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Quality Assurance in Healthcare Service Delivery, Nursing and Personalized Medicine

Technologies and Processes

Athina Lazakidou & Andriani Daskalaki

Quality Assurance in Healthcare Service Delivery, Nursing, and Personalized Medicine:

Technologies and Processes

Athina Lazakidou
University of Peloponnese, Greece

Andriani Daskalaki
Max Planck Institute for Molecular Genetics, Germany

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Preface

In general, outcome measurement has focused on a health gain or health maintenance score, or an overall wellbeing result. However, because quality of life is difficult to define and even more difficult to measure - particularly with physically and mentally vulnerable people - outcomes from nursing in continuing care are not easily articulated. The focus of the nursing assessment tool is therefore on increasing quality of life, rather than perceiving health gain simply as increased longevity.

Assessment is considered to be the first step in the process of individualized nursing care. It provides information that is critical to the development of a plan of action that enhances personal health status. It also decreases the potential for, or the severity of, chronic conditions and helps the individual to gain control over their health through self-care.

One of the great challenges in medicine is to deliver effective therapies tailored to each patient based on his molecular signatures. The so-called "personalized medicine" would involve tools of patient evaluation that would tell clinicians the correct drug and doses for the patient. Patient outcomes of a drug intervention are the result of conditional probabilistic interactions between complexes of drug-metabolizing enzyme genes, a range of metabolic regulatory genes, and environmental factors. Systems biology tools and concepts that integrate modelling of signalling pathways and regulatory networks at many levels of biological organization in the whole organism provide a help to personalized medicine.

The main goal of this new book is to provide innovative and creative ideas for improving the quality of care and to explore all new technologies in medical informatics and health care delivery systems as well as technological advances in personalized medicine.

The topics of this book cover useful areas of general knowledge including concepts of quality, quality assessment and quality assurance, risk management and quality of care, patient and nurse assessment in personalized medicine, role of information technology in healthcare quality assessment, quality in telemedicine services, tools and techniques in systems biology, ethical guidelines for the quality assessment of healthcare, health system policies, and service delivery.

The book covers basic concepts, best practices, techniques, investigative challenges in clinic and research.

Organization of the Book

The book is divided into four sections:

Section One: *"Clinical Diagnostic Methods"* introduces innovative concepts in medical diagnostics. Chapter 1 presents patient specific cardiovascular diagnosis. The novel method described in this chapter produces a distribution of physiologically interpretable models that allow the identification of disease

specific patterns that corresponded to clinical diagnoses, enabling a probabilistic assessment of human health condition. In this work a technique is presented to identify arterial stenosis and aneurisms from anomalous patterns in signal and parameter space. Chapter 2 introduces experience sharing of Cai's gynecology in Traditional Chinese Medicine. A novel system named TCM-PMES is demonstrated. This system preserves the diagnostic processes in a personalized way.

Section Two, *"Basic Research: A Bridge to Modern Medicine,*" serves as a comprehensive introduction to methods supporting basic research. The emphasis of chapter 3 is on new therapeutic approaches using functional cells derived from stem cells. This chapter provides a general overview on technologies applied on stem cell research. Chapter 4 gives an extended analytical consideration of mathematical modelling to the analysis of biological pathways perturbated in disease. Chapter 5 discusses the development of novel universal strategies for exogenous control of gene expression based on designer riboswitches that can function in the cell.

Section Three is titled "*Medical Treatment and Research: Ethics and Applications."* Chapter 6, entitled "Ethical Guidelines for the Quality Assessment of Healthcare," describes how ethical principles can be used as guidelines for the quality assessment of healthcare provision. In Chapter 7, the authors present the computational workflow to investigate the process of tumor progression, and present this approach through an example of childhood neoplasias.

Section Four, *"Healthcare Quality Assessment,*" is dedicated to methods applied in the healthcare service delivery. In chapter 8, the authors define ethical requirements in educational practice for healthcare quality. In chapter 9, the author describe the social role of technology in healthcare quality improvement. Chapter 10 presents a novel solution to improve quality assurance, in drugs delivery; i.e. reduce clinical errors caused by drug interaction and dose. Telemedicine services should meet the international quality requirements in order to accomplish quality assurance in healthcare provision. Technology advances have brought forward new and evolved services and technical infrastructure that promote and enhance quality healthcare services, such as telepresence and wearable technology. Nevertheless, there are several obstacles in telemedicine performance that need to be resolved. In Chapter 11 the authors try to define quality issues in Telemedicine Services.

The main purpose of the Chapter 12 is to represent an alternative effective model for measuring the quality of healthcare (SERVQUAL) considering the geographical location of the under examination healthcare sectors. Geographical Information Systems (GIS) play a major role in all areas of health research, especially for the understanding of spatial variations concerning disease monitoring. Chapter 13 describes the methodological approach for the development of a real time electronic health record, for the statistical analysis of geographic information and graphical representation for disease monitoring.

The purpose of the Chapter 14 is to provide innovative knowledge and creative ideas of improving quality of care and to explore how risk management and Knowledge transfer and quality assurance can improve health care. Under careful consideration, our purpose is to summarize which factors improve and promote the quality of care and which factors diminish quality. There are forms of ongoing effort to make performance better. Quality improvement must be a long- term continuous effort, reducing errors and providing a safe trust environment for health professionals and patients. After reading this chapter, the reader should know the answer to these questions: What role can risk management and knowledge transfer play in quality of care? How risk management and knowledge transfer can work together? What are the factors that improve risk management and quality assurance in health care? How does knowledge transfer support inform and improves care?

Quantifying and improving the quality of health care is an increasingly important goal in medicine. Because quality of life is difficult to define and even more difficult to measure - particularly with physically and mentally vulnerable people - outcomes from nursing in continuing care are not easily articulated. The focus of the nursing assessment tool is therefore on increasing quality of life, rather than perceiving health gain simply as increased longevity. Assessment is considered to be the first step in the process of individualized nursing care. It provides information that is critical to the development of a plan of action that enhances personal health status. It also decreases the potential for, or the severity of, chronic conditions and helps the individual to gain control over their health through self-care. In the Chapter 15 the authors try to describe how important is the role of Information and especially of the Information Technology in Healthcare Quality Assessment.

The book, "Quality Assurance in Healthcare Service Delivery, Nursing and Personalized Medicine: Technologies and Processes," contains text information, but also a glossary of terms and definitions, contributions from international experts, in-depth analysis of issues, concepts, new trends, and advanced technologies in Healthcare Service Delivery, in modern clinical diagnostics, and in advanced medical research.

The new book will be an excellent source of comprehensive knowledge and literature on the topic of quality assurance in healthcare service delivery, nursing and personalized medicine.

All of us who worked on the book hope that readers will find it useful.

Athina Lazakidou
University of Peloponnese, Greece

Andriani Daskalaki
Max Planck Institute for Molecular Genetics, Germany

Section 1

Clinical Diagnostic Methods

Chapter 1
From Non-Invasive Hemodynamic Measurements towards Patient-Specific Cardiovascular Diagnosis

Stefan Bernhard
Freie Universität Berlin, Germany

Kristine Al Zoukra
Freie Universität Berlin, Germany

Christof Schütte
Freie Universität Berlin, Germany

ABSTRACT

The past two decades have seen impressive success in medical technology, generating novel experimental data at an unexpected rate. However, current computational methods cannot sufficiently manage the data analysis for interpretation, so clinical application is hindered, and the benefit for the patient is still small. Even though numerous physiological models have been developed to describe complex dynamical mechanisms, their clinical application is limited, because parameterization is crucial, and most problems are ill-posed and do not have unique solutions. However, this information deficit is imminent to physiological data, because the measurement process always contains contamination like artifacts or noise and is limited by a finite measurement precision. The lack of information in hemodynamic data measured at the outlet of the left ventricle, for example, induces an infinite number of solutions to the hemodynamic inverse problem (possible vascular morphologies that can represent the hemodynamic conditions) (Quick, 2001). Within this work, we propose that, despite these problems, the assimilation of morphological constraints, and the usage of statistical prior knowledge from clinical observations, reveals diagnostically useful information. If the morphology of the vascular network, for example, is constrained by a set of time series measurements taken at specific places of the cardiovascular system, it is possible to solve the hemodynamic inverse problem by a carefully designed mathematical forward model in combination with a Bayesian inference technique.

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The proposed cardiovascular system identification procedure allows us to deduce patient-specific information that can be used to diagnose a variety of cardiovascular diseases in an early state. In contrast to traditional inversion approaches, the novel method produces a distribution of physiologically interpretable models (patient-specific parameters and model states) that allow the identification of disease specific patterns that correspond to clinical diagnoses, enabling a probabilistic assessment of human health condition on the basis of a broad patient population. In the ongoing work we use this technique to identify arterial stenosis and aneurisms from anomalous patterns in signal and parameter space. The novel data mining procedure provides useful clinical information about the location of vascular defects like aneurisms and stenosis. We conclude that the Bayesian inference approach is able to solve the cardiovascular inverse problem and to interpret clinical data to allow a patient-specific model-based diagnosis of cardiovascular diseases. We think that the information-based approach provides a useful link between mathematical physiology and clinical diagnoses and that it will become constituent in the medical decision process in near future.

INTRODUCTION

Diagnosis of cardiovascular diseases is an important and difficult task in medicine. Usually differential diagnostic approaches are applied, where several factors or symptoms are considered in an exclusion procedure. This procedure may lead to false assumptions that often result in misdiagnoses. Therefore, the attempt of using the knowledge and experience of experts collected in databases to support the diagnosis process by computational methods seems reasonable. This non-obvious task is valuable for decision making in today's medicine.

Typically various data mining frameworks are used to transform the data into therapeutically relevant knowledge for decision making. Several iterative subtasks process the data to discover the hidden information that diagnose diseases. Even though the amount and information content within clinical data has grown continuously and rapidly the number of accepted and reliable procedures for extracting diagnostically or therapeutically relevant information has not increased comparably.

On the one hand the difficulty in the process of information extraction of richer data sets into improved clinical therapies lies in the traditional deterministic view immanent in current mathematical models of physiological processes. On the other hand there is a lack of sufficient therapeutic options that can cope with advanced diagnostic information. The former problem traces back to the fact that current methods have limited ability to integrate the statistical nature of clinical data obtained from a broad patient population.

Within this work we bridge the gap to a more suitable interpretation of model parameters and states observed in a broad patient population, namely by statistical analysis and interpretation. Such statistical models have been developed for a range of problems in biomedical signal analysis to remove contaminants from EEG and ECG signals (Sameni, 2008) and recently to estimate parameters of the cardiovascular system from blood pressure signals (McNames, 2008). It was shown that the extraction of information about the health condition of subgroups of the population with such methods provides the potential to develop new individualized therapeutic strategies that bring true benefit to the patients. Resolving this incongruity will pave the way for a statistical interpretation of cardiovascular system models that optimally use the information of specific subpopulations for diagnostic purposes.

The Intention of the Angiology Project Carried Out in the Innovation Laboratory

"Mathematics for Diagnosis (M^4D)" is twofold: the collection of physiologic information and associated the probabilistic models for the use in data base driven information systems and medical devices and to improve the understanding of human cardiovascular physiology.

Within the project, there will therefore be many scientific subtasks concerning the parameterization of anatomical regions of the cardiovascular system, the data integration and mathematical model building. These include mechanical and hemodynamical aspects of the normal and diseased system.

