood Cancer Survivor Study (CCSS). The results of this study highlight the need for timely screening for these health-related complications and appropriate interventions.

While screening guidelines have been developed by the Center for International Blood and Marrow Transplant Research (CIBMTR), European Group for Blood and Marrow Transplantation (EBMT), American Society for Blood and Marrow Transplantation (ASBMT) [5–7], and other societies, these are not pediatric focused. In 2011, the NCI and NHLBI held the first international consensus conference on late effects after pediatric HSCT and recommended a coordinated effort through the Pediatric Blood and Marrow Transplant Consortium (PBMTC), the Children's Oncology Group (COG)-SCT committee, the Children's Cancer and Leukemia group (CCLG) BMT group, the EBMT Pediatric Diseases Working Group, and other pediatric-oriented HSCT-specific groups, to work alongside larger pediatric cancer late effects groups such as the COG Late Effects Task Force to formulate formal guidelines.

Secondary Malignancies After HSCT

One of the most devastating complications after HSCT is the development of a secondary malignancy. Many host and clinical factors are associated with the increased risk of secondary malignant neoplasms after HSCT. These risk factors are summarized in Table 27.1.

The risk of secondary malignancy for children undergoing HSCT is increased not only compared to age-matched controls but also

compared to patients undergoing HSCT at older ages [8]. Table 27.2 lists the most common types of secondary malignancies seen in post-HSCT recipients. Curtis, et al. showed that cancer survivors who were transplanted at less than 10 years of age had a risk of new malignant neoplasms 36.6 times higher than the general population [9]. This risk decreased to 4.6-fold for those transplanted between the ages of 10 and 29 years. Table 27.3 summarizes the causative agents and screening recommendations for secondary malignant neoplasms. These secondary malignant neoplasms are often due to exposure to alkylators, topoisomerase II inhibitors, and TBI.

Table 27.1 Risk factors for secondary malignancies after HSCT

• Age at HSCT
• Type of HSCT (autologous vs allogeneic)
• Pre-HSCT therapy (chemotherapy + radiation)
• Total body irradiation (TBI) as part of the conditioning regimen
• Immunosuppression after HSCT (duration)
• Infections (Epstein–Barr virus (EBV), hepatitis B, hepatitis C)
• Type of original cancer

Table 27.2 Most common types of secondary malignant neoplasms in HSCT patients

• Treatment-related myelodysplasia (t-MDS) and treatment-related acute myeloid leukemia (t-AML)
• Lymphoma
• Solid non-hematopoietic tumors
• Skin cancers
• Posttransplant lymphoproliferative disease (PTLD)
– Ranges from benign mononucleosis-like disease to fulminant lymphoma

Treatment-Related Myelodysplastic Syndrome/Acute Myelogenous Leukemia

Treatment-related myelodysplastic syndromes (t-MDS) and acute myeloid leukemia (t-AML) are major causes of non-relapse mortality of HSCT. These disorders have very poor prognoses with conventional antileukemia therapies with a median survival of 1 year or less [10]. This outcome is related to several factors including older age at HSCT, pre-HSCT therapy with alkylating agents, topoisomerase II inhibitors and radiation, HSC mobilization with etoposide, use of peripheral blood HSCs, TBI-containing conditioning regimens, the number of $CD34^+$ hematopoietic stem cells infused, and history of multiple HSCTs [11]. Patients presenting with t-MDS often progress to t-AML. Two types of t-MDS/t-AML (which are related to the therapeutic exposure) are generally identified: those due to alkylating agents/radiation and those due to topoisomerase II inhibitors. t-MDS/t-AML related to alkylating agents typically develops 4–7 years postexposure. Cytopenias are common

Table 27.3 Risk factors and screening recommendations for second malignant neoplasms (SMN)

Causative agents	SMN	Screening recommendations
• Etoposide • Teniposide • Anthracyclines • Alkylating drugs	• AML • AML/MDS	• CBC with differential yearly for 10 years after exposure
• Radiation therapy	• SMN in radiation field: – Skin, bone, soft tissues	• Annual history and physical exam
• Radiation therapy affecting thyroid (TBI)	• Thyroid cancer	• Yearly US
• Radiation therapy affecting breast (TBI)	• Breast cancer	• Physical exam until age 25, then mammography and breast MRI yearly starting 8 years after radiation or age 25 (whichever is later)

AML acute myeloid leukemia, *MDS* myelodysplastic syndrome, *SMN* second malignant neoplasm, *US* ultrasonography

and approximately 65% present with MDS. The remainder present with AML but have myelodysplastic features. Cytogenetic abnormalities associated with a poor prognosis in non-therapy-related MDS and AML, such as del (7/7q) or a complex karyotype, are common in t-MDS and t-AML [12]. AML secondary to topoisomerase II inhibitors often presents as overt AML. The latency is very short (6 months to 5 years), and there is usually no antecedent myelodysplastic phase and is associated with balanced translocations involving chromosome 11q23 or 21q22. These disorders are also described after autologous HSCT [13]. HSCT should be considered promptly after a diagnosis of t-AML/t-MDS is made. However, allogeneic HSCT for t-MDS/t-AML is associated with a high risk of treatment-related mortality.

Lymphoma

Posttransplant lymphoproliferative disorder (PTLD) represents a spectrum of Epstein–Barr virus-related (EBV) clinical diseases, ranging from a benign mononucleosis-like illness to a fulminant non-Hodgkin's lymphoma. In the setting of HSCT, PTLD is an often fatal complication with the highest chance of occurrence in the first 5 years after HSCT [14, 15] with 80% of the cases occurring within the first year of HSCT [15]. Risk factors for PTLD are summarized in Table 27.4. PTLD is significantly associated with T-cell depletion of the donor bone marrow, use of antithymocyte globulin (ATG), and unrelated or HLA-mismatched grafts. Older age at the time of HSCT, second HSCT, and GvHD (both acute and chronic) increase the risk of developing post-HSCT PTLD by several folds. T-cell depleted grafts decrease the risk of acute GvHD but increase the risk of PTLD. In the majority of cases, PTLD is associated with EBV infection of B cells, either as a consequence of reactivation of the virus post-HSCT or from primary EBV infection. Primary infection with EBV may be acquired from the donor graft or, less commonly, from environmental exposure. The majority of the PTLD cases are due to B-cell proliferation, with only 5% of cases being of T-cell or T/NK cell origin. A vast majority of the B-cell PTLD cases (approximately 70%) are EBV-related. It is believed that EBV most likely predisposes infected B cells to uncontrolled proliferation which may result in the accumulation of (epi) genetic aberrations. Also, T-cell dysfunction caused by immunosuppressive treatment further allows uncontrolled proliferation of the EBV-infected B cells. The 2008 World Health Organization (WHO) classification divides PTLD into four categories based on morphologic, immunophenotypic, and molecular criteria: [1] early lesions, [2] polymorphic PTLD, [3] monomorphic PTLD, and [4] Hodgkin lymphoma.

Table 27.4 Risk factors for PTLD

• T-cell depletion of donor bone marrow
• Antithymocyte globulin
• Unrelated/HLA mismatched grafts
• Age at time of HSCT
• ≥Second HSCT
• GvHD

Reduction of immunosuppression remains the cornerstone for treatment of EBV-driven B-cell PTLD. It allows the patient's natural immunity to recover and gain control over proliferating EBV-infected B cells. Additional therapeutic measures include immune-based therapies such as monoclonal antibodies like rituximab (anti-CD20 monoclonal antibody), EBV-specific donor T cells, IVIG, and alpha interferon.

Lymphomas distinct from PTLD, such as Hodgkin lymphoma, can occur late post-HSCT (usually >2.5 years) and are associated with moderate and severe chronic GvHD. These late-onset lymphomas do not have an association with the risk factors typically associated with PTLD [16].

Non-hematologically Derived Tumors

A study investigating the incidence of secondary malignancies in a cohort of 3182 children who underwent allogeneic HSCT for leukemia revealed the cumulative risk of invasive solid tumors to be 0.9%, 4.3%, and 11% at 5 years, 10 years, and 15 years post-HSCT, respectively [14]. The risk was highest among children transplanted under the

age of 5 years and those who received high-dose TBI with a significantly increased risk for developing tumors of the tongue, salivary glands, brain, thyroid, and skin/connective tissue. In patients exposed to radiation at less than 30 years of age, the risk of developing a non-squamous cell carcinoma is ninefold higher than that of the general population, while for those older than 30 years of age when exposed, the risk approaches that of the general population [17].

Skin cancer: Allogeneic HSCT recipients have an increased risk of developing basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Leisenring et al. demonstrated that the incidence of BCC and SCC is approximately 6.5% and 3.4% at 20 years post-HSCT, respectively [18]. Exposure to TBI increases the risk of BCC, particularly in younger children. GvHD is correlated with the development of secondary skin cancers. The risk of SCC is increased in patients with acute GvHD, while chronic GvHD is correlated with both BCC and SCC.

Breast cancer: Female survivors of HSCT are at increased risk of developing breast cancer with a 25-year cumulative incidence of 11% [19]. The risk is higher among those who received TBI (17%) compared to those who did not (3%). The increased risk is directly related to the patient's age at the time of HSCT (hazard ratio [HR] = 9.5 for HCT < 18 years), exposure to TBI, and time since HSCT with the median time to development of breast cancer being 12.5 years.

Thyroid cancer: Cohen et al. showed that HSCT patients have a 3.3-fold increased risk of developing thyroid cancer as compared to age- and sex-matched controls from the general population [20]. Young age at HSCT (<10 years) confers the strongest risk with neck irradiation, female gender, and chronic GvHD being other risk factors.

System-Based Health Complications

Cardiac Dysfunction

HSCT survivors are at risk for long-term cardiotoxicity due to a combination of factors. These include pre-HSCT therapeutic exposure (especially anthracycline dosage greater than 300 mg/m^2 [21, 22]), HSCT conditioning regimens with high-dose chemotherapy and TBI, and post-HSCT GvHD. It presents as either a structural (valvular abnormalities, coronary artery disease) or functional (e.g., cardiomyopathy, arrhythmias, congestive heart failure) problem.

A prospective study evaluating the outcome of cardiac late effects in 162 children who underwent an allogeneic HSCT showed that the 5-year cumulative incidence of cardiac impairment was 26%. TBI alone and TBI with pre-HSCT anthracycline exposure were significant risk factors for decreased cardiac function [23]. The cumulative risk of cardiac late effects increases over time. Female gender, exposure to anthracyclines at a young age, and mediastinal radiation with exposure to the heart are well-established modifying factors for cardiac health among survivors of childhood cancer [23]. TBI and prolonged immunosuppressive therapy post-HSCT are HSCT-specific unique risk factors that contribute to diabetes and hypertension in this population further modifying the risk of cardiac late effects.

Another serious long-term complication is the development of therapy-related cardiovascular disease (CVD). This includes cerebrovascular disease (stroke, transient ischemic attack, carotid artery occlusion) and coronary artery disease both of which have an increased incidence and early occurrence rate among survivors of HSCT. At 25 years after HSCT, the cumulative incidence of CVD approaches 23% in certain high-risk populations. Endothelial injury provoked by GvHD is thought to contribute to the atherosclerotic changes after HSCT that lead to premature cardiovascular events. Girls with estrogen deficiency resulting from gonadotoxic therapy used in HSCT lose the normally protective effects of estrogen against coronary artery disease. Hence, prompt hormonal replacement for gonadal dysfunction in the girls and women who underwent HSCT at a young age is important for heart health. A study of long-term HSCT survivors who had survived for one year or more after a HSCT identified the presence of two or more of the following risk factors: obesity, dyslipidemia, hypertension,

and diabetes to be associated with 4.6-fold risk of late CVD ($p < 0.01$). Chest radiation prior to HSCT was associated with a 9.3-fold risk of coronary artery disease [24].

Compared to the general population, HSCT survivors are at a 2.3- to 4.0-fold increased risk of death due to cardiac reasons [3], emphasizing the need for lifelong monitoring for cardiac late effects in this patient population. The Children's Oncology Group (COG) long-term follow-up guidelines give specific recommendations for echocardiographic screening of these individuals ranging from annual to every 5 years based on their total cumulative anthracycline exposure, age at exposure, and exposure to mediastinal radiation (see Tables 27.5 and 27.6). Patients who have received chest radiation should be screened for early onset atherosclerosis. Pregnant women with past exposure to anthracyclines should be monitored very closely as the markedly increased blood volume during the pregnancy, especially during the third trimester, can add considerable stress to the heart that has already received cardiotoxic exposure. Survivors should be encouraged to participate in a healthy exercise program with aerobic activity and avoid isometric exercises that put strain on the heart. They should be counseled on ways to maintain heart health including dietary guidance and timely and appropriate screening for hypertension, dyslipidemia, and diabetes.

Table 27.5 Children's Oncology Group (COG) long-term follow-up guidelines for frequency of cardiac monitoring

Recommended frequency of echocardiogram (or comparable cardiac imaging)			
Age at treatment[a]	Radiation with potential impact to the heart	Anthracycline dose[b]	Recommended frequency
<1 year old	Yes	Any	Every year
	No	< 200 mg/m^2	Every 2 years
		≥200 mg/m^2	Every year
1–4 years old	Yes	Any	Every year
	No	<100 mg/m^2	Every 5 years
		≥100 to <300 mg/m^2	Every 2 years
		≥300 mg/m^2	Every year
≥5 years old	Yes	<300 mg/m^2	Every 2 years
		≥300 mg/m^2	Every year
	No	<200 mg/m^2	Every 5 years
		≥200 to <300 mg/m^2	Every 2 years
		≥300 mg/m^2	Every year
Any age with decrease in serial function			Every year

[a]Age at time of first cardiotoxic therapy (anthracycline or radiation, whichever was given first)
[b]Based on doxorubicin isotoxic equivalent dose

Table 27.6 Recommended frequency of echocardiogram

Age at treatment[a]	Radiation dose	Anthracycline dose[b]	Recommended frequency
<5 years	Any	None	Every 2 years
		Any	Every year
>5 years	<30 Gy	None	Every 5 years
	≥30 Gy	None	Every 2 years
	Any	<300 mg/m^2	Every 2 years
		≥300 mg/m^2	Every year
Any age with decrease in serial function			Every year

[a]Age at time of first cardiotoxic therapy (anthracycline or radiation, whichever was given first)
[b]Based on doxorubicin isotoxic equivalent dose

Pulmonary Complications

Approximately 35–45% of HSCT survivors have abnormal pulmonary function test results and suboptimal lung function; however, very few have clinical disease [23, 25]. Pulmonary complications post-HSCT can be divided into two broad categories: late-onset infectious pulmonary complications (LOIPCs) and late-onset noninfectious pulmonary complications (LONIPCs).

The infectious complications are influenced by the immune suppression following HSCT. These patients are at an increased risk for fungal, bacterial, and viral infections. These are discussed in depth in Chaps. 17 and 21.

The noninfectious pulmonary complications include bronchiolitis obliterans syndrome (BOS), bronchiolitis obliterans with organizing pneumonia (BOOP), and idiopathic pneumonia syndrome (IPS). These noninfectious complications, which are addressed in much more depth in Chap. 21, usually appear after approximately 100 days from HSCT and are related to chronic GvHD. In a retrospective review of pediatric patients who had survived more than 3 months from an allogeneic HSCT, Nishio et al. found that the incidence rate of LONIPCs to be about 10.3% [26]. That study identified high risk underlying disease and extensive chronic GvHD to be significant risk factors associated with the development of the LONIPCs. Another study identified chronic GvHD and compromised pulmonary function existing prior to HSCT to be independently associated with a late decline in lung function. Patients with chronic GvHD and low pre-HSCT diffusion capacity for carbon monoxide (DLco) or low forced expiratory volume (FEV_1) in the first second were more affected [27]. LONIPCs have been associated with a significantly worse mortality rate, especially after unrelated donor allogeneic HSCT.

Bronchiolitis obliterans syndrome: Bronchiolitis obliterans syndrome (BOS) presents as nonspecific inflammatory injury to the small airways and is strongly associated with chronic GvHD. Its incidence is approximately 8% in allogeneic HSCT recipients but is increased to approximately 13% in those with chronic GvHD. It typically occurs within the first 2 years post-HSCT with a median of 1.5 years. BOS initially presents as an obstructive disease and then gradually progresses to a restrictive disease due to peribronchiolar fibrosis. Other factors associated with an increased risk of BOS are the use of peripheral blood stem cells, busulfan-based conditioning regimen, 14 months or greater interval from diagnosis to transplant, sex match of female donor to male recipient, past history of interstitial pneumonitis, and an episode of grade 2 or higher acute GvHD [28]. High-resolution CT scan of the chest with imaging in inspiration and expiration typically demonstrates air trapping. However, PFTs needed to establish the diagnosis demonstrating an FEV_1 decreased >20% from baseline. Patients at risk for developing BOS should have PFTs performed every 3 months for the first 2 years post-HSCT because changes in PFTs will occur before changes on CT scan or the development of clinical symptoms, and early intervention, particularly before a patient becomes symptomatic, may blunt the progression of BOS. See Chap. 21 for further discussion of BOS.

Bronchiolitis obliterans organizing pneumonia: Bronchiolitis obliterans organizing pneumonia (BOOP) has an incidence of less than 2%. It usually occurs in the first year following a HSCT and presents as an interstitial pneumonia with sudden onset cough, shortness of breath, and fever. It has a restrictive pattern on pulmonary function tests, and chest x-ray shows ground glass attenuation with nodular opacities. See Chap. 21 for further discussion of BOOP.

Idiopathic pneumonia syndrome: Idiopathic pneumonia syndrome occurs in the first 4 months after HSCT. Conditions which predispose patients to developing IPS are TBI, pretransplant chemotherapy, GvHD, and increasing age at the time of HSCT [29]. See Chap. 21 for further discussion of idiopathic pneumonia syndrome.

Risk factors associated with restrictive lung disease (RLD) include the conditioning regimen, indication for HSCT, scleroderma/contracture, and donor relation (sibling, parent/relative, unrelated, autologous). Patients with single fraction TBI have the highest risk of RLD. Risk factors for obstructive lung disease (OLD) include

chronic GvHD, time after HSCT, and the conditioning regimen [25].

All patients who have undergone a HSCT and have had exposure to bleomycin or pulmonary radiation are recommended to have baseline screening pulmonary function tests on entry to long-term follow-up. They should also be counseled about risk of smoking.

Endocrine-Related Complications

Common endocrine-related long-term complications of HSCT include thyroid dysfunction (most commonly hyperthyroidism and secondary thyroid cancers), metabolic syndrome, impairment of growth and development, and pubertal delay or failure. Table 27.7 summarizes the frequency and recommended screening for some of these common endocrine-related long-term complications.

Thyroid dysfunction: Thyroid dysfunction is a common problem seen following a HSCT. It often presents as subclinical or overt hypothyroidism. Subclinical hypothyroidism is defined as elevated thyroid stimulating hormone (TSH) but normal T4 levels, while overt hypothyroidism is reflected by low T4 levels and elevated TSH. Hypothyroidism is directly related to radiation of the thyroid gland (as part of neck/mediastinal radiation or TBI) [30]. A study of 791 patients who were transplanted before the age of 18 years demonstrated that age < 10 years and the use of Busulfan or TBI for conditioning are the greatest risk factors for development of hypothyroidism post-HSCT (see Table 27.8). In this study, 30% of the patients developed hypothyroidism with 20% needing thyroid hormone replacement [31]. Although the latency period is variable, the majority of patients will develop hypothyroidism within the first 2 years post-HSCT. There is some thought that a subclinical GvHD-like phenomenon may play a role in the development of some cases of thyroid dysfunction, as it has been observed that children who receive an unrelated donor HSCT are more likely to develop hypothyroidism than those who receive matched sibling donor HSCT (36% vs 9%) [30]. Other thyroid disorders such as hyperthyroidism, thyroiditis, and benign thyroid nodules can occur in some patients but are uncommon.

In addition to thyroid dysfunction survivors of HSCT are also at an increased risk of developing thyroid cancers. Though rare in absolute number, thyroid cancer is one of the most common second neoplasms after HSCT along with malignant tumors of the brain [14]. However, its prevalence is less than 1% [14]. The majority of patients with thyroid tumors have a history of TBI [31].

It is recommended that HSCT survivors be screened annually for thyroid disorders by checking thyroid function tests (TSH and free T4) and a thyroid ultrasound if a thyroid nodule is palpated.