Some of the major components of the cardiovascular system for which computational models have been developed recently include the hemodynamic conditions in the coronary and systemic vascular circulation. Of particular interest are lumped-parameter models describing transport of blood accounting for the spatio-temporal distribution of blood and uptake of nutrients and oxygen within the vasculature. Clinical data has been essential in this area, both for estimating the structural and kinetic parameters of mass transport and for providing independent measurements that are used to validate such models. These models should provide an opportunity to answer important questions in integrative cardiovascular physiology that have abscond intuitive understanding. One example discussed in this study is the detection of vascular defects like stenosis or aneurisms that influence perfusion and cause acute ischemia.

Following studies regarding the needs of clinical applications (Capova, 2003; Xiao, 2002; Wessel, 2000), we will have to combine physical and physiological aspects of pathological conditions in the cardiovascular system with patient-specific simulations based on non-invasively accessible data. Within our project we developed parametric computational techniques for modeling the mechanics, and transport processes, while incorporating kinetic and structural information from data measurements. In this approach a set of multi-transducer measurements, taken at several places of the vasculature, act as constraints to the hemodynamic inverse problem. Its solution allows to extract a variety of useful patient-specific diagnostic information like for example the network morphology including mechanical properties and the location of arterial stenosis and aneurisms, the stroke volume and ejection fraction of the heart and peripheral resistances.

In the following we will discuss several mathematical models generally used to simulate the dynamics of the cardiovascular system and we will introduce an electrical analogue model developed within the M^4D group. The model is used to show the success and failure in traditional parameter estimation procedures in example of an aortic aneurism. The failures lead us to a novel view of cardiovascular modeling where the hemodynamic inverse problem is stated as statistical inference problem. Finally we discuss the potential of the Bayesian inference technique in cardiovascular system identification and in the classification of diseased model states.

Mathematical Forward Models in Cardiovascular Physiology

The application of mathematical models of physiological mechanisms could sufficiently enhance the interpretation of clinical data. However, such models are generally nonlinear and complex, so that the identification of parameters and states that represent available data are difficult to determine and interpret.

Cardiovascular models have been widely used in physiology and medicine to support clinicians. Specifically, they provide insights on the behavior of blood pressures in the vasculature of the human circulatory system under normal and diseased conditions. Within this study we are especially interested in the modeling and detection

of pathologic defects like vascular stenosis and/or aneurisms.

Common Cardiovascular System Models

Several forward models of the cardiovascular system have been previously developed to describe and analyze the cardiovascular function on a theoretical basis. In one of the first theoretical investigations the arterial system was described by a Windkessel model, which reduces the system to a single elastic chamber that temporarily stores the blood (Stergiopulos, 1999). Although the benefits for calculation of cardiac output loomed large the model did not manage to describe the pulse wave propagation, because the Windkessel model cannot describe the spatial distribution of pressure and flow waves. This disadvantage is caused by the lumped description of the cardiovascular system as a single elastic chamber that cannot give a detailed description of all constituents (hundreds of arteries, arterioles and capillaries). The lumped description is however well satisfied and applicable as a boundary condition to model outflow conditions of the capillary bed (Quateroni, 2001).

Today's cardiovascular system models are based on the Navier-Stokes equation and the constitutive equation for the arterial wall or a set of equations resulting from the application of a variety of simplifying assumptions. For circular symmetric and linear elastic arteries blood flow is described by a set of nonlinear one-dimensional wave equations describing pressure and flow along the arteries (Womersley, 1955; Olufsen, 2000; Alastruey, 2009; Formaggia, 2009). Further reduction can be obtained by the long wave approximation, where the pressure variation along a vessel segment is assumed to be small, so that non-linearities can be removed. These equations can be solved by a semi-analytical approach if the vascular network is truncated after a few generations (Stergiopulos, 1992; Segers, 1998; Zamir, 2000; Pedley, 2008).

Anatomically detailed models describing propagation of pressure and flow waves by one-dimensional equations are derived in several places (Olufsen, 2000; Formaggia, 2009; Alastruey, 2009). The authors of (Matthys, 2007) have used experimental models to verify and test parameters and hemodynamic conditions in one-dimensional model systems. The accuracy of the pressure and flow within their one-dimensional formulation was quantified to be 4% relative error in the pressure difference compared to the experiments.

Further simplification of the one-dimensional system of partial differential equations (PDE) is obtained integrating out the spatial dependency. The resulting lumped system of zero-dimensional ordinary differential equations (ODE) (Olufsen, 2004) has similar structure as those used in electrical analog circuits. These models are often used as a Windkessel type boundary conditions in higher dimensional models (Quateroni, 2001), where they represent the dynamic peripheral impedance of the capillary bed (Stergiopulos, 1999), (Lee, 2004).

However they also find application in several detailed descriptions of the coronary (Wang, 1989) and systemic (Westerhof, 1969; Avolio, 1980; John, 2004) vasculature. In Wang (1989) for example electrical analogue elements are used to model pressure and flow in 14 coronary arteries and in John (2004) a detailed representation of the whole systemic circulation is given. Both model systems where used to simulate the hemodynamics under normal and stenotic arterial conditions. In John (2004) the option of inverse calculation to determine the mechanical properties from blood flow and pressure waveforms is raised, but no further details are given.

The Cardiovascular System Model Used in the Study

In the ongoing study we decided to set up a lumped parameter model of the cardiovascular system, because these models are known to reproduce the pulsatile pressure and flow in the systemic

vasculature quite realistically (Westerhof, 1969; Wang, 1989; John, 2004). The model is mathematically formulated in terms of an electric circuit analogue, where the electrical current is used as the analogue of the blood flow, F, and the electrical potential that of the pressure, P, in the circulatory system. Within the system of ordinary differential equations shown in Figure 1 (a) the pressure and flow in a vessel segment is modeled as RCL-circuit (three element Windkessel) consisting of a resistance, R, an inductor, L, in series as well as a capacity, C, in parallel (see Figure 1 (b)). The reader should be aware that the electrical circuit analogue is just for the sake of simple interpretation and graphical representation of the models to be discussed and does not mean that the cardiovascular system should be understood as a kind electrical circuit system.

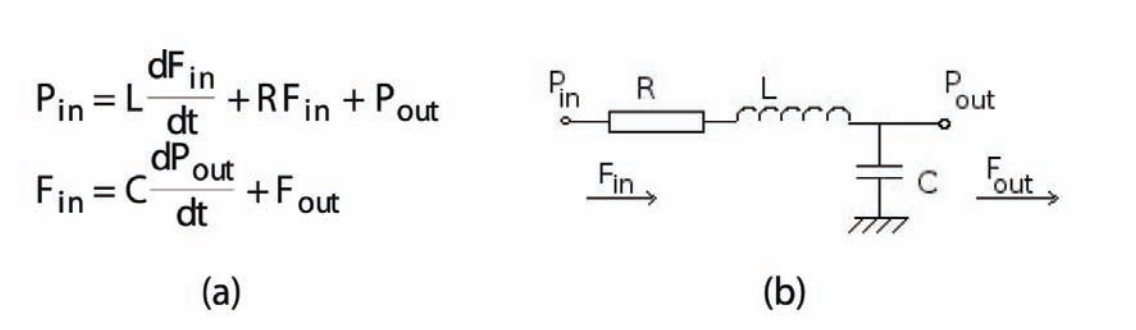
The pressure at the in- and outlet is denoted by P_{in} and P_{out} respectively, while the flow is denoted by F_{in} and F_{out}. The viscosity of the blood is modeled by a resistance, while the inductance comprises the inertia of the blood. The capacity in parallel models the linear elastic compliance of the arterial wall.

Most electrical analogue models of the cardiovascular system are based on the pioneering work of Noordergraaf (1963). However, several modifications where done to adapt the model behavior (Stergiopulos, 1999; Hassani, 2007; Alastruey, 2008). These comprise for example the inclusion of non-linearities or visco-elasticity of the vessel wall, derivation of elements to represent leakage flow, vascular defects or the inclusion of parameters that include additional vascular branches that are of particular interest in a study.

Parameterization of Cardiovascular Models

In the forward problem a set of given model parameters, such as the length, cross-sectional area and elasticity, lead to the pressure and flow at specified locations in the vascular network. In practice it is often not possible to determine the cross-sectional area or elasticity for most of the arteries by *direct* measurement, even the segmental length is difficult to measure directly (Jovanovic, 2004). However good estimates where obtained in experimental studies, that computed the desired values from indirect measurements (Dobrin, 1978; Tardy, 1999). The parameter values found are widely spread and properties significantly change with the health condition and age of the subject (Zanchi, 1997; Hoeks, 1999). The strong variance within these parameters makes it difficult to simulate patient-specific settings, because the measurement of values for individual patients is time-consuming (Manor, 1994).

Figure 1. (a) ODE system describing the pressure and flow within the three element Windkessel model shown in (b)



The model parameters used in this work characterize a system of 242 first order ordinary differential equations like the ones given in Figure 1 (a), that are coupled to represent the morphology of the human arterial tree as given in (Westerhof, 1969). Within the model the blood flow in the arterioles and capillaries is lumped into a peripheral resistance, Z, that characterizes the pressure-flow relation at the network outlets. Parameters for the peripheral resistances where taken from Matthys (2007)

Numerical Solution of Cardiovascular Models

The solution of the high-dimensional ODE system is conducted by common numerical solvers and allows us to predict the blood pressure and flow at arbitrary nodal points in the circulatory system. Within this study we are especially interested in the pressures at non-invasively accessible locations like the arteria radialis, the arterial carotis or the arteria brachials, because the signals can be directly compared with in-vivo pressure measurements to fit the model equations.

In the next section we employ traditional optimization methods to re-estimate a set of parameters from pressure and flow waves that have been generated from the model outputs. Taking the simulated waves as observation data, we are able test a variety of hypothesis about vascular defects to dis-/prove the existence of specific diseases. In the adjacent section, we propose a statistical method that applies hypothesis based on a disease specific subpopulation as prior information to classify the measurement data automatically.

Parameter Estimation and Inverse Modeling in Cardiovascular Physiology

The idea to use mathematical models to gain a better insight into the mechanisms of cardiovascular diseases, is not new but previous investigations concentrated on the solution of the forward problem (Westerhof, 1969; Olufsen, 2000). These models determine the blood and pressure distribution within the vasculature from specified structural and hemodynamical parameters to compare the results with in-vivo measurements. Inverse solutions have been seeked (Quick, 2001) but current methods only determine basic parameters like the total arterial compliance or the total peripheral resistance with sufficient accuracy. The general setting for model based data inversion in cardiovascular physiology is shown in Figure 2.