Metabolic syndrome: Metabolic syndrome is comprised of central obesity, insulin resistance, glucose intolerance, dyslipidemia, and hypertension. It is associated with an increased risk of developing type 2 diabetes mellitus and atherosclerotic cardiovascular disease. A study looking

Table 27.7 Screening for endocrine complications after HSCT

Endocrine complications		
Condition	Screening	Frequency
Hypothyroidism	TSH, Free T4 Thyroid Exam	Yearly Yearly
Impaired glucose metabolism/ diabetes mellitus	Fasting blood glucose or HbA1c	Every 2 years More frequently if clinically indicated
Dyslipidemia	Fasting lipid panel	Every 2 years More frequently if clinically indicated
Growth hormone deficiency	Height, weight, BMI Tanner Staging	Every 6 months till growth is completed and then yearly Every 6 months till sexually mature

Table 27.8 Risk factors for hypothyroidism after HSCT

- Age at time of HSCT (<10 years)
- TBI
- Busulfan conditioning

at the prevalence and risk factors for metabolic syndrome in young adult survivors of childhood leukemia treated both with and without HSCT showed that, among the HSCT recipients, the prevalence of metabolic syndrome was 5.9% among the patients who did not receive TBI and was 18.6% for those who did receive TBI [32]. Furthermore, HSCT with TBI was associated with a higher rate of hypertriglyceridemia, high-fasting glucose and a low level of high-density lipoprotein cholesterol, supporting that TBI is a major risk factor for the development of metabolic syndrome.

Direct damage to the vascular endothelium by both chemotherapy and radiation, insult to the hypothalamic-pituitary axis due to radiation with resultant deficiency of growth hormone and hypogonadism, and prolonged immune suppression post-HSCT all contribute to the development of metabolic syndrome. A study comparing the late effects for glucose and lipid metabolism in three patient populations (long-term survivors of HSCT who were 3–18 year post-HSCT for leukemia, a subset of leukemia patients who were in remission. And matched healthy controls) revealed that 39% of the HSCT survivors had core signs of metabolic syndrome as compared to 8% of the leukemia in remission controls and 0% of the healthy controls [33]. Fifty-two percentage of the HSCT patients had developed hyperinsulinemia, and 43% had abnormal glucose metabolism. Furthermore, this study showed that long-term survivors of HSCT are at a significantly increased risk of developing insulin resistance, glucose intolerance, and type 2 diabetes even at a normal weight and young age.

The Bone Marrow Transplant Survivor Study (BMT-SS) evaluated the prevalence of late occurrence of diabetes, hypertension, and cardiovascular disease in survivors of HSCT by self-report as compared to matched sibling controls. Survivors were required to be at least 2 year post-HSCT and off immune suppression. After adjusting for age, sex, race, and body mass index (BMI), survivors of allogeneic HSCT were 3.65 times more likely to report diabetes than their siblings and 2.06 times more likely to report hypertension [34]. Allogeneic HSCT survivors were also more likely to develop hypertension than autologous HSCT recipients. TBI exposure was associated with an increased risk of diabetes, supporting the notion that HSCT survivors have a higher age- and BMI-adjusted risk of diabetes and hypertension which could contribute to a higher than expected risk of cardiovascular events with age.

HSCT survivors should be screened periodically for cardiovascular risk factors, such as lipid abnormalities, and monitored for development of diabetes and hypertension.

Growth and development: Growth impairment is a frequent complication following HSCT. Insult to the hypothalamic-pituitary axis due to radiation (cranial radiation or TBI) is the primary cause with other modifying factors such as nutritional status, gonadal failure with impaired sex hormone production, hypothyroidism, prolonged exposure to corticosteroids for GvHD management, and genetic causes. TBI can have damaging effects on the epiphyseal growth plates causing direct impairment of growth. It can also impact growth secondarily by affecting growth hormone secretion or by causing gonadal failure leading to estrogen deficiency in girls or due to hypothyroidism. A study looking at 181 patients who underwent bone marrow transplantation for various hematologic disorders during childhood revealed that 80% of the patients attained an adult height within the normal range for a healthy population [35]. Irradiation, male gender, and younger age at the time of the bone marrow transplantation were directly related to long-term loss in height. Prior cranial radiation and single-dose, unfractionated TBI had the greatest negative effect on final height achievement. Fractionated TBI had significantly less effect on final adult height, and alternate conditioning with cyclophosphamide and busulfan had no effect on height loss.

The maximum benefit of growth hormone (GH) therapy has been demonstrated for patients transplanted before 10 years of age and with documented growth hormone deficiency [36]. Risk of relapse of the original cancer with growth hormone therapy has not been shown, but there is some suggestion that it may be linked with an increased risk of second malignancies [37].

Puberty and fertility: Gonadal failure, pubertal failure, and infertility are well-known late effects of HSCT and are related primarily to the high-dose alkylator therapy and radiation (TBI) used for HSCT conditioning.

Undergoing HSCT can result in pubertal delay or, rarely, complete failure due to disruption of the hypothalamic-pituitary-gonadal axis. Delayed or incomplete puberty occurs in approximately 57% of females and 53% of males [38]. High doses of radiation to the hypothalamus and pituitary cause impaired gonadotropin secretion and hypogonadism. Lower doses of radiation (<20 Gy), however, can lead to an earlier onset of puberty. Early puberty combined with impaired GH secretion can result in severe stunting of growth. Boys who receive >24 Gy testicular radiation have a very high risk of pubertal failure and often need testosterone replacement to develop secondary sexual characteristics. The risk of delayed puberty is related to the conditioning regimen used (see Table 27.9).

Pubertal development affects the self-esteem and the social integration of adolescents. Hence, it must be monitored appropriately with timely hormone replacement if pubertal signs are not occurring after 13 years of age in girls and after 15 years of age in boys.

Gonadal failure is related to the pubertal status at the time of HSCT. One of the first signs of impaired sex hormone production is delayed puberty in prepubertal patients, while the postpubertal patients may demonstrate incomplete pubertal development, primary or secondary amenorrhea, and infertility due to premature menopause or azoospermia.

Premature ovarian failure is observed in approximately 65–84% of females after HSCT [38]. Ovarian failure is considered partial when plasma estradiol level is normal and complete when the plasma estradiol is low. Ovarian failure impairs both fertility and estradiol production. Risk factors include pubertal development at the time of HSCT, busulfan-/cyclophosphamide-based conditioning regimens, and single-dose, unfractionated TBI. Prepubertal patients are more resistant to the gonadotoxic effects of cyclophosphamide and are likely to retain or recover ovarian function. Prepubescent females can tolerate as high as 25–30 g/m^2 of cyclophosphamide and retain ovarian function, while for women between 30 and 39 years of age, a dose of 9 mg/m^2 causes a similar effect [39]. Fertility is more likely to be preserved in patients who undergo HSCT at a young age and those who receive non-TBI-based conditioning regimens. Several cases have reported the resumption of ovarian function after initial ovarian failure following HSCT. HSCT survivors who do get pregnant have an increased risk of preterm delivery and delivery of low birth weight infants if they received TBI as part of conditioning due to the radiation-induced structural changes of the uterus.

Testicular failure is seen in 45–85% of males after HSCT [40]. Younger age offers protection for boys as well. Similar to females, the risk of gonadal failure is dependent upon the conditioning regimen and dose (cyclophosphamide and TBI are more toxic). The germinal epithelium of the testes is more vulnerable to chemotherapy and radiation than the Leydig cells. Spermatogenesis is also exquisitely sensitive to radiation and even 2–3 Gy can cause significant impairment in function. In a prospective study of 64 male patients undergoing HSCT with various conditioning regimens, the overall rate of azoospermia was about 70% [41]. Recovery of

Table 27.9 Risk of pubertal delay based on conditioning regimen

Conditioning regimen	Risk of delayed puberty	
	Males, %	Females, %
Cyclophosphamide alone (200 mg/kg)	14	16
Busulfan (16 mg/kg) + cyclophosphamide (120–200 mg/kg)	48	72
10 Gy single-exposure TBI	81	71
12-15.75 Gy TBI	58	57

spermatogenesis was directly related to the conditioning regimen. Among the patients who received cyclophosphamide alone, the recovery of spermatogenesis was seen in 90%, and those conditioned with cyclophosphamide plus busulfan or thiotepa, the recovery was seen in 50%. In contrast, for those who received cyclophosphamide plus TBI, spermatogenesis recovery was seen in just 17% of patients. The sperm quality and functional recovery time were better with cyclophosphamide alone as compared to other regimens. Thus, since the testosterone production is independent of spermatogenesis and even if fertility is impaired, the testosterone production may be normal.

Ovarian failure should be treated with hormone replacement therapy, and boys with Leydig cell failure should get testosterone replacement.

Pubertal stage should be assessed every 3–6 months until puberty is completed. The Children's Oncology Group (COG) long-term follow-up guidelines recommend baseline estradiol, FSH, and LH testing at age 13 years for girls and baseline testosterone, FSH and LH testing for boys at age 14 years, and then as clinically indicated. The high prevalence of infertility among HSCT survivors highlights the importance of discussion of options for fertility preservation with patients and families prior to HSCT. Embryo cryopreservation is the standard option for adult females with a committed partner, while oocyte cryopreservation and ovarian tissue cryopreservation are currently available experimental options for females without a partner. Sperm cryopreservation is the best option for adolescent and young adult males with cancer and/or plan to undergo HSCT.

Musculoskeletal-Related Complications and Bone Health Post-HSCT

Long-term survivors of HSCT are known to have musculoskeletal problems including decreased bone mineral density, avascular necrosis (AVN), and osteonecrosis. The major predisposing risk factors are radiation therapy (especially TBI), prolonged use of high-dose steroids, and low-estrogen secondary to therapy-related gonadal failure. Interplay of other modifying factors such as gender, age, physical activity status, nutritional status, race, family history, and intake of calcium and/or vitamin D plays an important role in overall bone health as well (see Table 27.10).

A prospective study involving children between 5 and 18 years of age who underwent HSCT showed that the incidence of osteopenia increased from 18% at baseline to 33% at 1 year post-HSCT. The most significant loss of bone density occurred in the first 6 months after HSCT. Bone-specific alkaline phosphatase decreased by 30% by day 100 post-HSCT and recovered to near baseline levels by 6 months, demonstrating that bone mineral density (BMD) can recover post-HSCT. Osteocalcin levels at day 100 post-HSCT predicted recovery of initial bone loss by 1-year post-HSCT [42]. Myeloablative therapy is known to affect the osteoprogenitor cells within the bone marrow and also cause a cytokine storm which stimulates bone resorption. As peak bone mineral accretion occurs in adolescence and young adulthood, children who were transplanted at a very young age should still be able to regain BMD.

Table 27.10 Risk factors for reduced bone mineral density (BMD) after HSCT

Patient related
• Age at transplant (younger age)
• Gender
• Family history
• Race (Caucasians)
• Lower weight and BMI
Therapy related
• Radiation therapy (TBI)
• Corticosteroids
• Cyclosporine
• Tacrolimus
• Gonadal failure
Health practices
• Nutrition (intake of calcium and vitamin D)
• Physical activity
• Smoking
• Alcohol use

AVN develops in approximately 4–10% of the HSCT survivors at a median of 12 months after allogeneic HSCT. It can cause significant morbidity and sometimes requires surgery including total joint replacement. A retrospective study of 1346 HSCT survivors revealed that the cumulative incidence of AVN was 2.9% at 10 years after an autologous HSCT, 5.4% after an allogeneic matched-related donor HSCT, and 15% after an unrelated donor HSCT. Among the allogeneic HSCT recipients, male sex, chronic GvHD, and exposure to immunosuppressants such as cyclosporine, tacrolimus, prednisone, and MMF (Cellcept) increase the risk of AVN, especially with exposure to three or more of these drugs [43]. In children, knees are the most common site of AVN followed by hips. Morbidity results from progressive joint damage and includes pain, decreased range of motion, arthritis, and articular collapse. If left untreated, joint destruction occurs within 1–5 years after onset of symptoms.

The pathogenesis of osteonecrosis is often multifactorial, and several mechanisms have been proposed including increased intraosseous pressure or intraluminal obliteration that can compromise intramedullary blood flow, causing marrow ischemia and then necrosis. Contributing mechanisms include defective bone repair due to damage to the bone marrow stroma, immunosuppression, and injury to the vessel wall and vasculitis related to radiation and drug.

MRI imaging has high sensitivity and specificity for detection of early lesions of AVN. Various interventions including Vitamin D and calcium supplementation, treatment with bisphosphonates, and hormone replacement therapy in females with gonadal failure are often implemented. Early referral to a pediatric orthopedic surgeon with expertise in surgery related to AVN is recommended for timely surgical intervention. Core decompression to relieve the intramedullary compartment syndrome is sometimes tried as a temporizing measure before joint replacement.

The COG long-term follow-up guidelines recommend a baseline DEXA (dual-emission x-ray absorptiometry) scan upon entry to long-term follow-up.

Renal Dysfunction

Nowadays, most children who undergo HSCT do not develop clinically significant renal dysfunction. A retrospective study of 121 long-term survivors of HSCT who were transplanted between 1991 and 1998 demonstrated a 24% prevalence rate of chronic renal failure (CRF) among these long-term survivors. Interestingly, their prospective cohort of patients who received a HSCT from 1998 to 2000 showed a lower prevalence of chronic renal failure (10%), reflecting the improvements in supportive care along with the less frequent use of nephrotoxic medications including amphotericin, aminoglycosides, and tighter control of cyclosporine A trough level targets. However, only 4–5% had GFR <70 ml/min/1.73 m^2 [44]. High serum creatinine pre-HSCT is a strong predictor of CRF, and acute renal failure in the first 3 months post-HSCT shows a trend toward predicting CRF. The previously believed contributing role of TBI toward CRF has not been confirmed in recent studies. This observation is most likely due to the use of high-dose fractionated TBI nowadays. 3–12% of children have been found to have proximal tubular dysfunction 5 years after HSCT, and approximately 9–13% of patients have mild distal tubular dysfunction. However, neither of them is clinically significant.

Renal function normally stabilizes about 1-year post-HSCT, but yearly serum creatinine monitoring in long-term survivors is essential as a screening test of renal function. Serum creatinine, blood urea nitrogen, and serum chemistry should be checked at baseline. Urinalysis and blood pressure measurements should be performed at baseline and then annually thereafter.

Ocular Late Effects

The development of cataracts is a common complication in survivors of childhood HSCT (see Chap. 24). A study of HSCT survivors who were transplanted during childhood or adolescence showed that the cumulative incidence of cataracts was 36% at 15-year posttransplant [45]. The use

of TBI for conditioning, cranial radiation, and GvHD is the greatest risk factor for cataract development. The cataracts from TBI are posterior and subcapsular which are different from the ones that occur with old age (nuclear cataracts). Hyperfractionated TBI has a lower incidence of cataract development than single-dose TBI (13% vs 21% $p < 0.01$) [46]. Other risk factors include age at the time of HSCT, steroid administration, and pre-HSCT cranial radiation.

Ocular surface disease such as dry eye syndrome (DES), blepharitis, infection, conjunctivitis, corneal ulceration, keratitis, and keratoconjunctivitis sicca syndrome (KCS) has been seen post-HSCT of which DES is the most common in children, likely due to the fast regeneration of conjunctival epithelial cells in children. Severe ocular GvHD can lead to vision-threatening lesions such as uveitis, corneal ulceration, and severe KCS. Supportive care strategies, including the use of preservative-free artificial tears, long-acting lubricants, and close follow-up with an ophthalmologist, are essential. Severe KCS not responsive to supportive therapy can be treated with custom-fitted, fluid-ventilated, and gas permeable scleral lenses.

Patients with exposure to TBI, cranial radiation, and corticosteroids need annual ophthalmologic evaluations.

Dental and Oral Complications

Teeth: Many of the oral and dental sequelae of chemotherapy and radiation are irreversible and have long-term implications. Structural anomalies like enamel hypoplasia, microdontia, tooth agenesis, root malformation, increased risk of dental carries, as well as abnormal salivary function and secondary oral malignancies are increasingly recognized after allogeneic HSCT [47]. HSCT conditioning regimens, specifically those containing TBI, may cause tooth agenesis and root anomalies [47, 48]. A study of long-term childhood cancer survivors who were treated before the age of 10 years found that children who underwent HSCT with a TBI-containing conditioning regimen had smaller tooth roots as compared to children treated with other conditioning modalities [49].

Salivary gland: Salivary gland dysfunction in HSCT recipients occurs as a secondary effect of the conditioning regimens or as an early symptom of chronic GvHD. 60% of the HSCT survivors exposed to a conditioning regimen with cyclophosphamide and a 10-Gy single dose of TBI have decreased salivary secretion rates as compared to 26% in those who received cyclophosphamide and busulfan [50]. Chronic GvHD-associated salivary dysfunction is seen in 75–85% of patients with chronic GvHD and is secondary to the lymphocyte-mediated attack on the salivary duct and acinar tissue. Decreased and thickened saliva predisposes patients to increased and recurrent infections, dental decay, and periodontitis.

Others: Squamous cell carcinoma and parotid gland cancers are frequent secondary solid tumors following HSCT. Leukoplakia that occurs more than 2–3 years after HSCT may be misdiagnosed as chronic GvHD, and, thus, suspicious lesions need to be monitored closely and biopsied periodically to exclude malignant transformation.

Early identification of oral and dental morbidity and early interventions can optimize health and quality of life. Patients should be encouraged to maintain good oral hygiene and should be counseled to avoid carcinogenic exposures like tobacco use and excessive sun exposure.

Neurocognitive Complications

TBI and prolonged immune suppression post-HSCT increase the HSCT survivor's risk for long-term neurocognitive complications (also see Chap. 24). The actual incidence of neurocognitive disabilities varies and is related to previous chemotherapeutic exposure (systemic and intrathecal), cranial radiation and age at the time of HSCT [51]. Several studies using neuropsychological testing have identified memory and attention deficits as the most prevalent and long-lasting neurocognitive impairments affecting adult HSCT survivors. Although some survivors have acute deficits in neurocognitive function that appear to improve over time, other

patients have progressive declines that are chronic. Phipps et al. found that children less than three years of age at the time of HSCT and those who received cranial radiation as part of prior therapy were at increased risk, especially those who received extra CNS radiation dose from TBI [52].

Patients and families should be counseled about possible cognitive impairment that may occur during and immediately after HSCT. Neurocognitive testing should ideally be performed prior to HSCT, and then as the child progresses through school, an individualized education plan should be generated for the patient as needed to help set the survivor up for success by allowing the patient's educational strengths to overcome any noted deficits.

Conclusion

The high burden of late effects resulting from the intensive regimens used for attaining cure highlight the need for alternative strategies to help decrease a child's cumulative exposure to chemotherapy and radiation. Pre-HSCT therapeutic exposure, conditioning regimens, immune suppression post-HSCT, and GvHD all contribute toward the development of chronic and sometimes debilitating health conditions. New advances with targeted therapies and promising results with chimeric antigen receptor T cells (CAR-T cells) [53, 54] (which are genetically modified, tumor directed T cells) offer novel approaches other than HSCT for attaining remission for relapsed and refractory disease. Research efforts are ongoing to explore reduced intensity conditioning regimens for various diseases which would significantly decrease the morbidity from these therapy-related late effects. Chronic GvHD remains a significant contributor to the chronic health conditions resulting from a HSCT, not only from the direct effects it has on multiple organ systems but also the toxic therapies that are needed to treat it. Determined attempts to find novel approaches to prevent and treat GvHD are of utmost importance and being actively investigated.

In addition, the timely and appropriate screening of patients for therapy-related late effects in long-term follow-up clinics dedicated to HSCT patients is critical due to the direct impact of these long-term effects on the morbidity and mortality of the survivors. It is essential to educate patients about their past therapy and possible late effects resulting from it to so that they are aware of the signs and symptoms and will be proactive about seeking out medical attention early on. Innovative therapies as well as risk-adapted and patient-specific timely screening for treatment-related effects will help decrease the burden of late effects in this unique pediatric population.

Key Points

- Hematopoietic stem cell transplant (HSCT) is gaining increasing prominence as a curative therapeutic option for patients with malignancies and many nonmalignant conditions.
- Two-thirds of HSCT survivors will develop at least one chronic condition.
- Mortality rates among 15-year survivors of HSCT remain twice as high as the general population.
- Alkylators and topoisomerase II inhibitors are major culprits for the development of treatment-related myelodysplastic syndrome (t-MDS).
- HSCT survivors are at a 2.3- to 4.0-fold increased risk of death due to cardiac-related causes compared to the general population.
- Hypothyroidism, metabolic syndrome, and growth impairment are common endocrine problems in patients who received a total body irradiation (TBI)-containing conditioning regimen.
- Gonadal failure is directly related to the age at HSCT and the conditioning regimen.
- Radiation therapy (especially TBI), high-dose steroids, and low estrogen secondary to gonadal failure are key factors for the development of decreased bone mineral density and avascular necrosis in HSCT patients.
- Risk-based and exposure-related screening for therapy-related late effects is critical in order to avoid long-term morbidity in this unique population.

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Part VI

Pharmacologic-Related Issues of Pediatric HSCT

Medications Commonly Used in Pediatric HSCT

28

Kevin M. Mulieri, Ashley Teusink-Cross, JoEllen Weilnau, Krisoula Spatz, and Katie S. Gatwood

Abstract

This chapter contains charts of medication classes most commonly used in pediatric hematopoietic stem cell transplantation (HSCT). These include chemotherapeutics, immunosuppressants, SOS/VOD medications, antibacterials, antifungals, antivirals, anti-pneumocystis agents, pain medications, and diuretics. This chapter is designed to be a quick reference guide, and thus the detailed references provided should be consulted for more complete information. While the dosing provided is pediatric-based, more comprehensive references should be reviewed for neonatal/young infant dosing.

Introduction

The following charts are designed to provide a quick reference of medication mechanisms of action, adverse effects, dosing, and suggested dosing adjustments for organ dysfunction for medications commonly utilized in pediatric hematopoietic stem cell transplantation. It is not designed to provide comprehensive medication information. The authors recommend consulting more detailed references provided for more complete information.

Additional Author Notes

1. Dosing provided includes common pediatric dosing of agents with maximum adult doses provided where applicable. More comprehensive references should be consulted for neonatal/young infant dosing.
2. Only relevant common and serious adverse effects are presented. Not all known adverse effects are presented.
3. For medications requiring dosage adjustments due to renal dysfunction, suggested dosage adjustments are based on creatinine clearances normalized to adult values in mL/min/1.73 m^2. For patients requiring renal replacement therapy (e.g., hemodialysis, continuous renal replacement therapy), more detailed references should be consulted.

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Chemotherapeutics

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Busulfan (Myleran®, Busulfex®)	Class: Alkylating agent (alkyl sulfonate group) Mechanism: Creates DNA cross-links that result in inhibition of DNA synthesis Cell cycle nonspecific	Common: • Nausea/vomiting and diarrhea • Mucositis • Seizures (prophylaxis with antiepileptics required) • Skin hyperpigmentation Serious: • Hepatotoxicity, including VOD/SOS • Pulmonary toxicity	<12 kg: 1.1 mg IV q6h × 2–4 days ≥12 kg: 3.2 mg/kg IV q24h OR 0.8 mg/kg IV q6h × 2–4 days IV dose is 70–80% of PO *Monitoring*: AUC or Cp_{ss} monitoring with first dose or via test dose Target Cp_{ss} 600–900 ng/mL Target AUC 900–1200 μM min	Renal: No adjustment necessary Hepatic: No adjustment necessary
Carboplatin (Paraplatin®)	Class: Platinum analogue Mechanism: Creates DNA cross-links that result in inhibition of DNA synthesis Cell cycle nonspecific	Common: • Delayed nausea/vomiting • Renal toxicity • Electrolyte wasting (K^+, Mg^{2+}, Ca^{2+}) • Peripheral neuropathy • Ototoxicity Serious: • Hypersensitivity reactions	<12 kg: 10–17 mg/kg/day IV × 2–4 days ≥12 kg: 400–700 mg/m²/day IV × 3–4 days OR target AUC = 7 × 1 dose	Renal: CrCl <50–100 mL/min: Utilize modified Calvert formula to calculate dose (CrCl cutoff for dose adjustment is variable between protocols) Hepatic: No adjustment necessary
Carmustine (BCNU)	Class: Alkylating agent (nitrosourea group) Mechanism: Creates DNA cross-links that result in inhibition of DNA synthesis Cell cycle nonspecific	Common: • Nausea/vomiting • Phlebitis during infusion • Facial flushing during infusion • CNS toxicity (dizziness, vision changes, etc.) during infusion • Transaminitis Serious: • Pulmonary toxicity	300–600 mg/m² IV × 1 dose	Renal: May consider: • CrCl 46–60 mL/min: Decrease dose by 20% • CrCl 31–45 mL/min: Decrease dose by 25% • CrCl ≤30 mL/min: Do not use Hepatic: No adjustment necessary
Clofarabine (Clolar®)	Class: Purine antimetabolite (second generation) Mechanism: Incorporates into DNA and inhibits DNA polymerase to terminate DNA synthesis Also disrupts mitochondrial membrane to induce apoptosis Cell cycle specific for S phase	Common: • Nausea/vomiting • Plantar-palmar erythrodysesthesia and rash • Hepatotoxicity (transient) Serious: • Capillary leak syndrome (requires premedication with a corticosteroid) • Stevens–Johnson syndrome and toxic epidermal necrolysis	30–40 mg/m²/day IV × 4–5 days	Renal: • CrCl 30–60 mL/min: Decrease dose by 50% • CrCl <30 mL/min: Use with caution Hepatic: No adjustment necessary

Cyclophosphamide (Cytoxan®)	Class: Alkylating agent (bischloroethylamine, or nitrogen mustard, group) Mechanism: Creates DNA cross-links that result in inhibition of DNA synthesis Cell cycle nonspecific	Common: • Nausea/vomiting • Hemorrhagic cystitis (requires hydration and MESNA with stem cell transplantation dosing) • Mucositis • SIADH Serious: • Cardiotoxicity • Hepatotoxicity, including VOD/SOS	50–100 mg/kg/day IV × 2–4 days	Renal: No adjustment necessary Hepatic: No recommendations but may consider dose reduction or avoidance of use if TBili >3–5 mg/dL or transaminases >5× ULN
Cytarabine (Ara-C)	Class: Pyrimidine antimetabolite Mechanism: Incorporates into DNA and inhibits DNA polymerase to terminate DNA synthesis Cell cycle specific for S phase	Common: • Nausea/vomiting • Hepatotoxicity (transient) Serious: • Neurotoxicity (slurred speech, gait disturbances, confusion, etc.) • Plantar-palmar erythrodysesthesia and rash	400 mg/m^2/day IV × 4 days	Renal: No adjustment necessary with non-high dose therapy (1–3 g/m^2/dose) Hepatic: No recommendations but may consider dose reduction if TBili >2 mg/dL
Etoposide (VePesid®, VP-16)	Class: Topoisomerase II inhibitor Mechanism: Inhibits topoisomerase II to prevent unwinding of DNA and inhibit DNA synthesis Cell cycle specific for S and G_2 phase	Common: • Mucositis • Nausea/vomiting • Hypotension during infusion • Plantar-palmar erythrodysesthesia Serious: • Hypersensitivity reactions	200 mg/m^2/day IV × 2–4 days	Renal: • CrCl 15–50 mL/min: Decrease dose by 25% • CrCl <15% mL/min: Decrease dose by 50% Hepatic: No recommendations but may consider 50% dose reduction if TBili >1.5–3 mg/dL or AST >3× ULN
Etoposide phosphate (Etopophos®)	Class: Topoisomerase II inhibitor Mechanism: Inhibits topoisomerase II to prevent unwinding of DNA and inhibit DNA synthesis Cell cycle specific for S and G_2 phase	Common: • Mucositis • Nausea/vomiting • Hypotension during infusion Serious: • Hypersensitivity reactions (less frequent than with etoposide)	200 mg/m^2/day IV × 2–4 days	Renal: • CrCl 15–50 mL/min: Decrease dose by 25% • CrCl <15% mL/min: Decrease dose by 50% Hepatic: No recommendations but may consider 50% dose reduction if TBili >1.5–3 mg/dL or AST >3× ULN

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Fludarabine (Fludara®)	Class: Purine antimetabolite Mechanism: Incorporates into DNA and inhibits DNA polymerase to terminate DNA synthesis and inhibit DNA repair Also induces apoptosis Both cell cycle specific for S phase and cell cycle nonspecific	Common: • Nausea/vomiting • Immunosuppression and increased infection risk Serious: • Neurotoxicity (confusion, somnolence, seizure, vision changes, etc.)	30–40 mg/m²/day IV × 4–5 days	Renal: • CrCl 30–50 mL/min: Decrease dose by 20% • CrCl <30 mL/min: Do not use Hepatic: No adjustment necessary
Melphalan (Alkeran®)	Class: Alkylating agent (bischloroethylamine, or nitrogen mustard, group) Mechanism: Creates DNA cross-links that result in inhibition of DNA synthesis Cell cycle nonspecific	Common: • Mucositis • Diarrhea (delayed) • Nausea/vomiting	140–200 mg/m² IV × 1 dose	Renal: BUN >30 mg/dL: Decrease dose by 50% Hepatic: No adjustment necessary
Thiotepa (Tepadina®)	Class: Alkylating agent (aziridine group) Mechanism: Creates DNA cross-links that result in inhibition of DNA synthesis Cell cycle nonspecific	Common: • Nausea/vomiting • Mucositis • Skin rash, burning, discoloration, flaking (requires special skin care to avoid severe toxicity) Serious: • Neurotoxicity (confusion, somnolence, etc.) • Hepatotoxicity, including VOD/SOS	5–15 mg/kg/day IV × 1–3 days Doses ≥10 mg/kg required for myeloablation	Renal: No adjustment necessary Hepatic: No adjustment necessary
Treosulfan (Ovastat®, Europe)	Class: Alkylating agent (alkyl sulfonate group) Mechanism: Creates DNA cross-links that result in inhibition of DNA synthesis Cell cycle nonspecific	Common: • Mucositis • Diarrhea • Nausea/vomiting Serious: • Hepatotoxicity (much lower risk than busulfan)	14 g/m²/day IV × 3 days	Renal: No adjustment necessary Hepatic: No adjustment necessary

Immunosuppressants

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Alemtuzumab (Campath®)	Class: Monoclonal antibody Mechanism: Binds to CD52 (surface antigen of B and T lymphocytes) causing cellular lysis	Common: • Fever • Headache • Rash • Lymphocytopenia • Increased risk of infection (herpes/fungal/urinary tract/respiratory) • Flushing • Hypertension/hypotension • Nausea/vomiting Serious: • Chest pain • Hypersensitivity	0.2–2 mg/kg/dose IV/subcutaneous daily for 5 days Max dose, 30 mg	Renal: No adjustments necessary Hepatic: No adjustments necessary
Antithymocyte immune globulin, horse (ATGAM®)	Class: Polyclonal antibody Mechanism: Elimination of antigen-reactive T lymphocytes (killer cells) in the peripheral blood or alteration in the function of T lymphocytes	Common: • Fever • Increased risk of infection • Leukopenia • Abnormal hepatic function tests • Hypertension/hypotension • Chills • Dizziness • Headache • Nausea/vomiting Serious: • Anaphylaxis	Aplastic anemia: 10–20 mg/kg/dose IV once daily for 8–14 days; additional every other day therapy can be administered up to a total of 21 doses in 28 days or 40 mg/kg/dose once daily IV for 4 days aGvHD treatment: 30 mg/kg/dose every other day for 6 doses or 15 mg/kg/dose twice daily for 10 doses aGvHD prophylaxis: 10–30 mg/kg/dose IV daily for 3–4 consecutive days	Renal: No adjustments necessary Hepatic: No adjustments necessary
Antithymocyte immune globulin, rabbit (Thymoglobulin®)	Class: Polyclonal antibody Mechanism: Acts on T-cell surface antigens and depletes CD4 lymphocytes	Common: • Fever • Increased risk of infection • Leukopenia and thrombocytopenia • Abnormal hepatic function tests • Hypertension/hypotension • Chills • Dizziness • Headache • Nausea/vomiting Serious: • Anaphylaxis	aGvHD prophylaxis: 1–3 mg/kg/dose IV once daily for 4 days before transplant aGvHD treatment: 1.5 mg/kg/dose IV once daily or every other day	Renal: No adjustments necessary Hepatic: No adjustments necessary

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Cyclosporine (Neoral®, Gengraf®)	Class: Calcineurin inhibitor Mechanism: Inhibition of production and release of interleukin-2 and inhibits interleukin-2-induced activation of resting T lymphocytes	Common: • Nausea/vomiting • Gingival hyperplasia, hirsutism • Hepatotoxicity • Hyperkalemia • Hypomagnesemia • Hypertension • Increase risk of infections • Hyperglycemia • Hyperlipidemia Serious: • Nephrotoxicity • Neurotoxicity	IV: 3–5 mg/kg/day divided every 8–12 h or 3 mg/kg/day as a continuous infusion Oral: 6–12.5 mg/kg/day divided every 8–12 h Titrate dose to desired trough concentration 100–400 ng/mL	Renal: Use with caution in significant renal dysfunction Hepatic: Significant hepatic elimination. May accumulate in hepatic dysfunction. Follow serum drug concentrations
Tacrolimus (Prograf®)	Class: Calcineurin inhibitor Mechanism: Inhibits T-lymphocyte activation by binding to an intracellular protein (FKBP-12) and complexes with calcineurin-dependent proteins to inhibit calcineurin phosphatase activity	Common: • Nausea/vomiting • Hepatotoxicity • Hyperkalemia • Hypomagnesemia • Hypertension • Increased risk of infections • Hyperglycemia • Hyperlipidemia Serious: • Nephrotoxicity • Neurotoxicity	IV: 0.03–0.05 mg/kg/day IV continuous infusion or 0.015 mg/kg IV every 12 h Oral: 0.15–0.2 mg/kg/day PO divided every 12 h Titrate dose to desired trough concentration 5–20 ng/mL	Renal: Use with caution in significant renal dysfunction Hepatic: Significant hepatic elimination. May accumulate in hepatic dysfunction. Follow serum drug concentrations
Methotrexate	Class: Antimetabolite Mechanism: Inhibits DNA synthesis, repair, and cellular replication by binding and inhibiting dihydrofolate reductase	Common/serious: • Nausea/vomiting • Mucositis • Diarrhea • Nephrotoxicity • Increased risk of infection • Neurotoxicity • Bone marrow suppression • Hepatotoxicity	IV: *GvHD prophylaxis*: ≤10 kg: 0.5 mg/kg/dose IV on day +1 and then 0.33 mg/kg/dose IV on days +3, +6, and +11 >10 kg: 15 mg/m^2/dose IV on day +1 and then 10 mg/m^2/dose IV on days +3, +6, and +11	Renal: • CrCl >50 mL/min: No adjustment necessary • CrCl 10–50 mL/min: Administer 50% of dose • CrCl <10 mL/min: Administer 30% of dose Hepatic: • Bilirubin 3.1–5 mg/dL *or* transaminases >3 times ULN: Administer 75% of dose • Bilirubin >5 mg/dL: Avoid use

Mycophenolate (CellCept®)	Class: Immunosuppressant Mechanism: Inhibits inosine monophosphate dehydrogenase which inhibits de novo guanosine nucleotide synthesis. T and B lymphocytes are dependent on this pathway for proliferation	Common/serious: • Hypertension • Peripheral edema • Abdominal pain, diarrhea, nausea • Bone marrow suppression • Increased risk of infections	IV/oral: 15 mg/kg/dose every 8 h up to 1000 mg every 8 h	Renal: For GFR < 25 mL/min, consider administering same dose but only twice daily Hepatic: No adjustment necessary
Prednisone/prednisolone (Prelone®, Orapred®)	Class: Corticosteroid Mechanism: Reverses capillary permeability and lysosomal stabilization preventing or controlling inflammation	*Common*: • Increased risk of infection • Increased appetite • Hypertension • Hyperglycemia • Hyperlipidemia • Confusion/psychosis/agitation • Edema/weight gain • Cushing appearance • HPA axis suppression *Severe*: • GI ulceration • Osteoporosis/avascular necrosis • Cataracts	Oral: 1–5 mg/kg/day divided once or twice daily	Renal: No adjustments necessary Hepatic: No adjustments necessary
Methylprednisolone (Solu-Medrol®, Medrol®)	Same as prednisone	Same as prednisone	IV/oral: 1–5 mg/kg/day divided every 6–24 h	Renal: No adjustments necessary Hepatic: No adjustments necessary
Beclomethasone (Beconase®)	Same as prednisone	Same as prednisone but less systemic absorption	Oral: For GI GvHD: 2 mg four times daily (8 mg/day)	Renal: No adjustments necessary Hepatic: No adjustments necessary
Budesonide (Entocort®)	Same as prednisone	Same as prednisone but less systemic absorption	Oral: For oral cGvHD: 3 mg dissolved in 5 mL of saline; swish and spit 2–3 times daily For GI GvHD: 3 mg once to three times daily	Renal: No adjustments necessary Hepatic: No adjustments necessary

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Clobetasol proprionate (Clobex®)	Same as prednisone	Common/serious: • Local irritation, burning • Adrenal suppression • Increased risk of infection	Oral: Oral cGvHD: Using 0.5% topical solution: Swish 5–10 mL for 1–5 min and then spit three times a day Consider using with nystatin	Renal: No adjustments necessary Hepatic: No adjustments necessary
Sirolimus (Rapamune®)	Class: mTOR kinase inhibitor Mechanism: Inhibits T-lymphocyte activation and proliferation in response to antigenic and cytokine stimulation and inhibits antibody production	Common: • Edema • Acne, rash • Hyperlipidemia • Abdominal pain, constipation, diarrhea, nausea • Stomatitis • Arthralgias • Dizziness • Impaired wound healing • Increased risk of infections Serious: • Chest pain • Hepatotoxicity • Nephrotoxicity	Oral: 7 mg/m^2 loading dose (maximum 12 mg), followed by 2.5 $mg/m^2/day$ (divided once or twice daily) maintenance dose (maximum 4 mg daily) until levels known Goal trough levels: (assay dependent) 3–12 ng/mL (mass spec) 5–14 ng/mL (CMIA)	Renal: No adjustment necessary Hepatic: Mild–moderate: Consider reducing initial doses by 1/3→ then follow therapeutic drug monitoring Moderate-severe: Consider reducing dose by 1/2→ then follow therapeutic drug monitoring
Infliximab (Remicade®)	Class: TNF blocker Mechanism: A monoclonal antibody with high-affinity binding to TNF-alpha receptors and neutralizing TNF-alpha activity	Common: • Rash • Abdominal pain, nausea • Headache • Cough, pharyngitis • Sinusitis Serious: • Increased risk of infection • Infusion reactions	IV: 10 mg/kg/dose once weekly	Renal: No adjustment necessary Hepatic: No adjustment necessary
Etanercept (Enbrel®)	Class: TNF blocker Mechanism: A recombinant DNA-derived protein which binds to TNF and blocks its interaction with cell surface receptors	Common: • Injection site reactions • Upper respiratory infections Serious: • Increased risk of infections	SubQ: 0.4 mg/kg/dose twice weekly (max 25 mg/dose)	Renal: No adjustment necessary Hepatic: No adjustment necessary

Rituximab (Rituxan®)	Class: Anti-CD20 monoclonal antibody Mechanism: A monoclonal antibody binding to the CD20 antigen on the cell surface of B lymphocytes, activating complement dependent B-cell cytotoxicity	Common: • Infusion reactions Serious: • Angioedema • Increased risk of infections	IV 375 mg/m2/dose once weekly	Renal: No adjustment necessary Hepatic: No adjustment necessary
Basiliximab (Simulect®)	Class: Immunosuppressant Mechanism: Monoclonal antibody against CD25 antigen; binds to IL-2 receptor of activated T cells, inhibiting activation and proliferation	Common: • Infusion reactions • Chills, fever • Hypertension • Rash Serious: • Increased risk of infections • Hypotension • Shortness of breath	IV: <35 kg: 10 mg once weekly ≥35 kg: 20 mg once weekly	Renal: No adjustment necessary Hepatic: No adjustment necessary
Thalidomide (Thalomid)	Class: Immunosuppressant Mechanism: Multiple immunosuppressive and immunomodulatory effects including inhibition of TNF-alpha, IL-6, IL-10, and IL-12 and increases in IL-2, IL-4, and IL-5 production	Common: • Edema • Sedation, dizziness • Dry skin, rash • Hypocalcemia • Weight gain/loss, constipation, diarrhea, nausea • Asthenia, neuropathies, tremor • Dyspnea Serious: • Seizures • Hepatotoxicity • Teratogenicity • Thromboembolism • Leukopenia	Oral: Doses gradually tapered up to 12 mg/kg/day (maximum of 1200 mg/day) in 3–4 divided doses	Renal: No adjustment necessary Hepatic: No adjustment necessary

VOD/SOS Medications

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Defibrotide (Defitelio®)	Class: Anticoagulant Mechanism: Enhances the enzymatic activity of plasmin to hydrolyze fibrin clots; increases plasminogen activator and thrombomodulin expression and decrease von Willebrand factor and plasminogen activator inhibitor expression	Common: • Nausea, vomiting, diarrhea Serious: • Hypotension • Hypersensitivity reaction • Increased risk of bleeding	IV: 6.25 mg/kg/dose every 6 h for at least 21 days	Renal: No adjustment necessary Hepatic: No adjustment necessary
Ursodiol (Actigall®)	Class: Gallstone dissolution agent Mechanism: Increases bile flow; reduces the detergent properties of the bile salts, thus reducing their cytotoxicity; and protects liver cells from damaging activity of toxic bile acid	Common: • Rash • Constipation, nausea, vomiting, flatulence • Dizziness • Backache	10 mg/kg/dose three times daily	Renal: No adjustment necessary Hepatic: No adjustment necessary