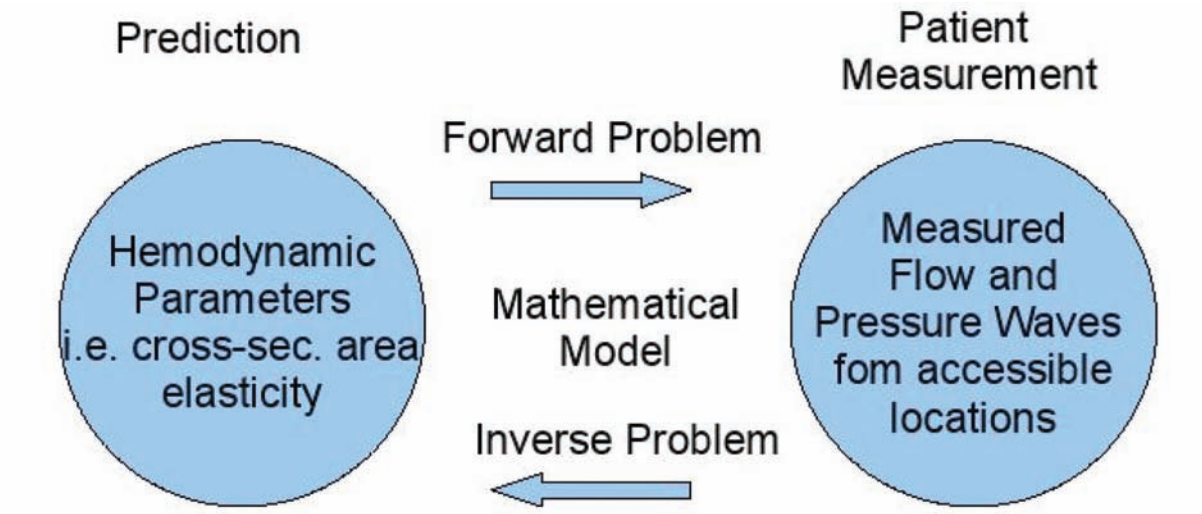
Conventionally, reduced arterial system models are fitted to data, and the resulting model parameters are assumed to represent pretended arterial properties. The achievable accuracy of the parameters depends critically the quality of the data and complexity of the model used. Basic parameters like the total arterial compliance are estimated with high accuracy using simple models, while highly specific parameters like the location of vascular defects may even not be estimated using complex models, because the amount of data available is insufficient. This suggests a multi-model setting in which several models of different complexity are used to solve the hemodynamic inverse problem for different levels of detail. This implies that detailed models are lumped into less detailed macromodels to obtain the basic parameters, while the highly specific information is obtained by the detailed models.

Success and Failures of Current Parameter Estimation Methods in Cardiovascular Medicine

In this section we will review some of the parameter estimation methods used in cardiovascular physiology. Subsequently we will resume what in our opinion is the main problem in cardiovascular parameter estimation.

In Lee (2004) an electrical analog model for the aorta was investigated, where the authors optimized the parameters of four electrical circuit

Figure 2. Schematic overview of the model based hemodynamic inverse problem



models to fit transfer functions calculated from invasively measured pressure waves taken at the end of the aortic segments (Lathem, 1985). They estimated all electrical parameters with high accuracy and the output response generated at the end element was almost identical with the measured wave.

Other methods try to reduce the order of the optimization problem to ensure the well-conditioning of the parameter estimation problem. In Samar (2005) a subset selection is therefore employed on a basic cardiovascular model consisting of left and right ventricles, systemic arteries and veins and pulmonary arteries and veins. A nonlinear least squares method was applied to identify the parameters. It was concluded, that the reduction of the parameter identification problem significantly improves the reliability of the estimates. Also in Heldt (2001) a subset of the entire parameter vector is determined by the Gauss-Newton method, matching simulated and measured data for a basic circulatory system.

The authors of Thore (2008) demonstrated the applicability of a detailed one-dimensional wave propagation approach in combination with a traditional parameter estimation method to identify patient-specific parameters. From a series of measurements they were able to reproduce the pressure wave in the abdominal aorta with high accuracy.

John (2006) developed an inverse electrical circuit problem for the lower limb, that was solved by a globally convergent modification of the Newton's method. Within the study the author's also examined pathological changes like the detection of stenosis where the radius was determined sufficiently accurate. However they do not explain the exact procedure.

Smith (2003) uses a six chamber closed loop model trying to simulate the cardiovascular system of a patient as accurately as possible. After detection of initial parameter values by trial and error parameters are approximated by unconstrained nonlinear optimization.

Due to the description of the cardiovascular system as analog circuits, parametric estimation methods used to determine the structure and parameters of electrical circuits are of interest. Within Timmer (2000) and Korovkin (2007) structural properties and parameters for electrical elements where reliably estimated from signal measurements.

From the above survey it becomes clear that, even though numerous investigators were able to determine selected parameters from measurements, a detailed description of methods that are able to determine parameters for the whole circulatory system are not available until now. We assume that this deficit is majorly provoked by insufficient data mining methods currently used in cardiovascular system identification.

Parameter Estimation Example in a Cardiovascular System with Aortic Aneurisms

Within this section we seek a set of parameters that represent a certain characteristic pressure or flow distribution measured for a specific disease. We imply that observable changes in the pressure distribution contain relevant information about a patient's health condition. However for true measurements the parameters are unknown and cannot be used to validate the method, thus we generated three data sets from the model outputs (with known parameters) that are used as ideally observed data throughout the section.

Data Generation and General Simulation Setup

Besides the normal parameterization of the systemic circulation model described in Westerhof (1969), we modified two aortic segments, (see segments *61* and *72* in Figure 8), to model either an thoracic aortic aneurism (TAA) or an abdominal aortic aneurism (AAA) in an ideal patient, respectively. The detailed parameters are given in Table 1.

The network structure that corresponds to the numbering of the segments is shown in Figure 8.

The simulated data sets comprise five pressure waveforms taken at the arteria vertebralis #8, arteria brachialis #14, arteria ulnaris #22, arteria femoralis #100 and arteria tibialis posterior #110 (see Figure 3 (a-c)). The results can be compared to pressure waves for the AAA obtained in (Segers, 2009). To assure that the system is in steady-state the simulation duration was chosen to be *25* seconds, but only the time series of the final two seconds were used in the optimization.

Optimization Setup

Due to the large number of variables we decided to use two constraint optimization algorithms: (i) a weighted variant of Levenberg-Marquardt nonlinear least squares algorithm that uses parameter sensitivities to control the step size (SENSOP) (Chan, 1999) and (ii) a non-linear steepest-descent algorithm (NLSD). For moderately sized models (of a few hundred parameters), the SENSOP is much faster than steepest-descent methods in a wide variety of problems. Even though the steepest descent algorithm is much slower, the implementation is simpler and the low storage requirement is advantageous for large problems. Finally, in some cases we employed a global optimization simulated annealing algorithm (SA).

The upper and lower constraints for optimized parameters are set to physically expectable values. These are C_{lb}=0 and C_{ub}=3 for the lower and upper bound of the compliance and L_{lb}, R_{lb}=0 and L_{li}, R_{li}=0.001 for the inductance and resistance respectively. The termination condition for the optimization is quantified by a threshold value for the root mean square deviation RMS_{tr}=0.001, computed for all data points in the simulated and measured time series.

Application of a Prior Hypothesis to Prove the Existence of Diseases

The detection of diseases in this section is either based on a prior hypothesis (i) applying expected parameters for a specific disease as initial condition or (ii) on the assumption of a defect location. In (i) a set of expected parameters for a particular

Table 1. Modified parameter set from Westerhof (1969), for the generation of idealized pressure waves under normal and diseased conditions in an ideal TAA and AAA (changes are underlined)

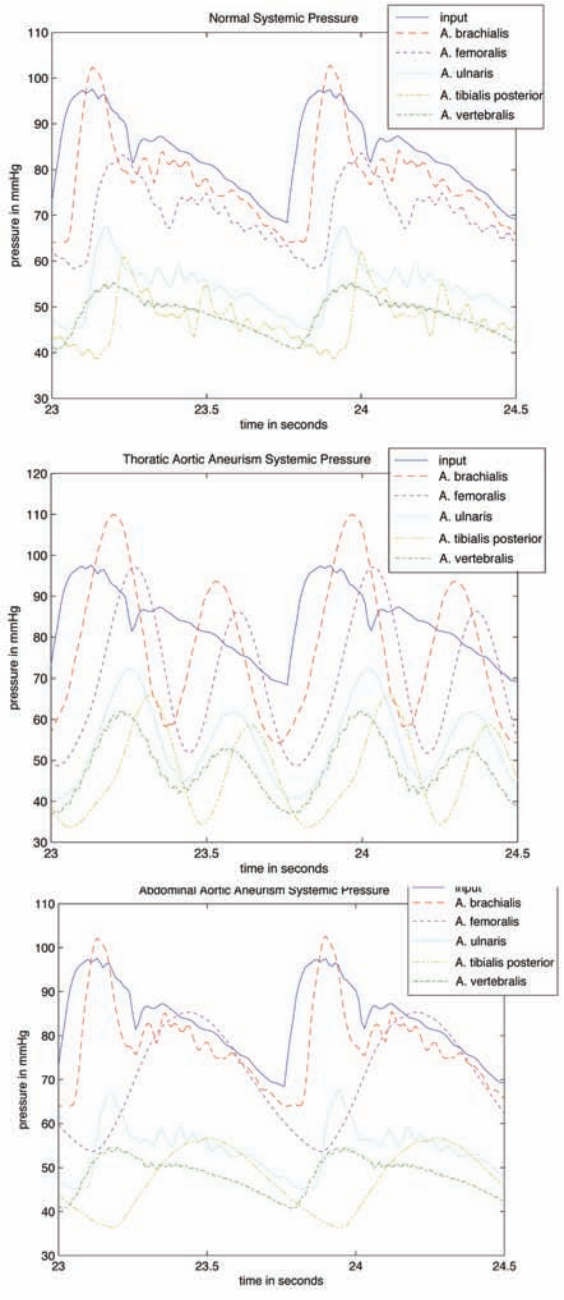
Normal	**61**	**62**	**63**	**64**	**69**	**71**	**72**
C $\left[\frac{ml}{\mathrm{mmHg}}\right]$	6.95E-02	7.96E-02	3.35E-02	3.00E-02	2.72E-02	2.41E-02	2.11E-02
L $\left[\frac{mmHg}{ml}s^2\right]$	9.00E-04	1.30E-03	2.90E-03	3.10E-03	3.50E-03	4.00E-03	4.50E-03
R $\left[\frac{mmHg}{ml}s\right]$	2.00E-04	3.00E-04	1.20E-03	1.70E-03	2.20E-03	2.70E-03	3.40E-03
TAA	**61**	**62**	**63**	**64**	**69**	**71**	**72**
C $\left[\frac{ml}{\mathrm{mmHg}}\right]$	2.78E+00	7.96E-02	3.35E-02	3.00E-02	2.72E-02	2.41E-02	2.11E-02
L $\left[\frac{mmHg}{ml}s^2\right]$	2.25E-04	1.30E-03	2.90E-03	3.10E-03	3.50E-03	4.00E-03	4.50E-03
R $\left[\frac{mmHg}{ml}s\right]$	1.25E-05	3.00E-04	1.20E-03	1.70E-03	2.20E-03	2.70E-03	3.40E-03
AAA	**61**	**62**	**63**	**64**	**69**	**71**	**72**
C $\left[\frac{ml}{\mathrm{mmHg}}\right]$	6.95E-02	7.96E-02	3.35E-02	3.00E-02	2.72E-02	2.41E-02	8.43E-01
L $\left[\frac{mmHg}{ml}s^2\right]$	9.00E-04	1.30E-03	2.90E-03	3.10E-03	3.50E-03	4.00E-03	1.10E-03
R $\left[\frac{mmHg}{ml}s\right]$	2.00E-04	3.00E-04	1.20E-03	1.70E-03	2.20E-03	2.70E-03	2.13E-03

disease is used to test the correlation to the measured data and in (ii) a set of normal parameters is used and the convergence of the optimization process in the assumed defect location is observed. Both approaches can be seen in analogy to the statistical classification procedure explained in the section “Future challenges”, except that the choice of locations and parameters are not single valued and entered by the user, but are expressed by prior probability distributions sampled from a subpopulation of patients affected by the supposed disease.