Antibacterials

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Cefepime (Maxipime®)	Class: Beta-lactam (cephalosporin, 4th generation) Mechanism: Inhibits bacterial cell wall	Common: • Headache • Fever • Pruritus • Nausea/vomiting/diarrhea • Elevated liver function tests Serious: • Hypersensitivity • Electrolyte abnormalities (hypophosphatemia, hypercalcemia, hyperkalemia)	IV: (Dosing for neutropenic fever, antipseudomonal coverage) 50 mg/kg/dose (max of 2000 mg) every 8 h	Renal: • CrCl >60 mL/min: No adjustment needed • CrCl 30–60 mL/min: 50 mg/kg/dose every 12 h • CrCl 11–29 mL/min: 50 mg/kg/dose every 24 h • CrCl <11 mL/min: 25 mg/kg/dose every 24 h Hepatic: No adjustments necessary

Ceftazidime (Fortaz®, Tazicef®)	Class: Beta-lactam (cephalosporin, 3rd generation) Mechanism: Inhibits bacterial cell wall	Common: • Headache • Elevated liver function tests • Phlebitis • Diarrhea Serious: • Hypersensitivity • Hemolytic anemia • Stevens–Johnsons	IV: Dosing for neutropenic fever, antipseudomonal coverage 50 mg/kg/dose every 8 h (max of 6 g/day)	Renal: • CrCl >50 mL/min: No adjustment required • CrCl 30–50 mL/min: 50 mg/kg/dose every 12 h • CrCl 10–29 mL/min: 50 mg/kg/dose every 24 h • CrCl ≤10 mL/min: 50 mg/kg/dose every 48 h Hepatic: No adjustments necessary
Piperacillin/tazobactam (Zosyn®)	Class: Beta-lactam (Penicillin/beta-lactamase inhibitor) Mechanism: Inhibits bacterial cell wall	Common: • Headache • Hypotension • Flushing • Diarrhea/nausea/vomiting • Pruritus • Rash • Fever • Increased liver function tests • Abnormal electrolytes (hyper- and hypoglycemia, hyper- and hyponatremia, hyper- and hypokalemia, hyper- and hypocalcemia) Serious: • Nephrotoxicity • Hypersensitivity • Hematologic toxicity • DRESS syndrome • Stevens–Johnson	Dosing is based on *piperacillin* component IV: Children and adolescents: 80–100 mg/kg/dose every 6–8 h (max of 16,000 mg/day)	Renal: • CrCl >50 mL/min: No adjustment required • CrCl 30–50 mL/min: 35–50 mg piperacillin/kg/dose every 6 h • CrCl <30 mL/min: 35–50 mg piperacillin/kg/dose every 8 h Hepatic: No adjustments necessary

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Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Imipenem and Cilastatin (Primaxin®)	Class: Beta-lactam (carbapenem) Mechanism: Inhibits bacterial cell wall	Common: • Headache • Pruritus • Diarrhea/nausea/vomiting • Rash • Hypertension/hypotension Serious: • Nephrotoxicity • Hepatotoxicity • Neurotoxicity • Hematologic toxicity	Dosing based on *imipenem* component IV: 15–25 mg/kg/dose every 6 h (max 4000 mg/day)	Renal: • CrCl 30–50 mL/min: Administer 7–13 mg/kg/dose every 8 h • CrCl 10–29 mL/min: Administer 7.5–12.5 mg/kg/dose every 12 h • CrCl <10 mL/min: Administer 7.5–12.5 mg/kg/dose every 24 h Hepatic: No adjustments necessary
Meropenem (Merrem®)	Class: Beta-lactam (carbapenem) Mechanism: Inhibits bacterial cell wall	Common: • Headache • Pruritus • Diarrhea/nausea/vomiting • Rash • Hypertension/hypotension Serious: • Hypersensitivity • Nephrotoxicity • Hepatotoxicity • Neurotoxicity	IV: 20–40 mg/kg/dose every 8 h (max of 6 g/day)	Renal: • CrCl >50 mL/min: No adjustment required • CrCl 30–50 mL/min: Administer 20–40 mg/kg/dose every 12 h • CrCl 10–29 mL/min: Administer 10–20 mg/kg/dose every 12 h • CrCl <10 mL/min: Administer 10–20 mg/kg/dose every 24 h Hepatic: No adjustments necessary
Aztreonam (Azactam®)	Class: Beta Lactam (monobactam) Mechanism: Inhibits bacterial cell wall	Common: • Phlebitis • Diarrhea/nausea/vomiting • Rash Fever • Increased liver function tests Serious: • Nephrotoxicity • Hypersensitivity • Hematologic toxicity • Flushing	IV: 30 mg/kg/dose every 6–8 h (max of 8000 mg/day)	Renal: • CrCl ≥30 mL/min: No adjustment required • CrCl 10–29 mL/min: 15–20 mg/kg every 8 h • CrCl <10 mL/min: 7.5–10 mg/kg every 12 h Hepatic: No adjustments necessary

Vancomycin (Vancocin®)	Class: Glycopeptide Mechanism: Inhibits bacterial cell wall	Common: • Headache • Diarrhea/nausea/vomiting • Red man syndrome • Chills • Fever • Hypotension Serious: • Nephrotoxicity • Ototoxicity • Stevens–Johnson • DRESS syndrome	IV: 15–20 mg/kg/dose every 6–8 h. Maximum initial dose of 4000 mg/day Obtain prior to 4th dose (steady state): *Trough levels*: 15–20 μg/mL: bacteremia, endocarditis, meningitis, and osteomyelitis and organisms with an MIC ≥1 μg/mL 10–15 μg/mL: other infections when organism has MIC <1 μg/mL Note: trough's serve as surrogate marker to achieve AUC/MIC of ≥400. More advanced serum drug monitoring may be conducted for patient-specific targets Note: Alternative pharmacokinetic dosing strategies exist Oral for *Clostridium difficile*: 40 mg/kg/*day* divided every 6–8 h (max of 2000 mg/day)	Renal: (initial dosing) • CrCl 30–50 mL/min: 10 mg/kg/dose every 12 h • CrCl 10–29 mL/min: 10 mg/kg/dose every 18–24 h • CrCl <10 mL/min: 10 mg/kg/dose; redose based on serum concentrations Hepatic: No adjustments necessary
Daptomycin (Cubicin®)	Class: Lipopeptide Mechanism: Binds to components of the cell membrane of susceptible organisms and causes rapid depolarization, inhibiting intracellular synthesis of DNA, RNA, and protein	Common: • Headache • Hypertension/hypotension • Dizziness • Diarrhea • Pruritus • Rash • Fever • Increased liver function tests Serious: • Nephrotoxicity • Tinnitus • Hypersensitivity • Hematologic toxicity • Stevens–Johnsons • Rhabdomyolysis	IV: 6–10 mg/kg/dose once daily	Renal: • CrCl >30 mL/min: Administer full dose • CrCl 10–29 mL/min: 4 mg/kg/dose every 24 h • CrCl <10 mL/min: 4 mg/kg/dose every 48 h Hepatic: No adjustments necessary

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Linezolid (Zyvox®)	Class: Oxazolidinone Mechanism: Inhibits bacterial protein synthesis by binding to bacterial 23S ribosomal RNA of the 50S subunit	Common: • Headache • Diarrhea/nausea/vomiting • Pruritus • Fever • Increased liver function tests Serious: • Nephrotoxicity • Serotonin syndrome • Stevens–Johnsons syndrome • Bone marrow suppression • Peripheral neuropathy • Optic neuropathy	IV/oral: <12 years old: 10 mg/kg/dose every 8 h (max of 600 mg) ≥12 years old: 10 mg/kg/dose (max of 600 mg) every 12 h or 600 mg every 12 h fixed dose	Renal: No adjustments necessary Hepatic: No adjustments necessary
Clindamycin (Cleocin®)	Class: Lincosamide Mechanism: Inhibits bacterial protein synthesis by binding to 50S ribosomal subunits preventing peptide bond formation	Common: • Hypotension • Metallic taste • Diarrhea/nausea/vomiting • Rash • Fever • Increased liver function tests Serious: • Nephrotoxicity • Hypersensitivity • Hematologic toxicity • DRESS syndrome • Stevens–Johnson	IV: 20–40 mg/kg/*day* divided every 6–8 h (max of 2700 mg/day) Oral: 10–40 mg/kg/*day* divided every 6–8 h (max of 1800 mg/day)	Renal: No adjustments necessary Hepatic: No adjustments necessary
Ciprofloxacin (Cipro®)	Class: Fluoroquinolone Mechanism: Inhibits DNA-gyrase, thereby inhibits relaxation of supercoiled DNA and promotes breakage of DNA strands	Common: • Edema • Dizziness • Pruritus • Headache • Nausea/vomiting • Constipation/diarrhea Serious: • Chest pain • QTc prolongation/torsades de pointes • Stevens–Johnson syndrome • Peripheral neuropathy • Tendon rupture	Oral: 10–20 mg/kg/dose twice daily (max of 750 mg/dose) IV: 10 mg/kg/dose every 8–12 h or 15 mg/kg/dose every 12 h (max of 400 mg/dose)	Renal: • CrCl ≥30 mL/min: No dosage adjustment necessary • CrCl 10–29 mL/min: 10–15 mg/kg/dose every 18 h • CrCl <10 mL/min: 10–15 mg/kg/dose every 24 h Hepatic: No adjustments necessary

Levofloxacin (Levaquin®)	Same as ciprofloxacin	• Same as ciprofloxacin	Oral and IV: 6 months to <5 years: 8–10 mg/kg/dose twice daily ≥5 years: 10 mg/kg/dose once daily (max of 750 mg/*day*)	Renal: • CrCl ≥30 mL/min: No dose adjustment necessary • CrCl 10–29 mL/min: 5–10 mg/kg/dose every 24 h • CrCl <10 mL/min: 5–10 mg/kg/dose every 48 h Hepatic: No adjustments necessary
Gentamicin	Class: Aminoglycoside Mechanism: Produces a defective bacterial cell wall by interfering with bacterial protein synthesis via binding to 30S and 50S ribosomal subunits	Common: • Edema • Fever • Electrolyte abnormalities (hypocalcemia, hypokalemia, hypomagnesemia, hyponatremia) Serious: • Nephrotoxicity • Ototoxicity	IV: *Traditional dosing*: 2.5 mg/kg/dose every 8 h *Gram-positive synergy*: 1–2 mg/kg/dose every 8 h Obtain levels around 3rd or 4th dose (steady state): *Peak*: Drawn 30 min after 30 min infusions Most infections: 6–10 μg/mL Gram-positive synergy: 3–5 μg/mL *Trough*: Drawn immediately before next dose due: ≤1 μg/mL *Once daily dosing*: 5–7 mg/kg/*day* Levels may be obtained after each dose: *Peak*: Drawn 60 min after 60 min infusion Most infections: 2–3 times the desired peak of traditional dosing *18–20 h post-infusion level*: <1 μg/mL but detectable *Trough*: Drawn immediately before next dose due: Not detectable *Note: Alternative pharmacokinetic dosing strategies exist	Renal (with traditional dosing, once daily dosing not recommended with renal dysfunction): • CrCl >50 mL/min: No adjustment required • CrCl 30–50 mL/min: Administer every 12–18 h • CrCl 10–29 mL/min: Administer every 18–24 h • CrCl <10 mL/min: Administer every 48–72 h Hepatic: No adjustments necessary

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Tobramycin	Same as gentamicin	Common/serious: • Same as gentamicin	IV: *Traditional dosing*: 2–2.5 mg/kg/dose every 6–8 h Obtain levels around 3rd or 4th dose (steady state): *Peak*: Drawn 30 min after 30 min infusions 6–10 μg/mL *Trough*: Drawn immediately before next dose due: ≤0.5–2 μg/mL *Once daily dosing*: 4.5–8.5 mg/kg/*day* Levels may be obtained after each dose: *Peak*: Drawn 60 min after 60 min infusion Most infections: 2–3 times the desired peak of traditional dosing *18–20 h post-infusion level*: <1 μg/mL but detectable *Trough*: Drawn immediately before next dose due: Not detectable *Note: Alternative pharmacokinetic dosing strategies exist	Renal (with traditional dosing, once daily dosing not recommended with renal dysfunction): • CrCl >50 mL/min: No adjustment required • CrCl 30–50 mL/min: Administer every 12–18 h • CrCl 10–29 mL/min: Administer every 18–24 h • CrCl <10 mL/min: Administer every 48–72 h Hepatic: No adjustments necessary

Amikacin	Same as gentamicin	Common: • Same as gentamicin	IV: *Traditional dosing*: Children and adolescents: 5–7.5 mg/kg/dose every 8 h Obtain levels around 3rd or 4th dose (steady state): *Peak*: Drawn 30 min after 30 min infusions 15–40 μg/mL *Trough*: Drawn immediately before next dose due: ≤1–8 μg/mL *Once daily dosing*: 15–20 mg/kg/*day* Levels may be obtained after each dose: *Peak*: Drawn 60 min after 60 min infusion Most infections: 40–60 μg/mL *Trough*: Drawn immediately before next dose due: < 1 μg/mL *Note: Alternative pharmacokinetic dosing strategies exist	Renal (with traditional dosing, once daily dosing not recommended with renal dysfunction): • CrCl >50 mL/min: No adjustment required • CrCl 30–50 mL/min: Administer every 12–18 h • CrCl 10–29 mL/min: Administer every 18–24 h • CrCl < 10 mL/min: Administer every 48–72 h Hepatic: No adjustments necessary

Antifungals

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Fluconazole (Diflucan®)	Class: Azole antifungal Mechanism: Interferes with fungal cytochrome P450 activity decreasing ergosterol synthesis and inhibiting cell membrane formation	Common: • Nausea, vomiting • Headache Serious: • Prolonged QTc • Hepatotoxicity	IV/oral: *Prophylaxis*: 6 mg/kg/dose every 24 h (max dose 400 mg) *Treatment*: 10–12 mg/kg/dose every 24 h (max 800 mg)	Renal: • CrCl 10–50 mL/min: Administer 50% of recommended dose at the normal interval • CrCl ≤10 mL/min: Administer 50% of recommended dose every 48 h Hepatic: No recommendations but use with caution
Voriconazole (Vfend®)	Class: Azole antifungal Mechanism: Interferes with fungal cytochrome P450 activity decreasing ergosterol synthesis and inhibiting cell membrane formation	Common: • Rash • Hallucinations • Visual disturbances Serious: • Prolonged QTc • Hepatotoxicity	IV/oral: *<12 years*: Prophylaxis: 5–7 mg/kg/dose every 12 h (max dose 400 mg/dose) Treatment: 9 mg/kg/dose every 12 h × 2 doses followed by a maintenance dose of 8–9 mg/kg/dose every 12 h *≥12 years*: Prophylaxis: 4 mg/kg/dose every 12 h (max dose 400 mg/dose) Treatment: 6 mg/kg/dose every 12 h × two doses followed by a maintenance dose of 4 mg/kg/dose every 12 h Check voriconazole troughs after 5–7 days: Prophylaxis: 1–6 μg/mL Treatment of 2–6 μg/mL	Renal: No recommendations, but use IV formulation with caution in patients with CrCl <50 mL/min Hepatic: • Mild–moderate dysfunction: Use standard loading regimen; then decrease maintenance dose by 50% • Severe hepatic impairment: Not recommended unless benefit > risk

Posaconazole (Noxafil®)	Class: Azole antifungal Mechanism: Interferes with fungal cytochrome P450 activity decreasing ergosterol synthesis and inhibiting cell membrane formation	Common: • Hypokalemia • Diarrhea, vomiting • Headache • Fever Serious: • Prolonged QTc • Hepatotoxicity	Oral: *Prophylaxis*: Suspension: 4 mg/kg/dose three times daily, up to 200 mg three times daily with a full meal Delayed release Tablets: Only for patients ≥13 yo: 300 mg twice a day for 1 day, then 300 mg once daily *Treatment*: Suspension: 21 mg/kg/day divided 3–4 times daily up to 400 mg twice daily IV: *Prophylaxis*: Only for patients ≥13 yo: 300 mg twice a day for 1 day, then 300 mg once daily Check posaconazole troughs after 7 days: Prophylaxis: >0.7 μg/mL Treatment: >1.25 μg/mL	Renal: No adjustment necessary Hepatic: No adjustment necessary
Isavuconazonium sulfate (Cresemba®)	Class: Azole antifungal Mechanism: Interferes with fungal cytochrome P450 activity decreasing ergosterol synthesis and inhibiting cell membrane formation	Common: • Peripheral edema • Hypokalemia • Constipation, diarrhea, nausea, vomiting • Headache • Cough, dyspnea Serious: • Hepatotoxicity	Oral/IV: Pediatric dosing not available Adult dosing: 372 mg every 8 h for six doses, then 12–24 h later begin 372 mg once daily	Renal: No adjustment necessary Hepatic: No adjustment necessary
Caspofungin (Cancidas®)	Class: Echinocandin Mechanism: Antifungal that inhibits beta (1,3) D-glucan synthesis	Common: • Rash, pruritis • Diarrhea • Fever, shivering • Hepatotoxicity Serious: • Hypotension	IV: 70 mg/m^2 (max of 70 mg) × 1 dose followed 24 h later by 50–70 mg/m^2 (max of 50–70 mg) every 24 h	Renal: No adjustment necessary Hepatic: Moderate impairment: Give normal loading dose, followed by 30% maintenance dose reduction

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Micafungin (Mycamine®)	Class: Echinocandin Mechanism: Antifungal that inhibits beta (1,3) D-glucan synthesis	Common: • Diarrhea, nausea, vomiting • Headache • Fever Serious: • Thrombocytopenia	IV: Prophylaxis: 1–3 mg/kg/dose every 24 h (max 50 mg) Treatment: 3 mg/kg/dose every 24 h (max 150 mg)	Renal: No adjustment necessary Hepatic: No adjustment necessary
Amphotericin B deoxycholate (Fungizone®)	Class: Antifungal Mechanism: Binds to ergosterol altering cell membrane permeability in susceptible fungi causing leakage of cell components with subsequent cell death	Common: • Nausea, vomiting, diarrhea • Arthralgia, myalgia • Headache • Fever, shivering, rigors Serious: • Nephrotoxicity • Hypokalemia, hypomagnesemia • Hypotension • Infusion reactions	IV: Treatment: 0.25–1 mg/kg every 24 h	Renal: If renal dysfunction is due to the drug, consider decreasing the dose by 50% or administering every other day Hepatic: No adjustment necessary
Amphotericin B lipid complex (Abelcet®)	Class: Antifungal Mechanism: Binds to ergosterol altering cell membrane permeability in susceptible fungi causing leakage of cell components with subsequent cell death	Common: • Nausea, vomiting, diarrhea • Infusion reactions • Fever, shivering, rigors Serious: • Nephrotoxicity • Hypokalemia, hypomagnesemia	IV: Treatment: 3–5 mg/kg every 24 h	Renal: CrCl < 10 mL/min: usual IV dose (every 24–36 h) Hepatic: No adjustment necessary
Amphotericin B liposomal (Ambisome®)	Class: Antifungal Mechanism: Binds to ergosterol altering cell membrane permeability in susceptible fungi causing leakage of cell components with subsequent cell death	Common: • Nausea, vomiting, diarrhea • Infusion reactions • Fever, shivering, rigors Serious: • Nephrotoxicity • Hypokalemia, hypomagnesemia	IV: Treatment: 3–5 mg/kg every 24 h	Renal: CrCl < 10 mL/min usual IV dose (every 24–36 h) Hepatic: No adjustment necessary
Nystatin	Class: Antifungal Mechanism: Binds to sterols in the fungal cell membrane resulting in the cells membrane's inability to function as a selective barrier, thus allowing loss of essential cellular constituents	Common: • Nausea, vomiting Serious: • Hypersensitivity reactions	Oral: Oral suspension 400,000–600,000 units orally 4 times a day	Renal: No adjustment necessary Hepatic: No adjustment necessary

Antivirals

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Acyclovir (Zovirax®)	Class: Antiviral Mechanism: A nucleoside analogue that competitively inhibits viral DNA polymerase; requires thymidine kinase and host cellular enzymes to become active acyclovir triphosphate	Common: • Headache • Nausea diarrhea • Rash Severe: • Nephrotoxicity • Bone marrow suppression • Hepatotoxicity	*HSV and VZV Prophylaxis*: Start with cytoreduction and continue until engraftment or approximately 30 days after transplant, may continue for one year after transplant if VZV seropositive, may continue until 6 months after discontinuation of immunosuppressive agents Pediatrics <40 kg: 250 mg/m^2 intravenous every 8 h or 125 mg/m^2 intravenous every 6 h (daily maximum: 80 mg/kg) Adolescents/adults ≥40 kg: 400–800 mg orally twice daily or 250 mg/m2 intravenous every 12 h *Treatment*: 5–20 mg/kg (or 250–500 mg/m^2) (divided every 8 h) IV 40–80 mg/kg/day (max of 4000 mg/day) divided 4–5 times daily	Renal: Oral: • CrCl >25 mL/min/1.73m^2: No adjustment necessary • CrCl 10–25 mL/min: – No adjustment for normal every 12 hour dosing – Decrease normal 4 or 5 times daily dosing to same dose every 8 h • CrCl <10 mL/min: – Decrease normal every 12 hour dosing by 50% – Decrease normal 4 or 5 times daily dosing to same dose every 8 h Intravenous: • CrCl >50 mL/min: No adjustment necessary • CrCl 25–50 mL/min: Administer every 12 h • CrCl 10–25 mL/min: Administer every 24 h • CrCl <10 mL/min: Administer 50% of dose every 24 h (4) Hepatic: No dose adjustment necessary
Valacyclovir (Valtrex®)	Class: Antiviral Mechanism: A prodrug of acyclovir that shares the same mechanism of action	Common: • Headache • Nausea diarrhea • Rash Severe: • Nephrotoxicity • Bone marrow suppression • Hepatotoxicity	15–30 mg/kg (max of 1000 mg) orally three times daily	Renal: • CrCl >50 mL/min: No dose adjustment necessary • CrCl 30–49 mL/min: Give same dose every 12 h • CrCl 10–29 mL/min: Give same dose every 24 h • CrCl <10 mL/min: Decrease dose by 50% and give every 24 h Hepatic: No adjustment necessary