In the following examples we set up a series of hypothesis concerning the non-/existence of TAA, AAA and normal health conditions. The optimization results give hints that may lead us to a verification/falsification of the hypothesis.

A. Allow parameters within one segment to be optimized, use normal starting parameters,

Figure 3. (a-c) Simulated ideal pressure waves for the five specified locations under normal (top) and diseased conditions for the TAA (middle) and AAA (bottom). Parameter modification is given in Table 1.



observe convergence and modified parameters to verify/falsify location hypothesis.

B. Allow parameters in several locations to be optimized within a specific range, use normal parameters as initial condition, observe convergence and modified parameters to generate hint for location and kind of the disease.

C. Assume diseased parameters as initial condition for a series of locations, observe initial correlation to verify/falsify diagnostic hypothesis.

The above hypotheses can be combined and repeated to test several diseases where good parameter estimates already exist. This methodology can be regarded as manual cardiovascular screening by comparing the measurements to simulations generated by parameter sets that are related to specific diagnoses.

RESULTS AND PROBLEMS OF THE PARAMETER ESTIMATION

Within this paragraph we show the results obtained by a variety of hypothesis to the measured data. Firstly we assume the defect within several locations of the network, i.e. either (a) single set of RCL parameters in segment *61, 62, 69, 71* or *72* is optimized, while the other segment parameters are constraint or (b) the parameters of multiple segments are optimized together. Finally (c) we apply a set of aneurism parameters to several segments *61, 62, 69, 71* and *72* to compare the correlation to the observed data generated for the TAA by RMS error.

A. The optimization results indicate that obviously the best correlation with RMS of *9.77 E-5* is obtained if only segment *61* is optimized, while all other parameters are held fixed. In contrast the hypothesis that the defect is in *62* or *72* yielded only a RMS of

Figure 4. Comparison of the original pressure wave for TAA taken at the arteria femoralis (segment #100) to optimization results for selected hypothesis given in Table 2. The optimized data curve for a defect hypothesis in segment 61 is congruent with the original TAA data, while for a defect hypothesis in segment 72 the optimization yields decreasing correlation.

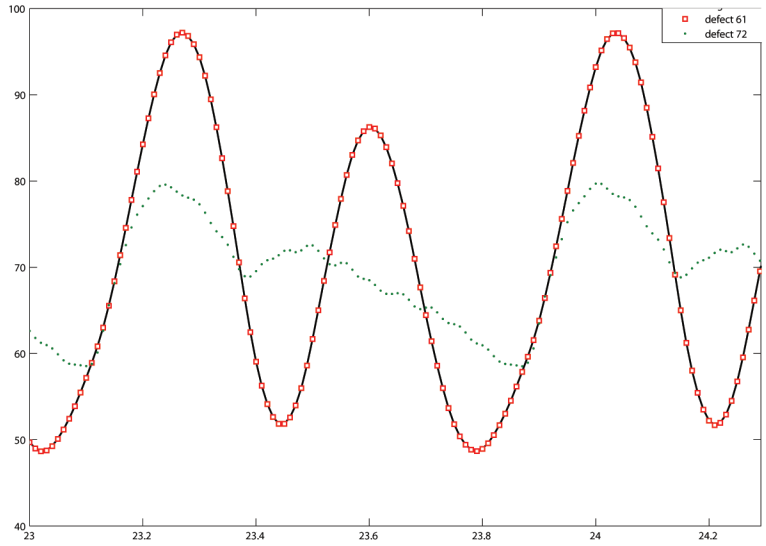


Table 2. Optimization for single segment hypothesis with SENSOP

Hypothesis	estimated C	estimated L	estimated R	RMS	Num. of iterations
61	2.78E+00	2.25E-04	1.24E-05	9.77E-05	89
62	1.58E+00	3.68E-05	0.00E+00	4.19E+00	84, no success
72	2.11E-02	4.50E-03	2.28E-03	1.01E+01	26, no success

4.19 and *10* respectively (see Figure 4 and Table 2). This indicates that the aneurism is in segment *61*.

B. The results and parameters for the optimization of the compliance in segments *61, 62, 69, 71* and *72*, indicate that an aneurism is located in segment *61*, because the value for *C61* is increased and close to the true value (see Figure 5 and Table 3)

C. The hypothesis that a set of chosen parameters for an aneurism are true/false for segments *61, 62, 69, 71* and *72* is done by comparing the RMS between the proposed locations. The RMS of *4.43* clearly indicates that the aneurism is in segment *61*, because all other locations have increased RMS error (see Figure 6 and Table 4).

Table 2 to 4 show the numerical results for the optimizations obtained for measurement data at locations *#8, #14, # 22, #100* and *#110* with hypothesis according to categories defined above. The number of iterations, RMS Error, true/estimated values are given to conclude the results.

Figure 5. According to the parameters in Table 3 the evidence that the defect is in segment 61 is assisted, because in either optimization over several parameters the values indicate that segment 61 has increased compliance.

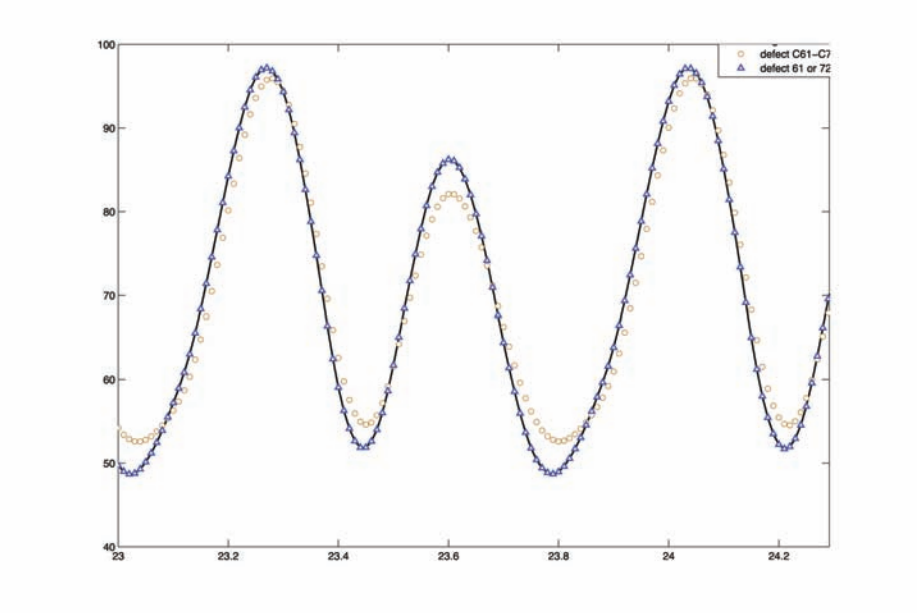


Table 3A. Optimization for multiple segment hypothesis (C61-C72 as free parameters) with NLSD after 484 iterations and RMS of 3.90. It is clearly seen that the value of C61 is increased and approximates the true value. This is a hint for an aneurism in segment 61.

Free parameter	Estimated Values	True Values
C61	1.75E+00	2.78E+00
C62	5.34E-02	7.96E-02
C69	6.20E-07	2.72E-02
C71	5.90E-02	2.41E-02
C72	5.47E-06	2.11E-02

Table 3B. Another hypothesis optimizing the RCL parameters of 61 and 72 yielded the same conclusion, because the NLSD found the true parameters with a RMS of 8.198E-4 in the signals. This indicates that the aneurism is more likely in segment 61.

Hypothesis	estimated C	estimated L	estimated R	RMS	Num. of iterations
61 / 72	2.778 / 2.104E-02	2.250E-04 / 4.499E-03	1.253E-05 / 3.394E-03	8.198E-04	1269, success

Figure 6. Comparison of the original pressure wave for TAA taken at the arteria femoralis (segment #100) to results for selected hypotheses about the location of the defect given a set of diseased parameters (see Table 4). The data curve for a defect hypothesis in segment 61 is most likely, because the RMS is smallest, while for segment 62, 69, 71 and 72 the RMS is increased.

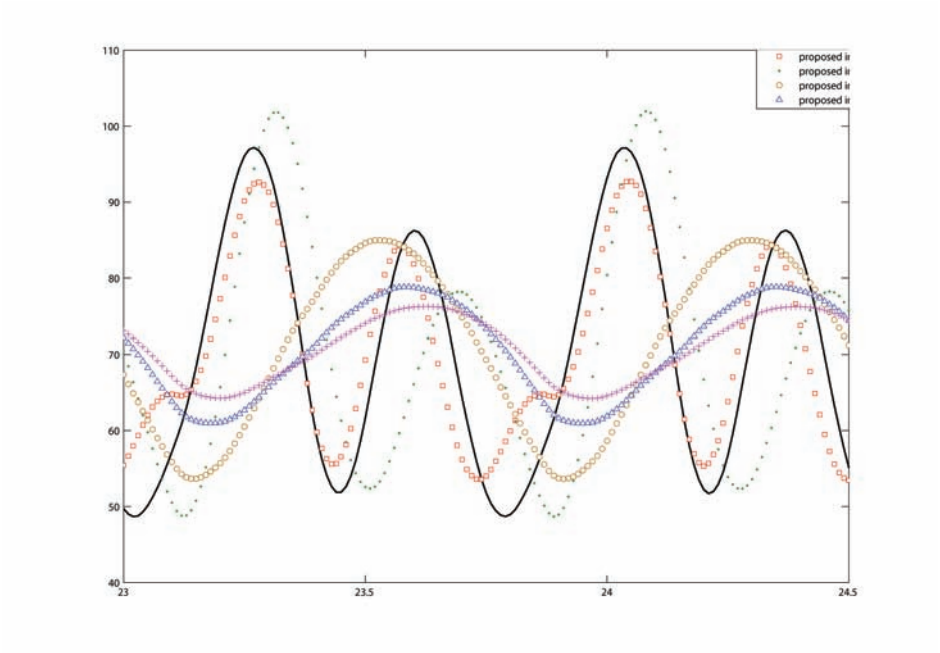


Table 4. RMS error obtained with diseased parameter hypothesis of C=2.0, L=3E-4 and R=1.5E-4 applied to segments 61, 62, 69, 71 and 72. The RMS error indicates that the defect is in segment 61.

Hypothesis	RMS error
61	4.43
62	9.10
69	10.89
71	10.40
72	10.20

Lessons Learnt so Far

From the above results is becomes clear that, even though, optimization over all parameters is not feasible, a series of well chosen hypothesis leads to a conclusion revealing the defect in segment *61*. However these results cannot be quantified into a one-to-one relation between defect and the parameters, but by a probabilistic relationship. The above examples are based on ideal data, i.e. complete time series, no noise, no variability of patients or other influencing conditions. For true measurements the information content is much lower and the above parameter estimation will lead to vague estimates that cannot be used for diagnosis. However this putative information deficit generally causing the ill-posedness of the inversion problem can be overcome by statisti-

cal methods that use prior assumptions observed within a broad patient population. A theoretical basis for the solution of such problems is given in the framework of Bayesian statistics, which we discuss in the next section.

The Hemodynamic Inverse Problem Expressed as Bayesian Inference Problem

Like in many fields of biological sciences and medicine, the methods that process signals obtained from measurements in cardiovascular physiology have to deal with uncertainty to obtain suitable results. The reason for the ill-posedness of model systems applied is manifold: (i) the number of measurements is limited, (ii) the measurements are imprecise, because they contain noise and artifacts and (iii) confounding variables (breathing, movement,...) are difficult to model and control. Indeed, each of these sources of uncertainty can be included in the data mining, if sufficient models for the underlying physiological phenomena exist and if the experimental conditions involved in the measuring process are known. In other words, problems in cardiovascular physiology are statistical in nature and their solution depends essentially on the quality of the data and the data mining routines used.