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Ganciclovir (Cytovene®)	Class: Antiviral Mechanism: An acyclic nucleoside analogue that interferes with viral replication; requires viral and cellular enzymes to convert ganciclovir to ganciclovir triphosphate, the active molecule	Common: • Nausea and vomiting • Diarrhea • Fever, chills Severe: • Myelosuppression • Nephrotoxicity	Induction: 5 mg/kg IV every 12 h for 7–14 days Maintenance: 5 mg/kg IV once daily	Renal: • CrCl ≥ 70 mL/min: No dose adjustment necessary • CrCl 50–69 mL/min: Induction: 2.5 mg/kg every 12 h. Maintenance: 2.5 mg/kg every 24 h • CrCl 25–49 mL/min: Induction: 2.5 mg/kg every 24 h. Maintenance: 1.25 mg/kg every 24 h • CrCl 10–24 mL/min: Induction: 1.25 mg/kg every 24 h. Maintenance: 0.625 mg/kg every 24 h • CrCl <10 mL/min: Induction: 1.25 mg/kg/dose 3 times per week. Maintenance: 0.625 mg/kg/dose 3 times per week Hepatic: No adjustment necessary
Valganciclovir (Valcyte®)	Class: Antiviral: Mechanism: Valganciclovir is converted to ganciclovir by intestinal and hepatic esterases and then shares the same mechanism of action	Common: • Nausea and vomiting • Diarrhea • Fever, chills Severe: • Myelosuppression • Nephrotoxicity	CMV induction for >40 kg: 900 mg orally twice daily 7–14 days CMV maintenance for >40 kg: 900 mg orally once daily CMV prevention in pediatrics: Dose (mg) = 7 × BSA × CrCl (modified Schwartz equation, with a maximum CrCl of 150 mL/min/1.73m^2) orally once daily	Renal: • CrCl ≥ 60 mL/min: No dose adjustment necessary • CrCl 40–59 mL/min: Induction: 450 mg twice daily. Maintenance: 450 mg once daily • CrCl 25–39 mL/min: Induction: 450 mg once daily. Maintenance: 450 mg every 2 days • CrCl 10–24 mL/min: Induction: 450 mg every 2 days. Maintenance: 450 mg twice weekly • CrCl <10 mL/min: Not recommended Hepatic: No adjustment necessary

Foscarnet (Foscavir®)	Class: Antiviral Mechanism: A pyrophosphate analogue that inhibits DNA polymerase of herpesviruses; does not require activation by thymidine kinase or other kinases	Common: • Hypocalcemia • Hypomagnesemia • Hypophosphatemia • Hypokalemia • Rash • Confusion Serious: • Seizures • Nephrotoxicity	CMV induction: 60 mg/kg IV every 8 h or 90 mg/kg IV every 12 h for 7–14 days CMV maintenance: 90–120 mg/kg IV once daily	Renal: Foscarnet requires dose adjustment and caution for patients with renal impairment. Adult data adjustments based on modified Cockcroft and Gault equation and then divided by body weight (kg) CMV equivalent to 60 mg/kg q8h >1.4 mL/min/kg: 60 mg/kg q8h >1.0–1.4 mL/min/kg: 45 mg/kg q8h >0.8–1.0 mL/min/kg: 50 mg/kg q12h >0.6–0.8 mL/min/kg: 40 mg/kg q12h >0.5–0.6 mL/min/kg: 60 mg/kg q24h >0.4–0.5 mL/min/kg: 50 mg/kg q24h <0.4 mL/min/kg: Not recommended CMV equivalent to 90 mg/kg q24h CrCl (mL/min/kg) >1.4 mL/min/kg: 90 mg/kg q24h >1.0–1.4 mL/min/kg: 70 mg/kg q24h >0.8–1.0 mL/min/kg: 50 mg/kg q24h >0.6–0.8 mL/min/kg: 80 mg/kg q48h >0.5–0.6 mL/min/kg: 60 mg/kg q48 h >0.4–0.5 mL/min/kg: 50 mg/kg q48h <0.4 mL/min/kg: Not recommended Hepatic: No adjustment necessary

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Cidofovir (Vistide®)	Class: Antiviral Mechanism: A nucleoside phosphonate analogue activated by intracellular kinases, which inhibits viral DNA synthesis	Common: • Nausea and vomiting • Rash Severe: • Nephrotoxicity (should be administered with hyper-hydration and probenecid due to rapid uptake by proximal tubular cells by organic anion transporters causing tubular necrosis) • Myelosuppression • Ocular toxicity • Metabolic acidosis	CMV induction: 5 mg/kg IV once weekly with prehydration and probenecid for 2 doses CMV all ages maintenance: 5 mg/kg IV every other week with prehydration and probenecid Adenovirus: 5 mg/kg IV once weekly or 1 mg/kg IV three times per week *Probenecid dosing*: • 25–40 mg/kg/dose or 1000–2000 mg/m^2/dose (max of 2000 mg) 3 h prior to cidofovir infusion • 10–20 mg/kg/dose or 500–1250 mg/m^2/dose (max of 1000 mg) 2 h and 8 h after completion of cidofovir infusion	Renal: (serum creatinine >1.5 mg/dl, creatinine clearance <90 mL/min/1.73m^2, proteinuria >2+) ≥ 6 months, children, and adolescents: • Adenovirus induction: 1 mg/kg/dose 3 times weekly (on alternate days for 2 consecutive days) • Adenovirus maintenance: 1 mg/kg/dose every other week Recommendations not available for use in CMV with renal dysfunction Hepatic: No adjustment necessary
Cytomegalovirus immunoglobulin (Cytogam®)	Class: Immunoglobulin Mechanism: Immunoglobulin G (human) containing a standardized amount of antibody to cytomegalovirus to attenuate or reduce the incidence of serious CMV disease	Common: • Flushing, fever, chills • Arthralgia • Nausea and vomiting • Hypertension Severe: • Aseptic meningitis syndrome	For treatment of CMV, in combination with antiviral medications, 100–400 mg/kg IV. Variety of dosing frequencies have been administered	Renal: No dosage adjustment available. Use with caution. Infuse at minimum rate possible Hepatic: No adjustment necessary

Anti-pneumocystis Agents

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Sulfamethoxazole/trimethoprim (Bactrim®, Septra®)	Class: Sulfonamide/antifolate Mechanism: Sulfamethoxazole inhibits bacterial synthesis of dihydrofolic acid by competing with para-aminobenzoic acid Trimethoprim reversibly binds to and inhibits dihydrofolate reductase, blocking the production of tetrahydrofolic acid from dihydrofolic acid	Common: • Bone marrow suppression • Nausea and vomiting Severe: • Anaphylaxis • Rash (maculopapular rash, rarely Stevens–Johnson syndrome) • Hepatitis • Interstitial nephritis	*Prophylaxis*: Infants ≥2 months and older: 150 mg trimethoprim/m^2/day or 5 mg trimethoprim/kg/day IV or oral divided twice daily for 3–7 days every week. Maximum daily dose: 320 mg trimethoprim per day *Treatment*: Infants ≥2 months and older: 15–20 mg trimethoprim/kg/day IV or oral in 3 or 4 divided doses for 21 days Neonates and young infants: Use should be avoided in patients <2 months old when possible due to risk of hyperbilirubinemia and kernicterus associated with sulfa antibiotics due to potential displacement of bilirubin from protein-binding sites	Renal: • CrCl >30 mL/min: No adjustment required • CrCl 15–30 mL/min: Administer 50% of recommended dose • CrCl <30 mL/min: Use is not recommended Hepatic: No adjustment necessary
Pentamidine (Pentam®)	Class: Antifungal/antiprotozoal Mechanism: Interferes with protozoal nuclear metabolism by inhibiting DNA, RNA, phospholipid, and protein synthesis	Common: • Nephrotoxicity • Blood glucose abnormalities • Hypotension Common (aerosolized): • Cough, sneezing, and bronchospasm (aerosolized only) Severe: • QTc interval prolongation (torsades de pointes) • Pancreatitis	*Prophylaxis*: Children >4 months: 300 mg inhaled once a month 4 mg/kg (max of 300 mg) IV every 2–4 weeks *Treatment*: 4 mg/kg IV once daily	Renal: • CrCl >30 mL/min: No adjustment required • CrCl 10–30 mL/min: Administer every 36 h • CrCl <30 mL/min: Administer every 48 h Hepatic: Use with caution

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Dapsone	Class: Sulfone Mechanism: An analogue of para-aminobenzoic acid, blocking microbial folic acid synthesis via inhibition of dihydropteroate synthetase activity	Common: • Rash • Headache Severe: • Hemolysis, agranulocytosis, aplastic anemia • Methemoglobinemia • Hypersensitivity reaction	*Prophylaxis*: 2 mg/kg/day (maximum total dosage of 100 mg daily) or 4 mg/kg/week (maximum of 200 mg weekly) orally	Renal: No adjustment necessary Hepatic: No adjustment necessary
Atovaquone (Mepron®)	Class: Antiprotozoal Mechanism: Interferes with pyrimidine synthesis, resulting in inhibition of mitochondrial electron transport	Common: • Skin rash • Nausea and vomiting • Diarrhea Severe: • Hepatotoxicity • Hypersensitivity reactions	*Prophylaxis*: Infants 1–3 months *and* patients >24 months: 30 mg/kg (max of 1500 mg) orally once daily with food Children 4–24 months: 45 mg/kg (max of 1500 mg) orally once daily with food *Treatment*: Children <3 months and >24 months: 30–40 mg/kg/day (max of 1500 mg/day) orally in two divided doses with food Children 3–24 months: 45 mg/kg/day (max of 1500 mg/day) orally in two divided doses with food Adolescents and adults: 750 mg orally twice daily with food	Renal: No adjustment necessary Hepatic: No adjustments available, but use with caution

Antiemetics

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Ondansetron (Zofran®)	Class: Selective 5-HT_3 receptor antagonist Mechanism: Works on serotonin 5-HT_3 receptors located both peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema	Common: • Headache, malaise/fatigue • Constipation • QTc interval prolongation Serious: • Serotonin syndrome, neuroleptic malignant syndrome-like events	5 mg/m^2/dose or 0.15 mg/kg/dose IV or PO pre-therapy x1 dose then every 8 h Maximum initial dose of 16 mg IV infused over 15 min. Do not exceed due to the dose dependent risk of QTc prolongation	Renal: No adjustment necessary Hepatic: In severe hepatic impairment (Child-Pugh[2] score $\geq$ 10) total daily dose of 8 mg should not be exceeded
Granisetron (Kytril®, Sancuso®)	Same as ondansetron	Common: • Headache, malaise/fatigue • Constipation • QTc interval prolongation • Application site reactions (patch only) Serious: • Serotonin syndrome; neuroleptic malignant syndrome-like events	40 µg/kg intravenous as a single daily dose or 40 µg/kg/dose oral q12h (max of 2 mg/dose) Transdermal patch: Apply 3.1 mg patch to the upper outer arm a minimum of 24 h (but no more than 48 h) before chemotherapy, and remove the patch a minimum of 24 h after completion of chemotherapy (patch can be worn up to 7 days; safety and efficacy not established in pediatric patients)	Renal: No adjustment necessary Hepatic: No recommendations, but consider that clearance may be decreased in patients with hepatic impairment
Palonosetron (Aloxi®)	Same as ondansetron	Common: • Headache, malaise/fatigue • Constipation • QTc interval prolongation Serious: • Serotonin syndrome; neuroleptic malignant syndrome-like events	20 µg/kg (max dose 1.5 mg) IV once approximately 30 min prior to starting chemotherapy Has been shown to be efficacious at fixed dosing of 0.25 mg in children 2–15 years of age	Renal: No adjustment necessary Hepatic: No adjustment necessary

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Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Dexamethasone (Decadron®)	Class: Corticosteroid Mechanism: Reverses capillary permeability and lysosomal stabilization preventing or controlling inflammation	Common: • Increased risk of infection • Increased appetite • Hypertension • Hyperglycemia • Hyperlipidemia • Confusion/psychosis/agitation • Edema/weight gain • Cushing appearance • HPA axis suppression Severe: • GI ulceration • Osteoporosis/avascular necrosis • Cataracts	IV/oral: 6 mg/m2/dose every 6–12 h Dose should be reduced by 50% if also receiving aprepitant/fosaprepitant	Renal: No adjustments necessary Hepatic: No adjustments necessary
Lorazepam (Ativan®)	Class: Benzodiazepine Mechanism: Binds to GABA receptors in the central nervous system resulting in antiemetic, anxiolytic, and amnesic properties	Common: • Drowsiness and fatigue • Hypotension • Potential for physical or psychological dependence Serious: • Respiratory depression	Up to 0.05 mg/kg IV or oral per dose (max of 2 mg) every 6 h as needed	Renal: Consider using lowest effective dose in patients with mild to moderate renal dysfunction Hepatic: Consider using lowest effective dose in patients with mild to moderate hepatic dysfunction
Chlorpromazine (Thorazine®)	Class: Phenothiazine Mechanism: Dopamine receptor antagonist with strong antiadrenergic and weaker peripheral anticholinergic activity with slight antihistaminic and antiserotonin activity	Common: • CNS depression, drowsiness • Dry mouth • Hypotension, EKG changes, and cardiac arrhythmias • Extrapyramidal effects (diphenhydramine or benztropine may help prevent)/tardive dyskinesia Serious: • Neuroleptic malignant syndrome • Leukopenia/neutropenia and agranulocytosis	0.5–1 mg/kg (max of 50 mg) IV or oral every 6 h	Renal: Use with caution Hepatic: Use with caution

Promethazine (Phenergan®)	Class: Phenothiazine Mechanism: Blocks dopamine, histamine, and muscarinic receptors	Common: • Sedation • Extrapyramidal symptoms • Hypotension • Dry mouth and secretions Serious: • Bone marrow suppression • Seizures • Respiratory depression; caution should be used for patients <2 years old	0.25–1 mg/kg (max of 25 mg) IV or oral every 6 h	Renal: No adjustment necessary Hepatic: Avoid use in pediatric patients whose signs and symptoms may suggest hepatic diseases
Metoclopramide (Reglan®)	Class: Prokinetic Mechanism: Dopamine receptor antagonist that blocks stimulation of the medullary chemoreceptor trigger zone. Also can block serotonin receptors at higher doses	Common: • Diarrhea • Akathisia, extrapyramidal reactions/tardive dyskinesia • Sedation Serious: • Neuroleptic malignant syndrome	1 mg/kg/dose, followed by 0.0375 mg/kg/dose IV or oral every 6 h Alternatively, 0.1–2 mg/kg/dose IV or oral every 6 h has been used Diphenhydramine should be used concurrently to prevent extrapyramidal adverse effects	Renal: For patients with CrCl <40 mL/min, initiate therapy at half the dose and may increase or decrease as appropriate Hepatic: No adjustment necessary
Diphenhydramine (Benadryl®)	Class: Antihistamine Mechanism: Competitive histamine antagonist possessing anticholinergic properties	Common: • Sedation, disturbed coordination, confusion • Dry mouth and secretions • Agitation in children Serious: • Tachycardia	1–1.25 mg/kg/dose (max of 50 mg) IV or oral every 6 h	Renal: No adjustment necessary Hepatic: No adjustment necessary
Olanzapine (Zyprexa®)	Class: Atypical antipsychotic Mechanism: Blocks multiple neurotransmitters, including dopamine at the D2 and serotonin at the 5-HT3 receptors	Common: • Sedation • Increased appetite, weight gain • Extrapyramidal symptoms • Hypotension, arrhythmias Serious: • Hepatotoxicity • Bone marrow suppression • Seizures	0.1 mg/kg/dose (max of 10 mg) orally once daily	Renal: No dosage adjustment required. Not removed by dialysis Hepatic: Use with caution. Dosage adjustment may be necessary; however, no specific recommendations exist

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Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Dronabinol (Marinol®)	Class: Cannabinoid Mechanism: Cannabinoid-1 receptor agonistic mechanism, indirectly inhibiting emetogenic neurotransmitter release	Common: • Psychotropic effects • Sedation, dizziness, ataxia • Nausea and vomiting Serious: • Paranoid reactions	2.5–5 mg/m^2 orally 1–3 h prior to chemotherapy then every 6 h as needed (max dose of 15 mg/m^2)	Renal: No dosage adjustment required Hepatic: No dosage adjustment required
Nabilone (Cesamet®)	Same as dronabinol	Common: • Psychotropic effects • Sedation, dizziness, ataxia • Nausea and vomiting Serious: • Paranoid reactions	<18 kg: 0.5 mg orally twice daily 18–30 kg: 1 mg orally twice daily >30 kg: 1 mg three times daily	Renal: No dosage adjustment required Hepatic: No dosage adjustment required
Scopolamine (Transderm Scop®)	Class: Anticholinergic Mechanism: Blocks histamine, muscarinic, and serotonergic receptors	Common: • Headache, sedation, confusion • Dry mouth and secretions Serious: • Hallucinations	For patients at least 20 kg: Apply 1 patch (1.5 mg patch delivers approximately 1 mg) transdermally every 72 h	Renal: No dosage adjustments available, but caution is recommended due to increased risks of adverse effects Hepatic: No dosage adjustments available but caution is recommended due to increased risks of adverse effects
Aprepitant (Emend®)	Class: Neurokinin-1 (NK1) receptor antagonist Mechanism: Blocks substance P from binding to the NK1 receptor, preventing acute and delayed emesis	Common: • Fatigue • Hiccups Serious: • Swelling	• 6 months to <12 years old 3 mg/kg up to 125 mg orally on day 1 and then 2 mg/kg up to 80 mg orally on days 2 and 3 • 12 years and older: 125 mg orally on day 1 and 80 mg orally on days 2 and 3	Renal: No adjustment necessary Hepatic: No adjustment necessary

Pain Medications

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Morphine (MS Contin®, Kadian®, Astramorph®, Duramorph®)	Class: Opioid agonist Mechanism: Opioid agonist, binding to opioid receptors in the central nervous system, inhibiting the transmission of pain and general central nervous system depression	Common: • Nausea/vomiting, constipation • Pruritis • Sedation • Urinary retention Serious: • Hypotension, bradycardia • Respiratory depression • Physical dependence • Psychological dependence	Dosing in opioid-naïve patients: Oral: 0.2–0.5 mg/kg/dose (max of 15 mg) every 3–4 h as needed IV: 0.05–0.1 mg/kg/dose (2–5 mg) every 2 to 4 h as needed Patient-controlled analgesia initial settings: Basal rate: 0–0.02 mg/kg/h (initial max of 1 mg/h) On demand: 0.015–0.02 mg/kg/dose every 5–15 min (initial max of 1 mg/dose)	Renal: M6G (active) and M3G (inactive but CNS toxic) metabolites can accumulate in renal dysfunction. Avoid use in severe renal impairment Hepatic: Use lower doses in hepatic dysfunction
Hydrocodone (Hysingla®, Zohydro®, Lortab®)	Same as morphine	Same as morphine	Opioid-naïve patients: Usually used in products combined with acetaminophen in pediatrics Oral: 0.1–0.2 mg/kg/dose (5–10 mg) every 4–6 h as needed	Renal: Use lower doses in moderate to severe renal impairment Hepatic: Use lower doses in hepatic dysfunction
Hydromorphone (Dilaudid®, Exalgo®)	Same as morphine	Same as morphine	Opioid-naïve patients: Oral: 0.03–0.08 mg/kg/dose (1–2 mg) every 3–4 h as needed IV: 0.01–0.015 mg/kg/dose (0.2–0.4 mg) every 2–4 h as needed Patient-controlled analgesia initial settings: Basal rate: 0–0.004 mg/kg/h (initial max of 0.2 mg/h) On demand: 0.003–0.004 mg/kg/dose every 5–15 min (initial max of 0.2 mg/dose)	Renal: Use lower doses in moderate to severe renal impairment Hepatic: Use lower doses in hepatic dysfunction

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Oxycodone (Oxycontin®)	Same as morphine	Same as morphine	Opioid-naïve patients: Oral: 0.05–0.2 mg/kg/dose (5–10 mg) every 4–6 h as needed	Renal: Use lower doses in moderate to severe renal impairment Hepatic: Use lower doses in hepatic dysfunction
Fentanyl (Duragesic®)	Same as morphine	Same as morphine Rare risk of chest wall rigidity	Opioid-naïve patients: IV: 0.5–2 µg/kg/dose (25–50 µg) every 30–60 min as needed Patient-controlled analgesia initial settings: Basal rate: 0–1 µg/kg/h (initial max of 50 µg/h) On demand: 0.25 µg/kg/dose every 5–10 min (initial max of 20 µg/dose) Transmucosal and transdermal dosage forms not appropriate in opioid-naïve patients	Renal: No adjustments necessary Hepatic: Use lower doses in hepatic dysfunction
Methadone (Dolophine®)	Same as morphine Also possesses N-methyl-D-aspartate antagonist activity which potentially may decrease tolerance and improve efficacy in neuropathic pain	Same as morphine Risk of QTc prolongation/torsades de pointes	Opioid-naïve patients: Oral: 0.05–0.1 mg/kg/dose (2.5–5 mg) every 6 h as needed IV: 0.05–0.1 mg/kg/dose (2.5–5 mg) every 6 h as needed Note: Oral and IV dosing will cause accumulation with repeat dosing	Renal: Use lower doses in moderate to severe renal impairment Hepatic: Avoid use in hepatic dysfunction
Naloxone (Narcan®)	Class: Opioid antagonist Mechanism: Opioid antagonist that displaces opioids from opioid receptors. At low doses can inhibit adverse opioid effects such as pruritis, nausea, and vomiting with minimal effects on analgesia	Common: • Symptoms of opioid withdrawal, e.g., diarrhea, sweating, tachycardia, hypertension, nausea, and vomiting	IV: Opioid reversal: 0.01–0.1 mg/kg/dose (0.4–1 mg) every 2–3 min as needed Opioid-induced pruritis: 0.25–0.5 µg/kg/h continuous infusion	Renal: No adjustments necessary Hepatic: No adjustments necessary

Tramadol (Ultram®)	Class: Opioid agonist Mechanism: Parent drug and its active metabolite are weak opioid agonists which bind to opioid receptors in the central nervous system, inhibiting the transmission of pain. Also inhibits the reuptake of serotonin and norepinephrine causing additional pain relief (potentially neuropathic)	Common: • Sedation, dizziness • Agitation, euphoria • Pruritis • Nausea and vomiting, constipation Serious: • Serotonin syndrome • Seizures	Oral: 1–2 mg/kg/dose (50–100 mg) every 4–6 h as needed Maximum of 8 mg/kg/day (400 mg)	Renal: CrCl <30 mL/min: Give no more frequently than every 12 h Hepatic: Hepatic impairment: 1 mg/kg (50 mg) no more frequently than every 12 h
Celecoxib (Celebrex®)	Class: NSAID Mechanism: Decreases cyclooxygenase-2, decreasing prostaglandin synthesis. Antipyretic, analgesic, and anti-inflammatory effects. Minimal effect on platelets	Common: • Peripheral edema • Nausea and vomiting Serious: • Thrombosis • GI ulcer • Hemorrhage • Renal failure	Oral: 10 kg to <25 kg: 50 mg bid 25 kg to <60 kg: 100 mg bid ≥60 kg: 100–200 mg daily or bid	Renal: Avoid use in renal dysfunction Hepatic: • Decrease dose by 50% in mild–moderate hepatic dysfunction • Avoid use in severe hepatic dysfunction
Choline Magnesium Trisalicylate (Trilisate®)	Class: NSAID, salicylate Mechanism: Decreases cyclooxygenase, decreasing prostaglandin synthesis. Antipyretic, analgesic, and anti-inflammatory effects. Minimal effect on platelets	Common: • Nausea and vomiting • Tinnitus Serious: • GI ulcer • Hemorrhage • Renal failure	Oral: 10–25 mg/kg (1500–3000 mg) every 8–12 h as needed	Renal: Avoid use in renal dysfunction Hepatic: Avoid use in hepatic dysfunction
Gabapentin (Neurontin®)	Class: Anticonvulsant Mechanism: Blocks L-type, voltage-dependent calcium channels in the central nervous system, decreasing the release of excitatory neurotransmitters, possesses analgesic and anticonvulsant properties	Common: • Sedation, ataxia • Peripheral edema • Rash Serious: • Behavior changes (aggression)	Oral: 10–40 mg/kg/day (900–3600 mg) divided three times daily. Daily doses usually titrated up to goal over 3–10 days	Renal: • CrCl 30–59 mL/min: Decrease daily dose by 60% • CrCl 15–29 mL/min: Decrease daily dose by 75% • CrCl <15 mL/min: Decrease daily dose by 90% Hepatic: No adjustments necessary
Acetaminophen (Tylenol®, Ofirmev®)	Class: Non-opioid analgesic Mechanism: Inhibits prostaglandins in the central nervous system, causing decreased pain transmission and antipyretic effects	Common: None Serious: • Hepatotoxicity	Oral/IV: 10–15 mg/kg/dose (325–1000 mg) every 4–6 h as needed. Max of 75 mg/kg/day (4000 mg)	Renal: • CrCl <10 mL/min: Give no more frequently than every 8 h Hepatic: Avoid use in hepatic dysfunction

Cardiac Medications

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Amlodipine (Norvasc®)	Class: Dihydropyridine calcium channel blocker Peripheral arterial vasodilation, decreased peripheral vascular resistance, and decreased blood pressure. Long acting	Common: • Peripheral edema, flushing • Fatigue Serious: • Interstitial nephritis	Oral: 0.06–0.3 mg/kg (max of 10 mg) once daily	Renal: No adjustments necessary Hepatic: Consider lower starting doses in hepatic impairment
Isradipine (Dynacirc®)	Same as amlodipine (intermediate duration)	Common: • Peripheral edema • Flushing • Fatigue • Tachycardia Serious: • None	Oral: 0.05–0.8 mg/kg/day (max of 20 mg) divided 2–4 times daily	Renal: Consider lower starting doses in renal impairment Hepatic: Consider lower starting doses in hepatic impairment
Nifedipine (Procardia®)	Same as amlodipine (short duration of action, unless extended release formulation)	Common: • Peripheral edema • Flushing • Fatigue • Tachycardia Serious: • Syncope, severe hypotension	Oral: Immediate release: 0.1–0.6 mg/kg/dose (max of 20 mg) every 4–6 h Extended release: 0.25–3 mg/kg/day (max of 120 mg) once daily or divided twice daily	Renal: No adjustments necessary Hepatic: Consider lower starting doses in hepatic impairment
Clonidine (Catapres®)	Class: Alpha-2 adrenergic agonist Mechanism: Decreases peripheral vascular resistance, blood pressure, and heart rate	Common: • Bradycardia • Dry mouth • Sedation, fatigue Serious: • Rebound hypertension (if stopped abruptly)	Oral: 5–25 μg/kg/day (max of 900 μg) divided 2–3 times daily Transdermal Patch: 5–25 μg/kg/day (max of 900 μg) applied once every 7 days	Renal: Consider lower starting doses in renal impairment Hepatic: No adjustments necessary

Enalapril (Vasotec®)	Class: Angiotensin-converting-enzyme inhibitor Mechanism: Decreases angiotensin-II-mediated vasoconstriction, renin, and aldosterone activity (moderate duration)	Common: • Cough • Fatigue • Rash • Hyperkalemia Serious: • Angioedema • Nephrotoxicity • Hepatotoxicity • Bone marrow suppression	Oral: 0.08–0.6 mg/kg/day (max of 40 mg) once daily	Renal: • CrCl 10–50 mL/min: Decrease daily dose by 25% • CrCl <10 mL/min: Decrease daily dose by 50% Hepatic: No adjustments necessary
Captopril (Capoten®)	Same as enalapril (short duration)	Same as enalapril	Oral: 0.3–6 mg/kg/day (max of 450 mg) divided 2–3 times daily	Renal: • CrCl 10–50 mL/min: Decrease daily dose by 25% • CrCl <10 mL/min: Decrease daily dose by 50% Hepatic: No adjustments necessary
Lisinopril (Prinivil®, Zestril®)	Same as enalapril (long duration)	Same as enalapril	Oral: 0.07–0.6 mg/kg/day (max of 40 mg) once daily	Renal: • CrCl 10–50 mL/min: Decrease daily dose by 50% • CrCl <10 mL/min: Decrease daily dose by 75% Hepatic: No adjustments necessary
Losartan (Cozaar®)	Class: Angiotensin-II receptor antagonist Mechanism: Decreases vasoconstriction and aldosterone activity	Common: • Fatigue • Hyperkalemia Serious: • Angioedema • Nephrotoxicity	Oral: 0.7–1.4 mg/kg/day (max of 100 mg) once daily	Renal: Avoid use if CrCl <30 mL/min Hepatic: Consider lower starting doses in hepatic impairment
Candesartan (Atacand®)	Same as losartan	Same as losartan	Oral: 0.2–0.4 mg/kg/day (max of 32 mg) divided once or twice daily	Renal: Avoid use if CrCl <30 mL/min Hepatic: Consider lower starting doses in hepatic impairment
Valsartan (Diovan®)	Same as losartan	Same as losartan	Oral: 1.3–2.7 mg/kg day (max of 320 mg) once daily	Renal: Avoid use if CrCl <30 mL/min Hepatic: Avoid use in hepatic dysfunction

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Propranolol (Inderal®)	Class: Beta adrenergic blocker Mechanism: Beta-1 and beta-2 adrenergic blockers, decreasing blood pressure, heart rate, and myocardial contractility	Common: • Bradycardia • Fatigue, nightmares, and insomnia • Peripheral vasoconstriction Serious: • Rebound tachycardia and hypertension if abruptly stopped • Masking and worsening symptoms of hypoglycemia • Worsening of preexisting heart failure • Bronchospasm	Oral: Immediate Release: 1–16 mg/kg/day (max 640 mg) divided twice or three times daily Sustained release capsules: 1–16 mg/kg/day (max 640 mg) once daily	Renal: Consider lower starting doses in renal impairment Hepatic: Consider lower starting doses in hepatic impairment
Atenolol (Tenormin®)	Class: Beta adrenergic blocker Mechanism: Beta-1-selective adrenergic blocker, decreasing blood pressure, heart rate, and myocardial contractility	Common: • Bradycardia • Fatigue Serious: • Rebound tachycardia and hypertension if abruptly stopped • Masking symptoms of hypoglycemia • Worsening of preexisting heart failure	Oral: 0.5–2 mg/kg/day (max of 100 mg) divided once or twice daily	Renal: • CrCl 30–50 mL/min: max of 1 mg/kg (50 mg) every 24 h • CrCl <30 mL/min: max of 1 mg/kg (50 mg) every 48 h Hepatic: No adjustments necessary
Metoprolol (Lopressor®, Toprol®)	Class: Beta adrenergic blocker Mechanism: Beta-1-selective adrenergic blocker, decreasing blood pressure, heart rate, and myocardial contractility	Common: • Bradycardia • Fatigue Serious: • Rebound tachycardia and hypertension if abruptly stopped • Masking symptoms of hypoglycemia • Worsening of preexisting heart failure	Oral: Tartrate (immediate release): 1–6 mg/kg/day (max of 200 mg) divided twice daily Succinate (extended release): 1–6 mg/kg/day (max of 200 mg) once daily	Renal: No adjustments necessary Hepatic: Consider lower starting doses in hepatic impairment

Labetalol (Trandate®, Normodyne®)	Class: Adrenergic blocker Mechanism: Alpha, beta-1, and beta-2 adrenergic blockers, decreasing blood pressure, heart rate, and myocardial contractility	Common: • Bradycardia (less than other beta adrenergic blockers) • Fatigue • Peripheral vasoconstriction Serious: • Rebound tachycardia and hypertension if abruptly stopped • Masking and worsening symptoms of hypoglycemia • Worsening of preexisting heart failure • Bronchospasm	Oral: 1–12 mg/kg/day (max of 1200 mg) divided twice daily IV: For severe hypertension: 0.2–1 mg/kg (max of 40 mg) every 10 min (max of 300 mg cumulative dose)	Renal: No adjustments necessary Hepatic: Consider lower starting doses and use with caution in hepatic impairment
Carvedilol (Coreg®)	Class: Adrenergic blocker Mechanism: Alpha, beta-1, and beta-2 adrenergic blockers, decreasing blood pressure, heart rate, and myocardial contractility.	Common: • Bradycardia • Fatigue • Peripheral vasoconstriction Serious: • Rebound tachycardia and hypertension if abruptly stopped • Masking and worsening symptoms of hypoglycemia • Worsening of preexisting heart failure • Bronchospasm	Oral: Immediate release: 0.1–0.5 mg/kg/day (max of 50 mg) divided twice daily Extended release: 0.1–0.5 mg/kg/day (max of 80 mg) once daily	Renal: Consider lower starting doses in severe renal disease Hepatic: • Consider lower starting doses and use with caution in mild–moderate hepatic impairment • Contraindicated in severe hepatic impairment
Hydralazine (Apresoline®)	Class: Vasodilator Mechanism: Arteriole vasodilator, decreasing blood pressure	Common: • Tachycardia • Rash, pruritis • Headache Serious: • Lupus-like syndrome • Bone marrow suppression	Oral: 0.25–7.5 mg/kg/day (max of 200 mg) divided 3–4 times daily IV: 0.1–0.6 mg/kg/dose (max of 20 mg/dose) every 4–6 h	Renal: • CrCl 10–50 mL/min: Give no more frequently than every 8 h • CrCl <10 mL/min: Give no more frequently than every 12 h Hepatic: Consider lower starting doses in hepatic impairment

Diuretics

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Furosemide (Lasix®)	Class: Loop diuretic Mechanism: Inhibits sodium and chloride reabsorption in the Loop of Henle and distal renal tubule. Results in increased secretion of sodium, chloride, water, magnesium, calcium, and potassium	Common: • Hypokalemia, hypomagnesemia, hypocalcemia, hypophosphatemia, hyponatremia, hypochloremia • Hypotension • Photosensitivity Serious: • Ototoxicity • Nephrotoxicity • Anaphylaxis	Oral: 1–2 mg/kg/dose (max of 80 mg) every 6–24 h as needed IV: 1–2 mg/kg/dose (max of 80 mg) every 6–24 h as needed *(IV is more potent than oral)*	Renal: May need higher doses in renal dysfunction. Avoid use if oliguric Hepatic: Decreased diuretic effect, with increased hypokalemia in hepatic dysfunction
Bumetanide (Bumex®)	Same as furosemide	Same as furosemide	Oral: 0.015–0.1 mg/kg/dose (max of 10 mg/day) every 6–24 h as needed IV: 0.015–0.1 mg/kg/dose (max of 10 mg/day) every 6–24 h as needed *(IV is more potent than oral)* Note: Approximately 40 times more potent than furosemide	Renal: May need higher doses in renal dysfunction. Avoid use if oliguric/anuria Hepatic: Avoid use in severe hepatic dysfunction
Hydrochlorothiazide (Microzide®)	Class: Thiazide diuretic Mechanism: Inhibits sodium reabsorption in the distal renal tubule. Results in increased secretion of sodium, water, and potassium	Common: • Hypokalemia, hypomagnesemia, hyponatremia • Hypercalcemia • Photosensitivity Serious: • Rare hypersensitivity	Oral: 1–3 mg/kg/day (max of 200 mg) divided once or twice daily	Renal: • CrCl 10–30 mL/min: Ineffective unless combined with a loop diuretic • CrCl <10 mL/min: Avoid use; ineffective Hepatic: Avoid use in severe hepatic dysfunction, can cause hyponatremia

Chlorothiazide (Diuril®)	Same as hydrochlorothiazide	Same as hydrochlorothiazide	Oral: 10–40 mg/kg/day (max of 2000 mg) divided once or twice daily IV: 5–20 mg/kg/day (max of 1000 mg) divided once or twice daily	Renal: • CrCl 10–30 mL/min: Ineffective unless combined with a loop diuretic • CrCl <10 mL/min: Avoid use; ineffective Hepatic: Avoid use in severe hepatic dysfunction, can cause hyponatremia
Metolazone (Zaroxolyn®)	Same as hydrochlorothiazide May be more effective than other thiazide diuretics in renal insufficiency	Same as hydrochlorothiazide	Oral: 0.05–0.4 mg/kg/day (max of 20 mg) divided once or twice daily	Renal: CrCl <10 mL/min: Avoid use Hepatic: Avoid use in severe hepatic dysfunction, can cause hyponatremia
Hydrochlorothiazide (Microzide®)	Class: Thiazide diuretic Mechanism: Inhibits sodium reabsorption in the distal renal tubule. Results in increased secretion of sodium, water, and potassium	Common: • Hypokalemia, hypomagnesemia, hyponatremia • Hypercalcemia • Photosensitivity Serious: • Rare hypersensitivity	Oral: 1–3 mg/kg/day (max of 200 mg) divided once or twice daily	Renal: • CrCl 10–30 mL/min: Ineffective unless combined with a loop diuretic • CrCl <10 mL/min: Avoid use; ineffective Hepatic: Avoid use in severe hepatic dysfunction, can cause hyponatremia
Chlorothiazide (Diuril®)	Same as hydrochlorothiazide	Same as hydrochlorothiazide	Oral: 10–40 mg/kg/day (max of 2000 mg) divided once or twice daily IV: 5–20 mg/kg/day (max of 1000 mg) divided once or twice daily	Renal: • CrCl 10–30 mL/min: Ineffective unless combined with a loop diuretic • CrCl <10 mL/min: Avoid use; ineffective Hepatic: Avoid use in severe hepatic dysfunction, can cause hyponatremia

(continued)

Medication (brand)	Mechanism of action	Common/serious toxicities	Common dosing/therapeutic drug monitoring	Renal/hepatic adjustments
Metolazone (Zaroxolyn®)	Same as hydrochlorothiazide May be more effective than other thiazide diuretics in renal insufficiency	Same as hydrochlorothiazide	Oral: 0.05–0.4 mg/kg/day (max of 20 mg) divided once or twice daily	Renal: CrCl <10 mL/min: Avoid use Hepatic: Avoid use in severe hepatic dysfunction, can cause hyponatremia
Chlorthalidone (Thalitone®)	Class: Thiazide diuretic Mechanism: Inhibits sodium and chloride reabsorption in the ascending loop of Henle. Results in increased secretion of sodium, water, and potassium	Same as hydrochlorothiazide	Oral: 0.3–0.2 mg/kg/day (max of 50 mg) once daily	Renal: • CrCl 10–30 mL/min: Ineffective unless combined with a loop diuretic • CrCl <10 mL/min: Avoid use; ineffective Hepatic: • Avoid use in severe hepatic dysfunction, can cause hyponatremia
Spironolactone (Aldactone®)	Class: Potassium-sparing diuretic Mechanism: Blocks effects of aldosterone in the distal renal tubule leading to increased sodium chloride and water excretion while retaining potassium	Common: • Hyperkalemia • Fatigue • Rash Serious: • Gynecomastia (irreversible)	Oral: 1–3.3 mg/kg/day (max of 200 mg) divided every 6–24 h	Renal: • CrCl: 10–50 mL/min: Administer no more frequently than every 12 h • CrCl <10 mL/min: Avoid use Hepatic: No adjustments necessary