From Traditional Data Inversion to Statistical Inference Problems

Traditional data inversion techniques have shown their success in well-posed deterministic problems that appear in many areas of sciences. Ill-posedness is conventionally, treated by regularization methods that improve the condition of the problem by more or less ad-hoc assumptions. Examples of common regularization methods are the truncated singular value decomposition, Tikhonov regularization or iterative methods like the Landweber-Friedman or Kaczmarz iteration and Krylov subspace methods (Kaipio, 2005). The basic idea behind the regularization is to transform the ill-posed problem such that a unique solution can be obtained by standard methods that are robust to small errors in the data (Kaipio, 2005). However these methods mostly exclude important information from the solution procedure, thus the result is degenerated and may not represent important features contained in the data. Furthermore the representation of patient's health condition by a single set of parameters is not suitable for diagnostic purposes, because it cannot describe the variances within a patient population.

In contrast to traditional deterministic methods the objective of statistical inversion methods is to assess all information contained in the variables, based on the model and the measurement process, to express the uncertainty in terms of probability distributions. The solution to the inverse problem is then obtained by the evaluation of the posterior probability distribution. In other words, instead of a single estimate for the unknowns, as obtained by traditional inversion methods, the statistical method produces a continuous range of values to which the distributions assign a probability density value for each realization. In the setting of statistical inversion theory the ill-posedness is thus removed by restating the inverse problem as a well-posed extension in a larger space of probability distributions (Kaipio, 2005). We note that a delta function as posterior distribution in the statistical setting is equivalent to a single estimate in traditional inversion theory.

The statistical basis of inversion techniques is given by Bayes formula, the problem is referred to as Bayesian inference problem. Bayesian statistical inference techniques have been used in several areas to extract useful information contained in physiological data sets (D'Argenio, 2006; Gerwinn, 2010; Hallander, 2010). In most applications concerning cardiovascular physiology the physical quantities and hence the measured data are functions of time and parameter estimation has to occur continuously. Such classes of inverse

problems are referred to as non-stationary inverse problems (Kaipio, 2005).

Non-Stationary Inverse Problems

Bayesian recursive approaches rely on a state-space formulation of the inverse problem and provide useful tools to give continuous parameter estimates. The most popular representative is the extended Kalman filter (EKF) that can simultaneously estimate the parameters and unobserved states from noisy data of nonlinear time-continuous systems (Sitz, 2002). In contrast to the full Bayesian formulation it is restricted to uni-modal probability density functions, non-linear transition and measurement processes and Gaussian measurement noise.

Recently (Zhang, 2004; McCombie, 2005; Swamy, 2007; Mukkamala, 2008) have proposed KF based methods to estimate the central aortic pressure waveform form two non-invasive peripheral pressure measurements. These methods are currently limited, because they cannot identify the pressure distribution within the rest of the vasculature. This deficit was discussed in (Thore, 2008) where the authors demonstrate the applicability of a one-dimensional wave propagation approach in combination with a traditional parameter estimation method to identify patient-specific parameters. Additionally to the achievements in (Zhang, 2004; McCombie, 2005; Swamy, 2007; Mukkamala, 2008), they where able to reproduce the abdominal aortic pressure waveform from non-invasive measurements with high accuracy. However, their method does neither include uncertainty inherent to measurements nor it is able to describe the variability of parameters found in a patient population.

The subjective of the following paragraph is to include these deficits into a framework that allows to combine the variety of cardiovascular forward models with statistical inference methods for parameter estimation. The novel approach allows us to model patient-specific dynamics of the cardiovascular system based on measurements and to extract characteristic waveforms and parameters that are statistically related to diseases that can be used for therapeutic purposes.

Problem Abstraction of the Multi-Measurement Setup

The constraint hemodynamic inverse problem can be described as blind channel estimation problem with a single input (pressure-flow wave ejected by the left ventricle) and multiple outputs (multi-transducer measurement) (SIMO). This problem is well known from wireless network communication problems (Tong, 1998) and has been successfully applied to problems in physiology to remove noise and artifacts from measurements (Sameni, 2006) or to estimate the central aortic pressure (Zhang, 2004; McCombie, 2005; Swamy, 2007; Mukkamala, 2008). In contrast to the classical input–output problem, where both input signal, s, and observed data, d, are available to estimate the transfer-function, the SIMO problem only relies on the observation signal, d, to identify both the input signal, s, and the parameters, λ, of the channels H_k. In Figure 7 we show a schematic of the SIMO estimation problem derived for the multi-measurement setup of the constraint hemodynamic inverse problem.

The K different channels, H_k, with common source are obtained by the definition of K measurement locations. Each channel contains a forward map A_k, that relates the unknown parameters λk to the ideal data d (see Fig. 8). We assume that the measurement data is incomplete and contaminated by noise, so that the measured signals are assumed to have the form

$$d_k^* = \xi_k A_k(\lambda) + \eta_k. \quad (1)$$

Here we denote the data loss by ξk and the measurement noise by ηk. We assume an additive white Gaussian noise (AWGN) model where

Figure 7. Statistical inversion scheme for the single input – multiple output problem arising by reformulation of the hemodynamic inverse problem as statistical inference problem.

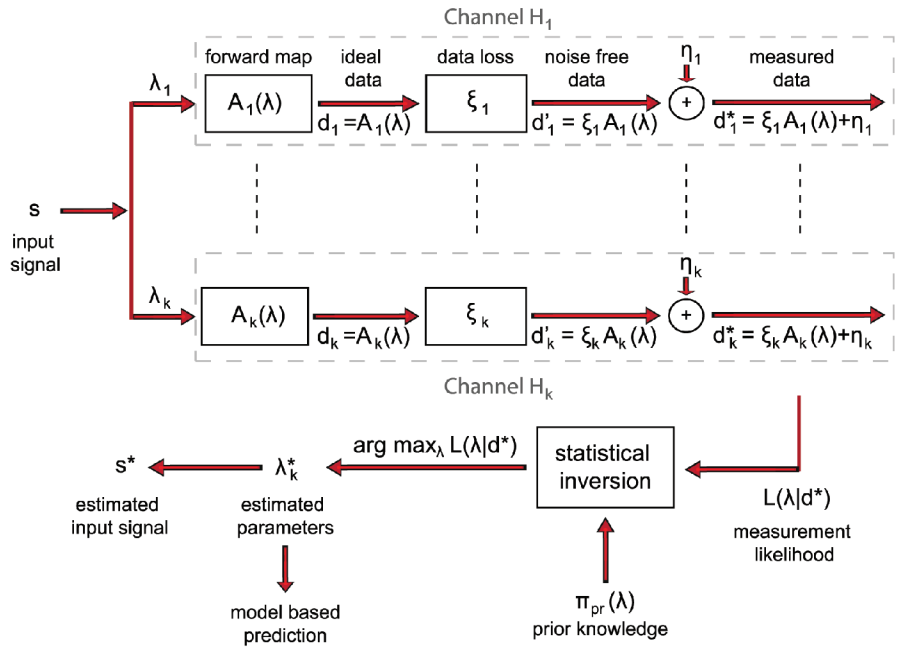
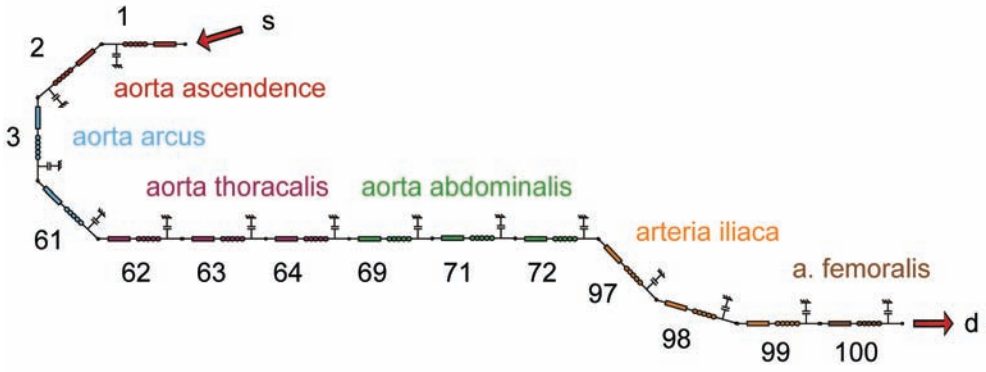


Figure 8. Deterministic forward map for the channel left ventricle – arteria femoralis. In this case the physical model is given by a series of RCL-circuits, where we have assumed insignificant data loss and noise.



$\eta_k \sim N(\mu_k, \sigma_k^2)$ with mean μk $_a$nd standard deviation σk.

In the underlying formulation the forward maps may contain either RCL-circuits, transmission line or one-dimensional wave propagation elements describing a subsystem of the vasculature. An example illustration for the channel from the heart to the arteria femoralis is given in Figure 8.

The parameters for the channels and properties of the input can be estimated by time series analysis of the outputs, if the input probability distribution and moments are known.

Problem Formulation in Terms of Bayesian Inference

The basis of statistical inversion techniques is given by Bayes formula

$$\pi_{post}(\lambda \mid d^*) = \frac{\pi_{pr}(\lambda)\pi(d^* \mid \lambda)}{\pi(d^*)}, \qquad (2)$$

that describes the statistical inference problem in terms of probability densities. $\pi_{pr}(\lambda)$ is the prior probability density for the parameters λ. It expresses what is known about the unknown parameter vector prior to the measurement, i.e. it contains prior information that does *not* depend on the actual measurement data d^*. The posterior probability density $\pi_{post}(\lambda|d^*)$ is the conditional probability density of λ, given the observed data d^*. The so called measurement likelihood, $\pi(d^*|\lambda)$, is the conditional probability of d^* given λ. It expresses the likelihood of different measurement outcomes given d^*. Finally the marginal probability $\pi(d^*)$ of d^* acts as normalization constant. The value indicates if the model is consistent with the experimental observation.

The solution to the statistical inverse problem is obtained by the determination of the parameters λ_k given a set of measurements d^*. It comprises three steps: (i) estimation of the prior probability density according to all prior information of λ, (ii) evaluation of the set of parameters where the measurement likelihood is maximal and (iii) computation of the posterior probability density.

Prior Modeling

Within the theory of statistical inversion the available prior information is sufficiently included in the inversion process. The prior knowledge helps to formulate the ill-posed problem as a well-posed inferential problem. In cardiovascular physiology such prior information consists of knowledge about the distributions of structural and biomechanical properties of the vasculature, like the vessel diameters, elasticities and lengths, but also the anatomic variants of the morphology of the network that is available prior to the measurement. The determination of appropriate model priors is challenging and consequently a variety different approaches have been introduced (Kaipio, 2005). In contrast to the specification of regularization functionals with either physical or ad-hoc assumptions, the construction of prior models involves the estimation of a probability distribution $\pi_{pr}(\lambda)$, that relates all values of the unknown λ to a probability value. Thus, values which are physically inadequate have a lower probability to appear than expectable ones. In the Bayesian approach model assumptions are typically proven by drawing several samples from the prior distribution and evaluating if the results are reasonable.

Initially a Gaussian distribution is appropriate as prior model, because the mean and standard deviation for the parameters λ can be estimated from biomechanical experiments found in literature (Dobrin, 1978; Manor, 1994; Zanchi, 1997; Hoeks, 1999). The structural priors that describe the morphology of the vasculature can be estimated from experimental studies that explore anatomical variants (Jovanovic, 2004). These initial prior models however are continuously improved by clinical measurements or simulation data obtained for a broad patient population.

Furthermore the definition of various sample-based priors, containing information about specific classes of diseases, serves as the basis for the design of a Bayesian classification algorithm (see later Section ÑFuture challenges“). Within the classification procedure these priors are referred to as representative ensemble that randomly generates samples that act as hypothesis to prove the existence of specific diseases.

Measurement Likelihood

The measurement likelihood expresses the distribution of the measurement signals if all parameters λ are known. It conversely can be used to generate simulated signals for a known parameter set that can be compared to the actual measurements. Within our problem the likelihood can be defined as

$$L(d^*_{1:I,1:K} \mid \lambda_{1:K}) = \prod_{i=1}^{I} \prod_{k=1}^{K} P[d^*_{i,k} \mid \lambda_k], \tag{3}$$

$$\lambda^* = \arg\max_{\lambda} L(d^* \mid \lambda)\,. \tag{4}$$

In other words, without assumptions about the parameter to estimate, one chooses the value that makes the output most likely. Within the context of Bayesian statistics this maximization problem can be solved even if the data is high-dimensional, incomplete and noisy. The general procedure is the expectation maximization (EM) algorithm discussed in the next paragraph.

Expectation Maximization Algorithm

Herman O. Hartley (Hartley, 1958) pioneered the research on the EM algorithm in the late 1950s, the mathematical basis was given by Dempster, Laird, and Rubin (Dempster, 1977) in the late 1970s. Over the years, the EM algorithm has found many applications in various domains and has become a powerful estimation tool (McLachlan, 1997; Duda, 2001). In order to explain the EM procedure we will follow Neal and Hinton (Neal, 1993; Neal, 1998; Minka, 1998) who presented the EM algorithm from the perspective of lower-bound maximization.

In order to apply MLE to our case, we formally assume a certain joint probability density function (PDF) $\pi(d^*,s|\lambda)$ between the observed and hidden random variables, and then marginalize over the hidden random variable s, i.e, we have to integrate the joint PDF over the hidden state space S_h of s. This is the key idea behind the EM algorithm. According to this strategy, we compute the optimal parameter estimates as optimizers of the marginalized log-likelihood, log$L$.

$$\lambda^* = \arg\max_{\lambda} \log L(d^* \mid \lambda) = \arg\max_{\lambda} \log\left(\int_{S_h} p(d^*, s \mid \lambda) ds\right) \tag{5}$$

In the case of a discrete, finite hidden state space the integral in (5) has to replaced by a sum.

The EM algorithm is an iterative optimization procedure. Starting with an initial param- eter estimate λ (0), it is guaranteed to monotonically increase the likelihood function and converge towards some maximum of the Likelihood function. This maximum typically is the gloabal one, if some good initial estimate $\lambda(0)$ is available. In our case, the initial estimate can be taken from the classical parameter estimation procedure.

Bayesian Classification as Concept in Patient-Specific Cardiovascular System Diagnosis

Bayesian data integration addresses the classification problem by learning the distribution of instances of specific diseases in a subpopulation. The basic idea of Bayesian data integration and classification is discussed in (Firedman, 2002; Ceci, 2003). Within this study we use a naive Bayesian classifier, since more complex models may reduce the performance of the learning and inference processes.

In order to outline the classification problem in the setting studied here, we have to start with the assumption that we have available the patient-specific measured signals $d^*_{i:I,k:K}$ for M different patients that in some appropriate sense form a sampling of the patient population. Let us write $d_1^*, \ddot{O}, d_M^*$ for the sequence of these measurements. Classification now means that we want to find out

which of the M patients belong to certain classes $C_1,\ddot{O},C_L$. These classes can be, e.g., the class of patients that are do not have any stenosis, C_0, and the classes of patients that have stenosis in segment number k of our model, C_k, which we will refer to as class k in the following. What we want to know is the probability that, conditional to the sequence of observed signals, patient j is in class k, i.e., we want to know

$$P\left(j \in C_k \mid d_1^*,...,d_M^*\right). \tag{6}$$

By using Bayes' formula again, this probability can be computed from

$$P\left(j \in C_k \mid d_1^*,...,d_M^*\right) = \frac{P\left[d_1^*,...,d_M^* \mid j \in C_k\right]P\left[j \in C_k\right]}{\sum_K P\left[d_1^*,...,d_M^* \mid j \in C_k\right]P\left[j \in C_k\right]} \tag{7}$$

where $P[j\in C_k]$ denotes the prior probability of patients of class k and $P[d_1*,\ddot{O},d_M*|j\in C_k]$ denotes the probability of measuring the signal from a patient of class k. While the former probability is a classical prior with which we have to deal as described above, the latter probability has to be estimated algorithmically from the sequence of observations $d_1*,\ddot{O},d_M*|$. Thus an appropriate classification algorithm has to perform two different estimations simultaneously:

1. Density estimation: Estimate the probability $[d_1*,\ddot{O},d_M*|j\in C_k]$ that a certain form of signal is observed from a patient of class k.
2. Classification: Find the hidden information whether patient j, from which signal d_j* originates, belongs to class k.

The combination of these two tasks in the sense of a joint likelihood optimization again leads to the EM algorithm. That is, in every step of the EM iteration first the density estimation is updated and then the classification probabilities. The iteration again converges if we chose appropriate initial values and results in the optimal densities and classifications based on the available observation. Consequently, the results become better and better when more and more signals are added to the available information.

FUTURE CHALLENGES

The aim of the above framework is to provide the basic tools that enable classification of measured data into classes with common properties – i.e. that are related to specific diseases. These classes can be used to classify unknown data measured at patients with unknown diagnosis by means of fuzzy probabilities. Such strategies have been applied in several areas of physiological sciences (Diamond, 2004; Noh, 2006; Srinivas, 2010). As obvious from the above description the Bayesian classification method comprises two steps:

First, in the learning period, the input of measurement data and known relationships to cardiovascular diseases (training data) are used to train the priors and densities needed in the EM algorithm. The input data can be any type of experimental or computational result that can be related to the model. The probability distributions over the values in these training datasets are learned from examples verified by the gold standard (valid diagnosis), thus allowing the generation of new relationships that describe disease specific classes. The gold standard relationships can include any properties of time series known to be related or unrelated to a particular disease. In general, these gold standards are formed by data obtained for a subpopulation of patients with known diagnosis.

Second, in the prediction phase, the classification probabilities are predicted by the classification algorithm based on newly acquired signals (testing data) without available valid diagnosis. The prediction is generally based on a network indicating how likely the observation of measurements fits to a specific class. If one considers this network as a connection matrix, it is just a collection of fuzzy

like measures, each representing a probability of functional relationship between the measurement and the class. According to the classification probabilities the procedure provides a diagnostic hint about the existence of afore characterized diseases. In other words, the algorithm sets up a series of hypothesis, that are based on the prior information of a subpopulation obtained in clinical observations, to classify the health condition of the patient by statistical inference.

The algorithmic classification procedure is as follows:

1. Use training data $d^*_{1:I,1:K}$ to determine optimal parameters λ^*, priors and density estimation via the EM algorithm
2. Classify testing data $d^{\dagger}_{1:I,1:K}$ via (fuzzy) classification probabilities $p^m(d^{\dagger}_{1:I,1:K})$.
3. Integrate testing data into training data set and re-optimize parameters.

In order to realize this approach for cardiovascular diseases we will have to train the algorithm on a significantly large population of patients which is the future challenge we will have to face. Then the statistical classification is a method that allows us to identify cardiovascular diseases in an early state that are followed by therapeutic intervention convenient for individual patients. In contrast to other methods, that determine a set of selected parameters with pretended relevance for diagnosis, the classification method automatically selects and quantifies all relevant parameters to prove a series of proposed diseases in the fashion of differential diagnosis.

CONCLUSION AND OUTLOOK

Within this work we have outlined the problems that may arise in the solution of the constraint hemodynamic inverse problem. We pointed out that ill-posedness is problem inherent and that a unique solution is hindered by (i) the lack of information contained in the finite number of measurements, (ii) the contamination by artifacts and noise and (iii) traditional models and data mining techniques generally used. We have shown that statistical inference methods provide various advantages over deterministic approaches such as quantitative parameter estimates, determination of confidence intervals, treatment of arbitrary forward maps, error estimates and parameter estimates given incomplete and noisy measurement data. These properties are proposed to be the basis in patient specific diagnoses, because they provide a statistical framework for the description of the cardiovascular system that allows therapeutic interventions in individual patients. In contrast to other methods the proposed classification automatically selects and quantifies all relevant parameters to prove a series of proposed diseases in the fashion of differential diagnosis

Although the interdisciplinary challenges involved in the ongoing project are daunting, it is important to recognize the potential gains for patient-specific cardiovascular diagnosis. However, up to now the progress has been inhibited by the lack of a broad data basis of non-invasive hemodynamic measurements, advanced inverse modeling tools and databases for large-scale model integration and data classification. Nevertheless we think that the new modeling tools will find several applications in today's medicine. The progress will depend on the level of support from funding and industry and the interest of clinicians.

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KEY TERMS AND DEFINITIONS

Hemodynamic Forward Problem: Within the forward problem the pressure and flow waveforms at places of interest are computed for the applied input pressure or flow using a set of model parameters for the circulatory system. This implies that the lengths, radii and compliance values of all vessel segments are known.

Hemodynamic Inverse Problem: Within the inverse or backward problem the model parameters of the circulatory system are estimated when pressure and flow at specific places are given.

Well-posed - Ill-posed: The mathematical term well-posed defines that a unique solution to a mathematical model of a physical phenomena exists, that depends continuously on the data. Problems that are not well-posed are termed ill-posed. Due to the lack of information in measured data inverse problems are often ill-posed.

Condition: Even if a problem is well-posed, it may be ill-conditioned, meaning that the results are strongly dependent (sensitive) on the initial data. An ill-conditioned problem is indicated by a large condition number.

Regularization: If the problem is not well-posed, it is generally re-formulated for numerical treatment. Typically this involves including additional assumptions, such as smoothness of solution. This process is known as regularization.

Bayesian Inference: is statistical inference in which observations are used to infer the probability that a hypothesis may be true.

Stenosis and Aneurism: A stenosis is a constriction of blood vessels mostly based on arteriosclerosis, while an aneurism is the term for a balloon-like dilation of a vessel. Both are vascular defects, the former can cause ischemia, while in the latter case the vessel may burst under normal pressure conditions.

Compliance: The term that quantifies the dispensability of a blood vessel for a specified pressure increment.

Chapter 2
Personalized Experience Sharing of Cai's TCM Gynecology

Guo-Zheng Li
Tongji University, China

Mingyu You
Tongji University, China

Liaoyu Xu
Shanghai Literature Institute of TCM, China

Suying Huang
Shanghai Literature Institute of TCM, China

ABSTRACT

This chapter introduces experience sharing of Cai's gynecology in Traditional Chinese Medicine (TCM). It argues that Cai's family in China are experts in TCM gynecology, whose seventh generation Xiaosun Cai is a TCM master. Therefore, inheriting experience of Xiaosun is a critical business in the present TCM field. The authors demonstrate a novel system (named TCM-PMES) for preserving the diagnostic processes of veteran practitioners like Xiaosun in a personalized way. Based on the summarized Cai's diagnostic template, a custom input system embracing the particular expressions of the specialist is set up in the platform. Unique and unfiltered experience of veteran practitioner is maintained as complete as possible. Various approaches on exploring relationships among TCM components and speculating syndrome types from clinical symptoms are also studied. This work provides a new way for keeping and researching the personalized factors in the diagnostic process of Cai's TCM gynecology.