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Index

A
ABO incompatibility, 126–127
  bidirectional, management of, 129
  donor/recipient, 287, 288
  hemolytic complications, 288
  management of, 127–129
  minor, management of, 129
Absolute neutrophil count (ANC), 188
Acetaminophen, 148, 212, 437
Acinus, 218
Acquired tolerance, 9
Acrolein, 236
Active immunity, 45
Acute graft-versus-host disease, 258, 264
  clinical features, 263
  diagnostic studies, 264
  grading scheme, 258
  management/prognosis, 264–265
  pathogenesis, 259
  prevention/prophylaxis, 259–263
  risk factors, 259
Acute irreversible leukoencephalopathy, 352
Acute kidney injury (AKI), 327, 328
Acute lymphoblastic leukemia (ALL), 12
  conditioning therapy, 153–154
  pretransplantation
    CR2, 58, 59
    CR3 and beyond, 59
    first complete remission (CR1), 58–59
    relapse, 59
Acute myeloid leukemia (AML), 86, 87
  conditioning therapy, 155
  pretransplantation, 59–60
Acute neurotoxicities, 347
Acute toxic leukoencephalopathy, 347
Acute tubular dysfunction, 329
Acyclovir, 348, 425
Acyclovir prophylaxis, 245
Adaptive immunity, 20, 40, 42
  cell-mediated immunity, 45
  humoral immunity, 44
  reconstitution of
    B cells, 377–378
    DCs, 377
    T cell, 375–377
Adenosine deaminase (ADA) deficiency, 67
Adenovirus, 236, 249, 329
Adult hematopoietic organs, 23
AIHA, *see* Autoimmune hemolytic anemia
Alanine transaminase (ALT), 216
Aldophosphamide, 148
Alemtuzumab, 130, 159–161, 347, 409
Alkylator chemotherapy, 147, 387
ALL, *see* Acute lymphoblastic leukemia
Alloantigens, 21, 45
Allogeneic, 46
Allogeneic hematopoietic stem cell transplantation, 78
Alloimmune hemolytic anemia, 284
Alloimmune hemolysis, 287
Alloimmunization, 290
Alloreactive T-cells, 271
Alloreactivity, 45
Allosensitization, 191
Alopecia, 364, 367
Alternative donors, 102
Amikacin, 421
Aminoglycosides, 329
AML, *see* Acute myeloid leukemia
Amlodipine, 438
Amphotericin B deoxycholate, 424
Amphotericin B lipid complex, 424
Amphotericin B liposomal, 424
Anthracyclines, 333
Antibacterial prophylaxis, 253
Antibacterials, 414–421
Antibodies, 44
Anticytokine therapy, 197
Antiemetics, 431–434
Antifibrinolytics, 292
Antifungal prophylaxis, 254
Antifungal therapy, 311
Antigen, 21, 44

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Antigen presentation, 45
Antigen-presenting cells (APC), 27, 259
  B-cells, 28
  macrophages, 28
  non-professional APCs, 28
Antimetabolite chemotherapy, 150
Anti-pneumocystis agents, 429–430
Anti-thymocyte globulin (ATG), 130, 192, 347, 375
Antithymocyte immune globulin, 409
Antiviral, 425–428
Antiviral prophylaxis, 253
Aorta-gonad-mesonephros (AGM) region, 22
APC, *see* Antigen-presenting cells (APC)
Apheresis, 113, 125, 127
Aplastic anemia, conditioning therapy, 158
Aprepitant, 434
Arrhythmias, 336
Ascites, 211
Aspartate aminotransferase (AST), 216
Aspergillosis, 310, 338
Aspergillus spp., 252
  fungal pneumonia, 309
  neurologic complications, 348–349
Atenolol, 440
Atovaquone, 430
Audiogram, 94
Audiometric testing, 356
Autoantibody, 285
Autoimmune hemolytic anemia (AIHA), 284
  alloimmune hemolysis, 287
  diagnosis and management, 285
  diagnostic criteria, 285
  differential diagnosis, 284
  etiology, 285
  risk factors, 285
  treatment, 286
Autologous, 45
Autologous donor, 119
Autologous stem cell transplant (ASCT), 61
Avascular necrosis (AVN), 395, 396
Azathioprine, 161, 276, 278
Aztreonam, 416

B

B cells, 377
Bacteremia, 244, 253
Bacteria, 244–245
Bacterial infection
  cardiac complications, 337
  neurologic complications, 349
Bacterial pneumonia, 308, 316
Bactrim, 323
Basiliximab, 413
Basophils, 27
B-cell, 28, 377
BCNU, etoposide, cytarabine, melphalan (BEAM), 143
Beclomethasone, 411
Benzodiazepines, 202
β-D-Glucan, 311
Beta-thalassemia major (BT), 68
Bidirectional ABO incompatibility, management of, 129
Bidirectional incompatibility, 127
BK polyomavirus virus, 236, 250, 330
Bladder inflammation, 235
Bladder irrigation, 238
Blood chemistries, 95
BOOP, *see* Bronchiolitis obliterans organizing pneumonia (BOOP)
Bone marrow, 23, 31
  harvesting, 122
  stroma, 31
Bone marrow transplant (BMT), 12, 373
Bone Marrow Transplant Survivor Study (BMT-SS), 386, 393
Bone mineral density (BMD), 395
Bradycardia, 336
Brain biopsy, 349
Brain tumors
  embryonal, 63
  GCT, 64
Breast cancer, 389
Brincidofovir, 250
Bronchiolitis obliterans (BO), 275
Bronchiolitis obliterans organizing pneumonia (BOOP), 275, 313, 314, 391
Bronchiolitis obliterans syndrome (BOS), 314, 315, 391
Bronchoalveolar lavage (BAL), 306
Budesonide, 411
Buffy coat, 123
Bumetanide, 442
Busulfan, 147, 148, 190, 222, 406

C

Calcineurin inhibitor, 263, 264, 276, 293, 329, 348
Candesartan, 439
Candida spp., 251
  fungal pneumonia, 310
Capillary leak syndrome (CLS), 151, 197
Captopril, 439
Carboplatin (Carbo), 152, 406
Cardiac complications associated with HSCT
  arrhythmias, 336
  bradycardia, 336
  cardiac diagnostic tests and monitoring, 335
  cardiac tamponade, 339
  chemotherapy-induced cardiotoxicity, 334–335
  etiologies, 334
  GvHD-induced cardiac complications, 337
  infection
    bacterial, 337
    fungal, 337–338
    viral, 338
  pericardial effusion, 338–339
  pulmonary hypertension, 339–340
  radiation-induced cardiotoxicity, 335–336

risk factors, 333, 334
tachycardia, 336–337
Cardiac dysfunction, 306, 329, 389
Cardiac medications, 438–441
Cardiac monitoring, long-term follow-up guidelines, 390
Cardiac tamponade, cardiac complications, 339
Cardiac troponins, 334
Cardiotoxicity, 389
Cardiovascular compromise/failure, 226
Cardiovascular disease (CVD), 389
Carmustine (BCNU), 150, 406
Carvedilol, 441
Caspofungin, 423
Cataracts, 357, 358, 396
CD34+ stem cells, 123, 192
Cefepime, 348, 414
Ceftazidime, 415
Celecoxib, 437
Cell-mediated/cellular immunity, 20, 43, 45
Cellular hypothesis, 9
Center for International Blood and Marrow Transplantation Research (CIBMTR), 143, 178
Central nervous system (CNS), 149, 150, 152, 344, 347
infection, neurologic complications, 348
Cerebral vascular events, 350
Chediak-Higashi syndrome, 68
Chemokines, 21, 39, 40
Chemotherapeutics, 406–408
Chemotherapy, 13, 329, 364
Chemotherapy-induced cardiotoxicity, 334
Chemotherapy-induced mucositis, 210
Childhood Cancer Survivor Study (CCSS), 386
Children's Oncology Group (COG) protocols, 78
Chimerism, 182
full versus mixed, 183
implications, 183–184
interventions, 184–185
monitoring time frame, 182–183
testing methods, 182
Chlorothiazide, 443
Chlorpromazine, 432
Chlorthalidone, 444
Choline magnesium trisalicylate, 437
Chromosome 6, 99
Chronic graft-versus-host disease
clinical features, 271–275
diagnostic studies and grading, 275, 276
differential diagnosis, 271
immunosuppressants, 277–279
incidence, 270–271
management and outcome, 276–277
pathophysiology, 271
risk factors, 271
sclerodermatous skin manifestation of, 274
steroid-refractory, treatment, 277–280
supportive measures, 280–281
Chronic granulomatous disease (CGD), 68
Chronic kidney disease (CKD), 327
Chronic leukoencephalopathy, 352
Chronic myelogenous leukemia (CML), 11, 13, 61
Chronic renal failure (CRF), 396
Cidofovir, 238, 250, 329, 331, 428
Cilastatin, 416
Ciprofloxacin, 250, 418
Class switching, 44, 377
Clindamycin, 309, 418
Clobetasol proprionate, 412
Clofarabine (Clo), 151, 152, 406
Clonazepam, 280
Clonidine, 438
Cluster of differentiation (CD) nomenclature, 21
CMV, *see* Cytomegalovirus
Cold agglutinin, 284
Common lymphoid progenitors (CLP), 23
Common myeloid progenitors (CMP), 23
Community-acquired respiratory viral infections
adenoviruses, 321
influenza virus, 319
parainfluenza virus, 320
RSV, 320
Complement, 329
Complete blood cell count (CBC), 95
Complete remission (CR), 77
Conditioning regimen, 345
Conditioning therapy, 139–140
alkylator chemotherapy, 147–150
antimetabolite chemotherapy, 150–152
chemotherapeutic agents, 152
common pediatric conditions, preparative regimens
ALL, 153
AML, 154–156
idiopathic aplastic anemia, 158–159
neuroblastoma, 156–158
SCID, 162–165
sickle cell disease, 159–162
conditioning regimens, classification, 142–145
high-dose chemotherapy, 146
rationale and GVM effect, 141–142
requirement, 140–141
TBI, 145
Consciousness, level of, 348
Contact dermatitis, 364
Cord blood transplantation, 376
Corrected count increment (CCI), 291
Creatinine, 328, 329
Creatinine clearance, 94
Cryopreservation, 131, 181
sperm, 95
stem cell products, 131
thawing, stem cells, 132
CXC-chemokine ligand 12 (CXCL12)-abundant reticular (CAR) cells, 34
CXCL12, 32–34, 36, 37
CXCR4, 32, 37, 125
Cyclophosphamide (Cy), 148, 149, 154, 236, 333, 407
Cyclophosphamide-induced cardiac damage, 334
Cyclophosphamide-induced cardiotoxicity, 334
Cyclosporine, 280, 410

Cyclosporine A, 347
Cystatin C, 328
Cystectomy, 238
Cystitis, 236
Cystoscopy, 238
Cytarabine, 151, 407
Cytokine, 20, 21, 24
  engraftment syndrome, 196
  storm, 181, 196
Cytomegalovirus (CMV), 95, 112, 236, 245, 318, 329
  immunoglobulin, 428

D
Dapsone, 430
Daptomycin, 417
Deep nasal pharyngeal swab, 95
Deferoxamine, 217
Defibrotide, 226–229, 293, 414
Dehydration, 329
Dendritic cells (DCs), 20, 23, 27, 40, 46, 281, 377
Density gradient media, 128
Dental assessment, 94
Dental complications, 397
Dexamethasone, 432
Diarrhea, 210, 264, 365
Diffuse alveolar hemorrhage (DAH), 307, 308
Diffuse intrinsic pontine glioma (DIPG), 65
Diffusing capacity of the lungs for carbon monoxide (DLCO), 94
Dimethyl sulfoxide (DMSO), 131, 132, 336
Diphenhydramine, 202, 433
Direct antiglobulin test (DAT), 284
Disease-free survival (DFS), 107, 153
Diuretics, 227, 306, 442–444
DLI, *see* Donor lymphocyte infusion
DMSO, *see* Dimethyl sulfoxide
Dog leukocyte antigen (DLA) system, 10
Donor eligibility, 112, 132
Donor lymphocyte infusion (DLI), 13, 142, 184, 270
Donor selection
  American Academy of Pediatrics Committee on Bioethics, 120
  autologous donor, 119
  consent, issue of, 119
  eligibility, suitability, and safety, 112–119
  informed consent, 119
  laboratory evaluation and, 113, 119
Donor suitability, 112, 133
Donor-derived T-cells, 270
Donor-related immune-mediated graft rejection, 191
Donor-specific antibodies (DSAs), 192
Doppler, 223, 224
Dronabinol, 434
Drug allergy, 364
Drug-induced renal toxicity, 329
Dry eye syndrome (DES), 357, 397
Dysgeusia, 359
Dysuria, 235

E
EBV-posttransplant lymphoproliferative disease, 250
Echinocandin, 310
Echocardiogram (EHCO), 93, 334
Eculizumab, 293, 330
Effector cell, 21, 36
Ejection fraction, 334, 336
Electrocardiogram (ECG), 94, 335
Electroencephalogram (EEG), 351
Electrolyte, 95
Electrolyte wasting, 328
Embryonal brain tumors, pretransplantation, 63–64
Enalapril, 439
Encephalopathy, 351
Endocarditis, 337
Endocrine-related complications, 392
Endocrine-related supportive care, 281
Engraftment, 103, 105, 107, 178
  factors
    bone marrow microenvironment of recipient, 181
    clinical manifestations, 181
    graft manipulation, 180–181
    GvHD, 181
    HSC dose, 180
    HSC source, 179
  implications, 181
  mechanism, 178–179
  neutrophil and platelet, 179
  timing of, 179
Engraftment syndrome (ES), 196, 306, 307, 364
  cytokine profile, 196–197
  management, 197
  pathophysiology of, 196
Enormous antigenic diversity, 112
Enteral nutrition, 205
Eosinophils, 27
Ependymoma, 64
Epstein–Barr virus (EBV), 95, 250, 388
Erythroderma, 162, 196
ES, *see* Engraftment syndrome
Etanercept, 313, 412
Etoposide (VP-16), 152, 407
Etoposide phosphate, 407
European Society for Blood and Marrow Transplantation (EBMT), 8
Ex vivo cell manipulation, 129
Ex vivo T-cell depletion, 376
Extracellular matrix (ECM), 31, 33, 37
Extracorporeal photopheresis (ECP), 264, 279
Eyes, chronic GvHD, 274

F
Fentanyl, 436
Fertility, 394
  preservation, 95
Ficoll-Hypaque, 128
Filgrastim, 178
Flow cytometry, 58, 78, 79, 101, 378

Flt-3 ligand, 25, 27
Fluconazole, 248, 310, 311, 422
Fludarabine (Flu), 142, 151, 347, 408
Fluid balance, 197
Flu-like syndrome, 181
Fluorescence in situ hybridization (FISH), 182
Folliculitis, 364
Foscarnet, 250, 329, 349, 427
Foundation for the Accreditation of Cellular Therapy (FACT), 178
Fulguration, 238
Fungal infection
  cardiac complications, 337
  neurologic complications, 348
Fungal pneumonia, 309
  Aspergillus, 309–310
  Candida, 310–311
  Zygomycetes, 311
Fungal pulmonary infections, 322
Fungus, 251–253
  Aspergillus spp., 252
  Candida spp., 251–252
  Zygomycetes spp., 252–253
Furosemide, 227, 442

G
Gabapentin, 437
Galactomannan, 254
Ganciclovir, 318, 349, 426
Gastrointestinal (GI) tract
  acute GvHD, 258, 263, 264
  chronic GvHD, 275
Gentamicin, 419
Germ cell tumors (GCT), 64–65
Glomerular disease, 331
Glomerular filtration rate (GFR), 328
Glutamine, 211
Gonadal failure, 95, 393, 394
Graft engineering, 129–131
Graft failure, 105, 188
  age, 190
  diagnostic studies, 192
  donor HSC source, 189–190
  etiologies, 188
  graft rejection, 191–192
  HSC dose and graft manipulation, 190
  incidence, 188
  interventions and outcomes, 192–193
  risk factors, 188, 189
  strategies, decrease risk of, 192
  underlying disease/disorder, 190–191
Graft rejection, 4, 9, 47, 48, 160, 190, 191
Graft versus leukemia effect, 13
Graft-versus-host disease (GvHD), 47, 148, 210, 270
  cardiac complications, 337
  hyperacute and acute, 307
  neurologic complications, 347
  nutrition, 205
  prophylaxis, 181
  renal complications, 331
  skin complication, 364
Graft-versus-malignancy (GVM) effect, 141, 373, 374
Gram-negative organisms, 245
Gram-positive (GP) organisms, 245
Granisetron, 431
Granulocyte colony-stimulating factor (G-CSF), 25, 178
Granulocyte-macrophage colony-stimulating factor (GM-CSF), 25
Growth factor, 24, 37, 178, 181
Growth hormone (GH) therapy, 393
Growth impairment, 393
GvHD, *see* Graft-versus-host disease

H
HA, *see* Hemolytic anemia (HA)
Hair complications, 367
Haploidentical donor, 103
Haploidentical HSCT, 107
Haploidentical-related (Haplo) HSCT, 376
Haplotypes, 100, 112
HC, *see* Hemorrhagic cystitis
Hearing loss, 353
  incidence, 355
  management and outcomes, 355
  risk factors and etiologies, 354
Hematologic complications associated with HSCT
  HA, 284
    alloimmune hemolysis, 287
    diagnosis and management, 285
    differential diagnosis, 284
    etiology, 285
  thrombocytopenia, 289–290
    etiology, 290–291
    management, 291–292
    TAM, 292
    thromboembolism, 293–294
Hematopoiesis, 4, 21–23, 371
Hematopoietic stem cell (HSC), 10
  bone marrow harvest, 122–123
  cell differentiation, 22
  infusions, 132–133
  niche, 31, 33, 35
  peripheral blood stem cell collection
    practical issues, 124–126
    theory, 123–124
  post-collection care of donor, 126
  source, 347
  source selection, advantages and disadvantages, 121
Hematopoietic stem cell transplantation (HSCT), 4, 385
  complications, 5
  course, 4
  immunotherapy, 4
  medical issues, 5
  medications and agents, comprehensive table, 5
  peri-HSCT period, 4–5
  pre-HSCT period, 4

Hematuria, 235
Hemoglobinopathy, 68, 190
Hemolysis, 129, 287–289, 292, 293
Hemolytic anemia (HA)
  alloimmune hemolysis, 287–289
  diagnosis and management, 285–287
  differential diagnosis, 284, 285
  etiology, 285
Hemolytic uremic syndrome (HUS), 292
Hemophagocytic lymphohistiocytosis (HLH), 68
Hemorrhage, 348, 350
Hemorrhagic cystitis (HC), 211, 235–237
  etiologies, 236–237
  grading system, 237
  pathophysiology, 236
  risk factors, 237
  treatment, 237–238
Hepatic acinus, 218
Hepatocytes, 218, 219
Hepatomegaly, 190, 211
Hepatorenal syndrome, 219, 227
Hepatotoxicity
  SOS, 217–218
    clinical features, 222
    complications, 226–227
    differential diagnosis, 222, 223
    grading, 224, 225
    incidence, 219
    management, 227–228
    outcomes, 228–229
    pathophysiology, 218–220
    prevention and prophylaxis, 225–226
    risk factors, 219–222
    surveillance, diagnostic studies, and diagnostic criteria, 222–224
  transaminitis, 216
    diagnostic studies, 216
    iron overload, 216
    management and outcomes, 216
    risk factors and etiologies, 216
Herpes simplex virus (HSV), 95, 245
HHV-6, 250
High endothelial venules (HEV), 39
High-dose chemotherapy, 62, 63, 119, 139, 146
Histoincompatibility, 270
HIV-1, 95
HIV-2, 95
Hodgkin lymphoma (HL), 62
Homeostasis, 20
Host (recipient)-related immune-mediated graft rejection, 192
HSC, *see* Hematopoietic stem cell
HSCT, *see* Hematopoietic stem cell transplantation
HTLV-1, 95
HTLV-2, 95
Human leukocyte antigen (HLA), 21, 271
  actual adult donors, attrition of, 106
  cluster, 112
  compatibility, 112
  donor availability, 104–108
  HSC source options and selection, 108
  matched sibling donors versus alternative donors, 102–104
  nomenclature, 98–101
  selection of alternative HSC donors, algorithm for, 104
  typing, 100–102
  unrelated adult donor and umbilical cord blood availability, 105
Human metapneumovirus, 249
Humoral hypothesis, 9
Humoral immunity, 20, 43, 44
Hydralazine, 441
Hydration, 204
Hydrochlorothiazide, 442, 443
Hydrocodone, 435
Hydromorphone, 435
Hydroxychloroquine, 276
Hydroxyethyl starch (HES), 128
Hyperacute GvHD, 196, 307
Hyperbaric oxygen therapy (HBO), 238
Hyperfractionated TBI, 153, 397
Hyperhydration, 237
Hyperpigmentation, 148, 364
Hypertension, 219, 328, 339, 340, 389, 393
Hypoalbuminemia, 226
Hypocalcemia, 126
Hypogeusia, 359
Hypopigmentation, 364, 367
Hypothyroidism, 392, 393

I
Idiopathic aplastic anemia, 158
Idiopathic interstitial pneumonia syndrome (IPS), 312, 313, 391
Imipenem, 416
Immobility, 366
Immune reconstitution, 49, 105
  assessments, 378–380
Immune responses, 20
  adaptive immunity, 41–44
    cell-mediated immunity, 45
    humoral immunity, 44–45
  innate immunity, 41
Immune revaccination assessments, 378
Immune system
  cells of, 25–31
  tissues of, 31
    bone marrow and hematopoietic stem cell niche, 31–37
    immune tissue regions, 40
    lymph nodes, 38–39
    spleen, 39–40
    thymus, 37–38
Immune tolerance, 9
Immune-mediated hemolytic anemia (IHA), 284
Immune-mediated neurologic complications, 352
Immunity, 20, 40, 41