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INTRODUCTION

Gynecology is so complicated, that doctors often say that illness of women is ten times harder to cure than that of men (Cai, Huang & Mo, 2000). Different from men, illness of women is related with menstruation, pregnancy, vaginal discharge and labor. Due to the variance of women with different conditions, the pathological mechanism and treatment prescription are not the same in TCM (Traditional Chinese Medicine) gynecology. Even for cold headache or pharyngalgia, if the patient is in the period of menstruation, we need to consider whether she is in disorder of menstruation, the quantity or abdominalgia. The prescription should not only release the exterior or disturb the menstruation, but also accord to the therapeutic principle of considering the root and tip in total. If the same disease takes place in another pregnant woman, some herbs hurting the foetus must not be used in principle, but may be used in specific cases. If the ill women newly gave birth, they have much spontaneous perspiration. The doctor should consider they are blood deficiency and need to discharge the lochia completely. So relieving the exterior with pungent and pool need to be improved with other measures. In TCM gynecology, we further need to consider their physical type, old or young, living conditions, working status and so on of the patients.

Cai's gynecology started from 250 years ago, the present existing successor is the 7th, whose name is Xiaosun Cai. Xiaosun was born in 1923, he has dedicated to medical care for more than 60 years. Xiaosun was elected as the national tutor for inheriting the maters' experience in 1992 and included in the medical who's who published by Cambridge Press in 1995. Now he has several students, one of which is Suying Huang, one of the authors. Xiaosun has inherited and accumulated ample experience in his career, he still takes front line jobs now. TCM masters like Xiaosun are high years old, and their experience may be lost some day, which are inherited from their family or accumulated in their work throughout their lives. These experiences are valuable, and hard to be summarized into knowledge directly now due to the complex TCM principles. Therefore, collecting their medical records and mining their experience is a critical work in the TCM field, which is also a policy of the Chinese government.

As a complete medical system, TCM plays an indispensable role in medical care in China for more than five millenniums. It is a treasure of Chinese culture. Different from the Reductionism thinking mode of Western Medicine (WM), TCM is based on the holistic and systematic ideas. Classification of TCM syndrome should be based on overall analysis of symptoms and signs of human body. Symptoms or signs in different areas of body may contribute to the same kind of syndrome. Even the relationship between disease and syndrome might be many-to-one. Based on the methodology of holism, TCM plays a unique role in health care and disease treatment. Increasing usage of Chinese natural herbal medicine and acupuncture world widely is a good indication of effectiveness of TCM (Yamashita, Tsukayama, & Sugishita, 2002; Honda & Jacobson, 2005; Thomas, Nicholl, & Coleman, 2001).

Medical knowledge and understanding of TCM veteran practitioner is the practical and creative achievement of TCM principles. They are all valuable assets to the TCM system. Knowledge of veteran practitioner on TCM clinical diagnosis and Chinese herbal medicine sometimes cannot be found in literatures. Traditionally, the precious medical knowledge and philosophical view of veteran practitioner is followed by his apprentices (C. Zhou, 2004). The number of juniors who have the honor to learn from a great master is limited. Furthermore, whether the juniors can grasp the soul of the knowledge system of the veteran practitioner totally depends on his personal ability, sometimes even gift. As most of the famous TCM practitioners getting older, preserving and researching into their clinical records, books or publications becomes urgent and important.

Preserving the knowledge of the TCM veteran practitioner includes understanding, systematical sorting and standardized inputting the available materials from the specialist. A powerful platform is the basis for literature and data gathering. Many TCM online systems are reported in published works. TCMMDB (X.-Z. Zhou, Wu, & Lu, 2001) is a unified web accessible multi-database query system, which has already integrated more than 50 databases. TCM-Grid (Chen, Wu, Huang, & Xu, 2003) is a grid-based TCM system. It is also extended to a semantic-based database grid. X. Zhou et al. (2004) have developed a large-scale ontology-based Unified TCM Language System to support concept-based information retrieval and information integration. These three systems mainly contribute to the collection of Chinese drug database or Chinese medical literature. They pay little attention to the clinical records of TCM practitioners, which are practical experience of TCM principles. Zhang et al. (2008) designs a platform for analyzing and mining knowledge of TCM veteran practitioner. The system is set up with a business software BO. All the platforms or systems mentioned above provide uniform input items for different veteran practitioners. Different from strict standardization of WM, clinical records from TCM practitioners, especially veteran practitioners, have obvious and splendid personal focus and expression. The symptoms and signs one practitioner cares might be different from the other in most parts. The herbal medicines and their combination that veteran practitioner prefers also have distinctly personal factors. All the personal items in the clinical process may be the embodiment of the TCM veteran practitioner's thinking mode and knowledge system. To preserve the symptoms, signs, formula and so on in their original taste and flavor is an interesting and significant task.

Upon the numerous TCM databases, different researches are conducted to discover the implied knowledge. Chinese Medical Formula (CMF) is composed of compound drugs with suitable doses. The potency of a single Chinese herb is limited. Application of several natural herbs ensures a full play of their advantages and inhibiting some side effects. Among the combinations, when two kinds of herbs are frequently used together, they are likely to be paired herbs. The number in paired herbs may be extended to three, four, etc. Yao, Ai, Yuan, and Qiao (2002) use the classical association rule method to study 106 formulae for treating diabetes. The results indicate that different experts have similar ideas and principles for this disease. Zhu et al. (2007) also adopt association rule to analyze the medical records on asthma. The incidence relation among pathogeny, location of disease and syndrome with four kinds of symptoms, the relationship among pathogeny, location of disease, syndrome, four kinds of symptoms and the use of TCM, the relationship among the TCM, are all summarized. Besides association rules, different methods and models are provided to discover the knowledge for TCM clinical diagnosis. Qin, Mao, and Deng (2007) use rough set in the diagnosis of rheumatoid arthritis. The results show that the diagnostic accuracy achieved by rough set for rheumatoid arthritis is greatly higher than that of fuzzy set. A novel self-learning expert system for diagnosis in TCM is constructed by using a hybrid system composing of a Bayesian network learning algorithm, naïve-bayes classifiers with a novel score-based strategy for feature selection and a method for mining constrained association rules. The learned knowledge is provided in multiple forms including causal diagram, association rule and reasoning rules derived from classifiers (Wang, Qu, Liu, & Cheng, 2004).

Supported by the Shanghai government, we launched a project to collect 10 TCM masters' medical records, where these masters are all over 75 years old. We designed a personalized TCM platform for Maters' Experience Sharing (named TCM-PMES) (You, Ge, Li, Xu, & Huang, 2009; You et al., 2008). This chapter aims to provide TCM-PEMS to preserve the precious materials of Cai' gynecology. We further introduce the sum-

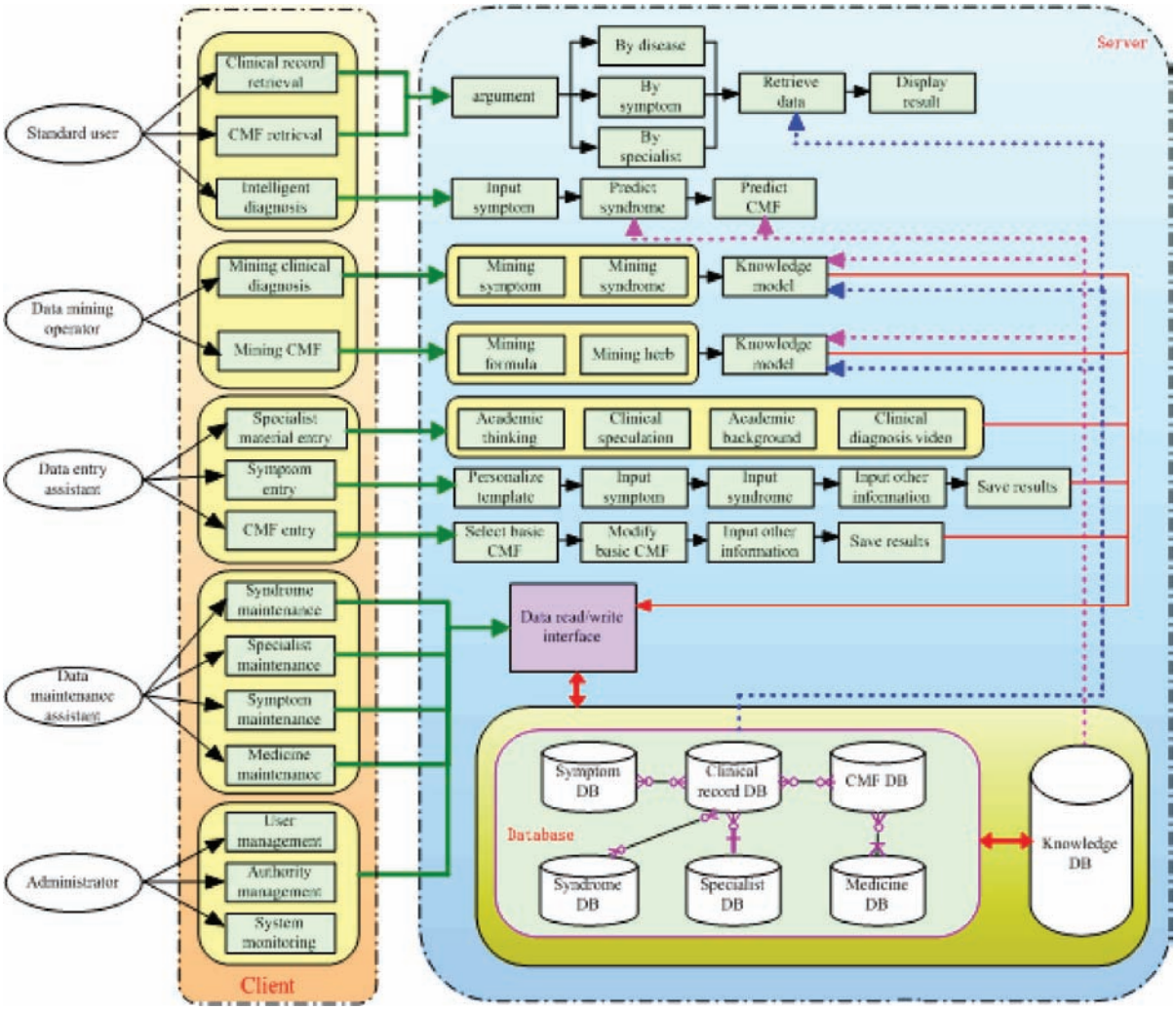
mery of Cai's experience. A brief introduction of the main functions and structure of TCM-PMES is presented in Section 2. Clinical records from Xiaosun Cai are included to illustrate the process of summarizing the clinical template and setting up the personalized system in Section 3. In Section 4, several interfaces of the set up system are explained. In Section 5, experience summarized from the above medical records are presented and discussed. Finally, the chapter concludes on the future work in Section 6.