Cell-mediated/cellular immunity
Immunodeficiency, 49
Immunosuppressants, 409–413
Impaired oxygenation, 366
Impaired skin integrity, 364, 365
In vivo T-cell depletion, 130
Infection, 330, 364
Infection prophylaxis, 280
Infectious complications
  bacteria, 244
  bacterial, viral, and fungal infection, risk of, 243
  early post-engraftment phase, 244
  fungus, 251
  immune system deficiency and recovery, 242
  infection control
    pharmacological preventive strategies, 247, 248, 253, 254
    procedures, 253
  infection risk, 242
  late post-engraftment phase, 244
  opportunistic and life-threatening infections, prevention, 253
  pre-engraftment phase, 243
  risk factors, 244
  viruses, 245
    adenovirus, 249
    BK virus, 250
    CMV, 245–246
    EBV and EBV-PTLD, 250–251
    HHV-6, 250
    HSV1/2, 245
    human metapneumovirus, 249
    parainfluenza, 249
    rhinovirus, influenza, 249
    RSV, 249
    VZV, 246–249
Infectious disease markers (IDM), 113
Infectious disease titers, 95
Infertility, 95, 394, 395
Infliximab, 313, 412
Influenza, 249–250
Influenza virus, 319
Inherited bone marrow failure syndromes (IBMFSs), 66
Inherited metabolic disorders, 69
Innate immune system, 20, 40, 41, 373, 374
  reconstitution of
    monocytes, 374
    neutrophil, 373
    NK cells, 373
  recovery, 374
Innate lymphoid cells, 30
Inotropes, 340
Interferon gamma (IFN-γ), 196
Interleukin-1 (IL-1), 196
Interleukin-3 (IL-3), 25
Interleukin-7 (IL-7), 25
International Bone Marrow Transplant Registry (IBMTR), 4
Intracranial hemorrhage, 346, 347
Intravascular depletion, 197
Intravenous immune globulin (IVIg), 287
Iron overload, 68, 216, 217
Irradiation, 9, 13
Isavuconazonium sulfate, 423
Isohemagglutinins, 127, 129, 288
Isolated extramedullary relapse (IEM), 59
Isradipine, 438

J
Janus kinase 1 (JAK1), 281
Juvenile myelomonocytic leukemia (JMML), 61

K
Keppra, 345
Keratoconjunctivitis sicca syndrome (KCS), 397

L
Labetalol, 441
Legionella pneumonia, 308
Leukoencephalopathy, 351
Levofloxacin, 419
Lichenoid, 274, 364
Linear banding of nails, 367
Linezolid, 418
Lipid-based amphotericin B, 329
Lisinopril, 439
Liver
  biopsy, 264
  chronic GvHD, 275
Lorazepam, 432
Losartan, 439
Low-molecular-weight heparin (LMWH), 294
Lung injury, 302, 311
Lymph nodes, 28, 38, 39
Lymphocyte, 28, 29
  B-cell, 28–30
  repertoire, 28
  T-cell, 30–31
Lymphoid-related immunodeficiencies, 67
Lymphoma, 388
  HL, 62
  NHL, 62

M
Macrophages, 25–28
Major histocompatibility complex (MHC), 21, 98
  class I, 98, 99
  class II, 98, 99
Malignant disorders, 65
  ALL, 58
  AML, 59
  CML, 61
  JMML, 61
  MDS, 60

Malignant gliomas, 64
Marginal zone, 27, 30, 40
Mast cells, 27
Matched sibling donor (MSD), 102, 119
  bone marrow transplantation, 373
Matched unrelated donor (MUD), 66
Medulloblastoma, 63
Melphalan (Mel), 149, 408
Membranous glomerulonephritis, 331
Mental status changes, 345
Meropenem, 416
Mesna, 237
Metabolic disorder, 69, 344, 345
Metabolic encephalopathy, 351
Metabolic syndrome, 392
Metaiodobenzylguanidine (mIBG), 157
Methadone, 436
Methotrexate (MTX), 263, 277, 410
Methylprednisolone, 228, 411
Metoclopramide, 433
Metolazone, 443, 444
Metoprolol, 440
Metronidazole, 148, 348
MHC, *see* Major histocompatibility complex
Micafungin, 424
Microangiopathic hemolytic anemia, 329
Mineral deficiencies, 364
Minimal change disease, 331
Minimal residual disease (MRD)
  ALL, 80–84
  allogeneic HSCT, 78
  assessment, 78, 79
Minor ABO incompatibility, 129
Mismatched sibling donor (mMSD), 102, 164
Mismatched unrelated (MMUD), 376
Mixed lymphocyte reaction (MLR), 47
Mobilization agents, 32
Monocyte colony-stimulating factor (M-CSF), 25
Monocytes, 25, 27, 28, 374
Morphine, 212, 435
Mouth, chronic GvHD, 274
MRD, *see* Minimal residual disease
MSD, *see* Matched sibling donor
Mucor, 252
Mucormycosis, 252–253, 310
Mucosal barrier injury laboratory-confirmed BSI (MBI-LCBSI), 244
Mucositis, 210
  supportive therapies, 211
Multichannel flow cytometry (MFC), 78
Multiple-gated acquisition (MUGA) scan, 94
Murine leukemia, 10
Musculoskeletal supportive care, 280
Musculoskeletal-related complications and bone health post-HSCT, 395
Mycobacteria pneumonia, 317
Mycophenolate, 411
Mycophenolate mofetil (MMF), 279, 287
Myeloablative conditioning (MAC), 142
  regimen, 131
Myelodysplastic syndrome (MDS), 60, 61, 183, 191
Myeloid-related immunodeficiencies, 68
Myeloproliferative disorders (MPD), 191
Myocarditis, 337, 338
Myositis, 275, 352

N

Nabilone, 434
N-Acetylcysteine (NAC), 228
Nail complications, 367
Naloxone, 436
National Marrow Donor Program (NMDP), 9, 104, 143
Natural killer (NK) cells, 373–374
Nephritis, 236
Nephrotic syndrome, 331
Nephrotoxicity, 329, 331
Neuroblastoma (NB), 156
  conditioning therapy, 156
  pretransplantation, 63
Neurocognitive complications, 397
Neurologic complications associated with HSCT
  acute irreversible and chronic leukoencephalopathy, 352
  cerebral vascular events, 350–351
  immune-mediated neurologic complications, 352
  metabolic encephalopathy and leukoencephalopathy, 351–352
  neurocognitive consequences, 352–353
  PRES, 350–352
  risk factors and etiologies, 344, 345
    age of recipient, 344
    bacterial, 349–350
    CNS infection, 348–349
    conditioning regimen, 345–347
    fungal etiologies, 348
    GvHD, 347–348
    HSC, 347–348
    HSCT agents and associated neurotoxicities, 346–347
    miscellaneous infectious, 349–350
    prior treatment, 345
    protozoal, 349
    underlying disease, 344, 345
    viral, 349
  seizures, 350
Neuropsychological testing, 94
Neurotoxicity, 211
Neutrophil, 25, 373
  engraftment, 179, 373
Next-generation sequence (NGS)-MRD, 79
Nifedipine, 438

NMDP, *see* National Marrow Donor Program
Nocardia asteroides, 349
Noncellular components, 36
Non-hematologically derived tumors, 388–389
Non-Hodgkin lymphoma (NHL), 62–63
Nonmalignant disorder, 65
  hemoglobinopathy, 68
  HLH, 68
  IBMFSs, 66
  inherited metabolic disorders, 69
  primary immunodeficiency, 67
  SAA, 65
Non-myeloablative (NMA) conditioning regimen, 14, 142, 160
Non-professional APCs, 28
Non-steroidal anti-inflammatory drugs (NSAIDs), 212
Non-tubercular *Mycobacterium* (NTM), 317
Nutrition
  assessment
    anthropometric, 204
    biochemical markers and methods, 203
    factors, impact serum levels, 203, 204
    pediatric malnutrition, 203
  gut and liver GvHD, 205–206
  hydration, 204
  impaired intake, 202
  metabolic alterations, 203
  nutrition support modalities, 204–205
  nutritional status monitoring, 206
  poor GI absorption, 202
  protein needs, 204
  REE, 204
  side effects and impact, 202
Nutritional status, 94
Nutritional supportive care, 280
Nystatin, 424

O
Ocular complications, 356, 359
Ocular late effects, 396, 397
Ocular supportive care, 280
Olanzapine, 433
Omenn's syndrome, 67
Ondansetron, 202, 431
Opportunistic infections, 11, 253, 357
Oral complications, 397
Osteonecrosis, 396
Osteopetrosis, 69
Ototoxic agents, 354
Ototoxicity, 353
  incidence, 355
  management and outcomes, 355
  risk factors and etiologies, 354
Ovarian failure, 95, 395
Oxycodone, 436

P
Pain, 209
  etiologies of, 210
  hemorrhagic cystitis, 211
  neurotoxicity, 211
  non-opioid agents, 212
  opioid agents, 212, 213
Palifermin, 211
Palonosetron, 431
Pancytopenia, 142, 158, 178
Paracortex, 38
Parainfluenza, 249, 318
Parainfluenza virus, 320–321
Parenteral nutrition, 205, 206
Parotid gland cancers, 397
Passenger lymphocyte syndrome (PLS), 289
Passive immunity, 45, 49
Patient-controlled analgesia (PCA), 212
Pediatric allogeneic transplantation, 146
Pediatric HSCT
  association, 8
  clinical perspective
    1980s to present, 13–15
    late 1960s to late 1970s, 11–12
    mid-1970s to late 1970s, 12–13
    post-World War II to mid-1960s, 11
  EBMT, 8
  NMDP, 9
  scientific and preclinical perspective
    1970s, 10
    late 1950s to late 1960s, 10
    post-World War II to mid-1950s, 9–10
    pre-world war II to mid-1940s, 9
Pentamidine, 429
Periarteriolar lymphoid sheaths, 40
Pericardial effusion, 197, 198
  cardiac complications, 338
Pericardiocentesis, 335, 336, 339
Pericarditis, 337, 339
Peripheral blood mononuclear cells (PBMCs), 375
Peripheral blood stem cell collection
  practical issues, 124
  theory, 123
Peripheral blood stem cell transplantation (PBSCT), 373
Phagocytes, 20, 25, 28, 41, 42, 45, 68
Phosphodiesterase-5 inhibitors, 340
Physical therapy, 274
Phytohemagglutinin (PHA), 375
Pilocarpine, 280
Piperacillin, 415
Plerixafor, 32, 125
Pleural effusion, 197, 302, 338
Pneumocystis jiroveci pneumonia (PJP), 322
Pokeweed mitogen (PWM), 375
Polymerase chain reaction (PCR), 78
Polymorphonuclear leukocytes, 25
Posaconazole, 252, 423

Posterior reversible encephalopathy syndrome (PRES), 351
Posttransplant lymphoproliferative disorder (PTLD), 250, 388
prednisolone, 411
Prednisone, 276, 411
Pregnancy, 95
Premature ovarian failure, 394
Pretransplantation
ASCT, 61
brain tumors, 63
embryonal, 63
GCT, 64
evaluation
confirmatory HLA typing, 93–94
disease assessment and restaging, 92, 93
drugs with their cumulative doses, 95
fertility preservation, 95–96
infection evaluation, 95
laboratory evaluation, 94–95
testing, 92
lymphoma
HL, 62
NHL, 62
malignant disorders
ALL, 58–59
AML, 59
CML, 61
JMML, 61
MDS, 60
neuroblastoma, 63
nonmalignant disorder, 65
hemoglobinopathy, 68
HLH, 68
IBMFSs, 66
inherited metabolic disorders, 69
primary immunodeficiency, 67
SAA, 65
Primary immunodeficiency, 67–68
Primitive neuroectodermal tumors (PNET), 63, 64
Probenecid, 329
Progressive multifocal leukoencephalopathy (PML), 349
Promethazine, 202, 433
Prophylaxis, 246
acyclovir, 245
antibacterial, 253
antifungal, 254
antiviral, 253
fluconazole, 251
infection, 280
SOS, 220
Propranolol, 440
Protein, nutrition, 204
Proteinuria, 328
Protozoa lung infections
PJP, 322
Toxoplasmosis gondii, 322–323
Protozoal infections, neurologic complications, 349
Protozoan, 243
Proximal tubular necrosis, 329
Psoralen and ultraviolet A radiation (PUVA) therapy, 279
Pubertal delay, 394
Pubertal development, 394
Puberty, 394
Pulmonary arterial hypertension (PAH), 339
Pulmonary complications, 391
early post-engraftment, 311–312
BOOP, 313–314
IPS, 312–313
late post-engraftment, 314–315
bacterial pneumonia, 316–317
BOS, 315–316
fungal pulmonary infections, 322
mycobacteria pneumonia, 317–318
protozoa lung infections, 322–323
viral pneumonia, 318–322
pre-engraftment period, 304, 305
bacterial pneumonia, 308–309
DAH, 307
engraftment syndrome, 306
fungal pneumonia, 309–311
hyperacute and acute GvHD, 307
pulmonary edema, 304
pulmonary infections, 308
Pulmonary edema, 196, 198, 304–306
Pulmonary function testing (PFT), 94
Pulmonary hypertension, cardiac complications, 339
Pulmonary infections, 308, 322
Pulmonary veno-occlusive disease (PVOD), 339

R

Radiation chimera, 9
Radiation-induced HC, 236
Radiation therapy, 63, 210, 329, 393
Real-time quantitative (RT-Q) PCR, 79
Recombinase-activating gene deficiencies (RAG-1 or RAG-2), 67
Red cell antigen, 284
Red pulp, 23, 26, 39
Reduced-intensity conditioning (RIC) regimen, 14, 142
Reduced-toxicity conditioning (RTC), 143
Re-immunization, 380
Renal complications associated with HSCT
causes of, 328
drug-induced renal toxicity, 329
GvHD and glomerular disease, 331
renal dysfunction, 327–331
TMA, 329
Renal dysfunction, 226, 238, 396
detection and diagnosis, 328
infection and, 330
prevalence of, 327
Renal toxicity, 236, 237
HC, 235
hemorrhagic cystitis

etiologies, 236
grading system, 237
pathophysiology, 236
risk factors, 237
treatment, 237
Respiratory compromise/failure, 226
Respiratory syncytial virus (RSV), 249, 320
Resting energy expenditure (REE), 204
Restrictive lung disease (RLD), 148, 313, 391
Revaccination, 287, 378, 379
Rhinovirus, 249
Rhizomucor, 252
Rhizopus, 252, 311
Rituximab, 287, 329, 330, 413
Ruxolitinib, 264, 281

S
Salivary gland dysfunction, 274, 397
Sargramostim, 178
SCID, *see* Severe combined immunodeficiency
Sclerodermatous, 274, 275, 364
Scopolamine, 434
Secondary disease, 10
Secondary malignancies, 386–387
risk factors, 387
screening recommendations, 387
types of, 387
Seizures, 148, 350
Sensory complications
HSCT-associated taste disturbances and dysfunction, 359
ototoxicity, 353–354
incidence, 355
management and outcomes, 355–356
risk factors and etiologies, 354–355
visual- and ocular, 356–359
Sequence-specific primer (SSP), 101
Serotherapy, 143, 375
Serotonin (5HT-3) antagonist, 202
Severe aplastic anemia (SAA), 65–66
Severe combined immunodeficiency (SCID), 11, 15, 67, 162–165, 376
Short tandem repeat (STR), 182
Sickle cell disease (SCD), 13, 68, 159–162, 190, 345
Signaling lymphocytic activation molecules (SLAM), 37
Sinus tachycardia, 337
Sinusitis, 275
Sinusoidal obstruction syndrome (SOS), 211, 217, 290
clinical features, 222
complications, 226
differential diagnosis, 222, 223
grading, 224
incidence, 219
management, 227
outcomes, 228
pathophysiology, 218, 220
prevention and prophylaxis, 225
risk factors, 219, 221
surveillance, diagnostic studies, and diagnostic criteria, 222, 224
Sirolimus, 412
Skin
biopsies, 264
cancer, 389
chronic GvHD, 274
infections, 365
complications, 363
diagnosis and evaluation, 366
risk factors, etiologies, and clinical manifestations, 364–366
supportive care, management, and outcomes, 366
SOS, *see* Sinusoidal obstruction syndrome
Spironolactone, 227, 444
Spleen, 23, 39
Splenectomy, 287
Squamous cell carcinoma, 397
Stem cell factor (SCF), 23, 31, 37
Stroke, 350
Sulfamethoxazole, 316, 349, 429
Superconducting quantum interference device (SQUID), 217
Supportive skin care, 281
Supratentorial PNET (sPNET), 64
Supravesical urinary diversion (SUD), 238
Syndrome of inappropriate antidiuretic hormone (SIADH), 149, 345
Syngeneic HSCTs, 45, 141, 142
Syphilis, 95
System-based health complications
cardiac dysfunction, 389–391
dental and oral complications, 397
endocrine-related complications, 392–395
musculoskeletal-related complications and bone health post-HSCT, 395–396
neurocognitive complications, 397–398
ocular late effects, 396–397
pulmonary complications, 391–392
renal dysfunction, 396

T
Tachycardia, 336, 337
Tacrolimus, 280, 329, 347, 410
TAM, *see* Transplant-associated microangiopathy
Tamponade, 339
Taste disturbances and dysfunction, 359
Tazobactam, 415
TBI, *see* Total body irradiation
T cell, 30, 163, 271, 375
T-cell receptor excision circles (TRECs), 375
Testicular failure, 394
Thalassemia, 13, 190
Thalidomide, 277, 279, 413
Thiotepa (Thio), 149, 150, 364, 408

Thrombocytopenia, 289
causes of, 290
etiology, 290
management, 291
TAM, 292
thromboembolism, 293
Thromboembolism, 293, 294, 337, 338
Thrombosis, 294, 329
Thrombotic microangiopathy (TMA), 329–330
Thrombotic thrombocytopenic purpura (TTP), 292, 329
Thymocytes, 37
Thymus, 28, 37, 43, 49
Thyroid cancer, 389, 392
Thyroid dysfunction, 392
Tobramycin, 420
Tolerance, 20, 45, 49
Topoisomerase II inhibitors, 387
Total body irradiation (TBI), 13, 94, 202, 365
conditioning therapy, 145–146
hyperfractionated, 153, 397
Total nucleated cells (TNCs), 180
Total parenteral nutrition (TPN), 221
Toxoplasma gondii, 323, 349
Toxoplasmosis, 95, 349
Tramadol, 437
Transaminitis, 216
diagnostic studies, 216
iron overload, 216
management and outcomes, 216
risk factors and etiologies, 216
Transplant-associated microangiopathy (TAM), 290, 292, 293
Transplantation immunology
alloreactivity, alloantigens, antigen presentation, and tolerance, 45–47
graft rejection and graft versus host disease, 47–48
immunodeficiency, 49
tolerance and immune reconstitution, 49
Transplantation-associated thrombotic microangiopathy (TA-TMA), 352
Transplantation-related morbidity and mortality (TRM), 281
Transplant-related mortality (TRM), 225
Treatment-related acute myeloid leukemia (t-AML), 387
Treatment-related morbidity and mortality (TRM), 107
Treatment-related myelodysplastic syndromes (t-MDS), 387–388
Treosulfan (Treo), 150, 408
Trimethoprim, 316, 429
Trimethoprim-sulfamethoxazole, 349
Tumor necrosis factor alpha (TNF-α), 196
inhibition, 264

U
Ultrasound, 223, 392
Umbilical cord blood (UCB), 14, 101, 123
Umbilical cord blood transplantation (UCBT), 127, 188, 373
Underlying disease, 344, 345
Urethritis, 236
Urinary obstruction, 235, 236
Urokinases, 238
Ursodeoxycholic acid, 225
Ursodiol, 225, 414

V
Valacyclovir, 425
Valganciclovir, 426
Valsartan, 439
Vancomycin, 329, 417
Varicella zoster virus (VZV), 95, 244, 246, 365
CNS infections, 349
Veno-occlusive disease (VOD), 148, 211, 217, 290, 291, 414
Venous thromboembolism (VTE), 293
Viral infection, 236, 243, 302, 315, 318, 365
cardiac complications, 338
neurologic complications, 349
Viral pneumonia
CMV, 318–319
community-acquired respiratory viral infections
adenoviruses, 321–322
influenza virus, 319
parainfluenza virus, 320
RSV, 320
Viral reactivation, 236
Viral-induced HC, 236
Viremia, 245, 249, 318
Viruses, 245
adenovirus, 236, 249, 329
BK virus, 236, 250, 330
CMV, 95, 112, 236, 245, 318, 329
EBV and EBV-PTLD, 250
HHV-6, 250
HSV1/2, 245
human metapneumovirus, 249
parainfluenza, 249
rhinovirus, influenza, 249
RSV, 249, 320
VZV, 95, 246, 365
Visual complications, 356, 357
Vitamin deficiencies, 364
Vitritis, 358
Voriconazole, 252, 310, 348, 422

W
Wasting syndrome, 10, 12, 275, 280
Weight gain, 196, 197
White pulp, 23, 39
Wiskott-Aldrich syndrome (WAS), 67

X
Xenograft, 46

Z
Zika virus, 113
Zygomycetes spp., 248, 252
lung infections, 311