TCM-PEMS: A Personalized TCM Platform for Masters' Experience Sharing

Cai's gynecology came from 250 years ago, it has many unique terms to describe the disease, the symptoms, cause of disease, mechanism of disease, diagnostic methods, prescription or medical formula, which are different the general terms normalized by the government or international societies. We design a personalized TCM Platform for Maters' Experience Sharing (TCM-PEMS), whose functional diagram is shown in Figure 1. The whole system is based on B/S architecture. Users visit the client by web browser. The server is composed of logic layer and database. The database stores specialist, herbs, syndrome, symptom, clinical record and CMF (Chinese Medical Formula) information. The discovered knowledge is also stored in the database. The client supports five kinds of user roles, and different role user has different authority. Standard user retrieves clinical record and CMF by given arguments, such as disease or symptom. Standard user also predicts the most possible syndrome and CMF from the symptom by leveraging the discovered knowledge saved in database. The knowledge models summarize the occurrence frequencies of symptoms, syndromes and herbs. The knowledge models also abstract the relationship between symptoms and syndromes, the relationship between syndrome and herbs, the relationship between special symptoms and combination of basic formulas, and the internal relationship of herbs. Data mining methods (Li, 2010) have been already implemented in PTCMS include association rule analysis, support vector machine and so on. Knowledge models are trained by data mining operators, who have the authority of mining clinical records and CMF. The training data is input and preprocessed by data entry assistant. The data maintenance assistant maintains the database. Administrator manages users and monitoring the whole system.

The platform is composed of five main layers in function (Figure 2). Data layer contains databases storing information of medicine, CMF, symptom, syndrome, clinical record and specialist. Knowledge models and learning algorithms are saved in the knowledge DB in data layer. The clinical record DB saves personalization template and clinical data. The syndrome DB stores the records of disease, syndrome and therapies with TCM. The specialist DB records the specialist information of individual, learning experience, academic background, academic thinking and clinical speculation. Symptom DB, CMF DB and medicine DB include essential information of symptom, CMF and medicine, respectively. In persistence layer, hibernate is an ORM (object-relational mapping) library for the Java language, which solves object-relational impedance mismatch problems by replacing direct persistence-related database accesses with high-layer object handling functions. The top three layers comprising business, action and view, are managed with the Model-View-Controller (MVC) pattern. The business layer manages the behavior and data in the application domain. There are three parts of application domains in the platform. Firstly, in the domain of statistic and analysis for data, the system accounts the frequencies of occurrence of symptoms, syndromes and herbs, analyzes the prescribing habits of specialists. Besides, the system abstracts the relationships among symp-

Figure 1. Functional diagram of TCM-PMES

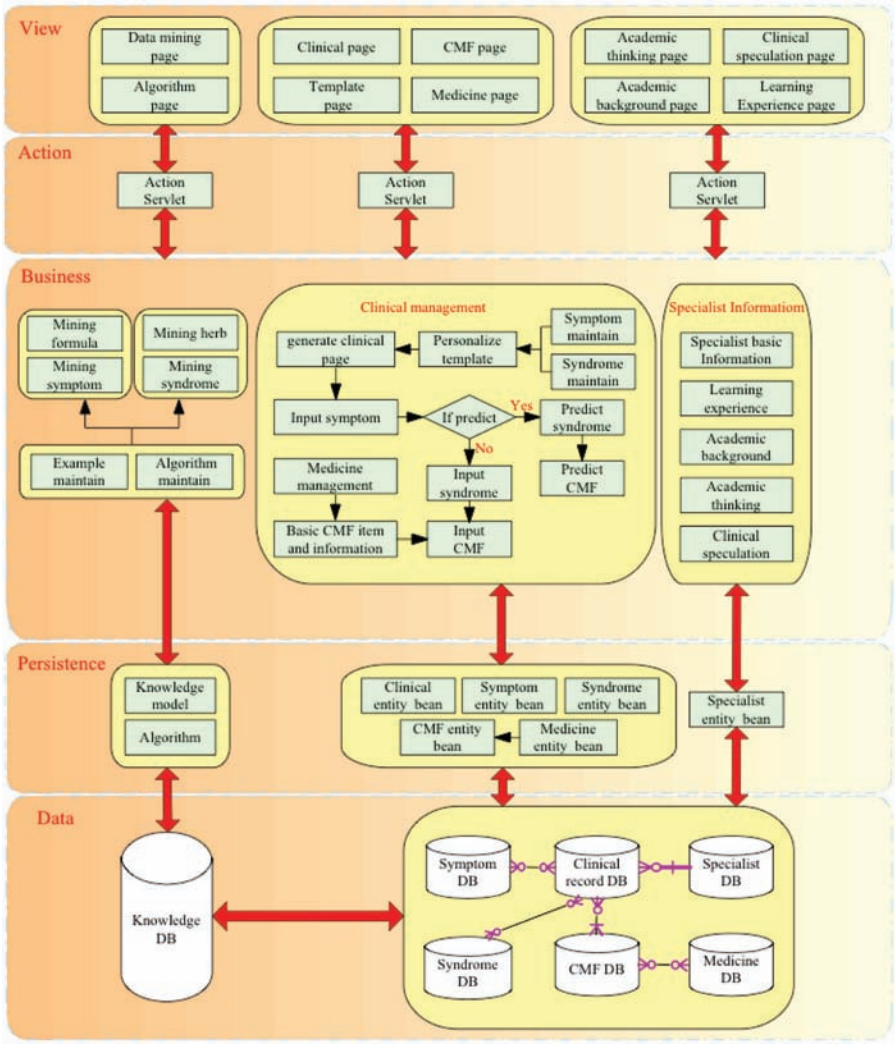


toms, syndromes, herbs and CMF by knowledge models. Secondly, in the clinical management domain, the information of symptom, syndrome, medicine and CMF is maintained. Clinical records including symptom, syndrome, and CMF can be input into the clinical page, which is generated according to the personalized template. Besides data recording, the knowledge models can predict the most possible syndrome from the symptoms of a patient and suggest a suitable CMF. Finally, the content of the specialist domain is a range of specialist information, such as learning experience, academic background, academic thinking and clinical speculation. The action layer interprets the mouse and keyboard inputs from the user, informing the business layer and the view layer to react appropriately. The view layer manages the display of different information.

Diagnostic Template for Cai's Gynecology

Due to the unique terms in Cai's Gynecology, we design a personalized diagnostic template. Cai's Gynecology is famous in China, even in the world, whose representative is Mr. Xiaosun

Figure 2. System architecture of TCM-PMES



Cai in present. Xiaosun is now 87 years old, he is the seventh generation of "Cai's Gynecology". The characteristics of Cai's Gynecology is that the four diagnostic methods: inspection, listening and smelling, inquiry, and palpation are all very important, which need a comprehensive consideration of all four methods (Cai,2000).

"Nine Inspection" is summarized as inspecting the face color, tongue color, lip color, nail, menstrual color and quality, leucorrhea, lochia, hair, as well as others like philtrum. For facial color, if one is blood deficiency, she will be chlorosis; if one is qi deficiency, she will be pallor or superficial; if one is blood stasis, she will be dark blue. If her face is red in cheek, she is yin deficiency. Tongue color and fur are also important, if tongue color is fresh red, she is blood heat; else if color is light, she is blood deficiency; else if color is yellow and fur is slimy in its root, she has dampness and heat in her lower energizer; else if there are purple points in margin of tongue, then she is blood stasis. Lip color reflects the status of spleen, if it is red and purple, she is blood heat; else if it is fresh red and crack, she is yin deficiency

with effulgent fire; else if it is light, she is spleen deficiency and blood deficiency; else if it is deep blue, she has pain, while slight blue means she is deficiency and cold.

Menstruation is a critical factor, if its color is fresh red or deep red, she is blood heat; else if its color is slight red, she is deficiency; else if its color is dark purple; she is blood stasis. If its property is thick, she has heat; else if it is thin, she is deficiency with cold; else if it is with blocks of blood, she is blood stasis. Virginal discharge reflects the status of one's reproductive system. If it is white and thin property, she is spleen deficiency; else if it is yellow with thick property, she is dampness and heat in lower energizer; else if it is blue with thin property, she is kidney deficiency; else if different colors appear, she has tumor in large possibility. In TCM, philtrum is an important position to reflect the status of reproductive system. If it is flat, her womb may not well cultured; else if it is narrow, her womb cervix is also narrow; else if it is short, her womb cervix may be short; else if there is acne on it, there is blood stasis in her reproductive system.

"Listening and Smelling" means ausculating the voices and other sounds and sniffing the tastes. If her voice is low and thin, she is qi deficiency; else if she often sigh, she is qi depression; else if her menstruation is foul smelling, she is blood heat; else if her virginal discharge is foul smelling, she is dampness and heat in lower energizer.

"Eight Inquiring" includes inquiring the age/marriage status, main symptoms, medical history, menstrual cycle, leucorrhea, pregnancy reaction, post partum, and professional life. The age and marriage status are important information. If there is irregular bleeding, she is functional womb bleeding for a girl, while she is malign tumor for a old woman. Some women is shame to say her symptoms or her status, e.g. sex life. Unmarried girl says she is delayed menstruation. But if she is ectopic pregnant, it is danger. As we can see in the above, we need to know the quantity, color and property of menstruation and virginal discharge. We also need to know her professional life, whether she has over nervous work or study. We need to know her diet and spirits, etc.

"Nine palpation" comprises palpating the menstrual pulse, leucorrhea pulse, pregnancy pulse, parturient pulse, postpartum pulse, skin, head and limb, breast, abdomen. If one is during the menstruation, her pulse should be more slippery and surging than usual. If the pulse is hollow, it means her qi and blood drop suddenly, which occurs when flooding or giving birth. For the pulse of a woman with virginal discharge, if it is spring like and racing, she is dampness and heat; else if it is sunken and thin, she is kidney deficiency; else if it is spring like and slippery, she has tumor or inflammation. We should touch the skin of patients, if it is not warm in four limbs, she is qi and yang deficiency; else if it is hot in hands and foots, she is yang and heat excess; else if it is hot in the center of hands and foots, she is yin deficiency and heat interior. On the abdomen, we need to know whether the napes is soft or hard, warm or cool, whether there is blocks and their location, size and property. If the block is hard and painful, she is blood stasis; else if there is no block or she is painless, she is blood deficiency; else if the block disappears when be pushed, she is qi stasis.

The above four diagnostic methods are principles of "Cai's Gynecology" in hundreds of years. Now they claim to acquire an overall and deep knowledge of the disease from macro to micro, combining the modern scientific instruments with the four techniques. For the gynecology examination, the vulva is important to know the quality, color, smell of the secretion and others. The inner reproductive system is also needed examination by considering both the vagina and abdomen.

This study summarizes a basic template from 320 clinical cases of Xiaosun. It is demonstrated from Table 1 to Table 6. We separate the information of patients into five parts, i.e. basic part, cardinal part, general menstrual part, local menstrual part and WM normal examination part. There are

Table 1. Basic information part in the personalized template of Cai's Gynecology

Patient Name	
Gender	Male/Female
Age	
Medical Record No.	
First Visit Date	
Menstrual History	Age of Menarche; Menstrual Period; Menstrual Duration
Marriage Status	Single/Married (YOM year of marriage)
Child Birth History	Times of pregnancy; Times of Miscarriage; Times of Abortion; Number of Children
Contraception	Yes (Condom/Oral Contraceptive Pills/Intrauterine Device)/No (How Long)
LMP (last Menstruation Period)	
PMP (Preparatory Menstruation Period)	
BBT (Basal Body Temperature)	Single Phase/ Two Phase/ Two Phase but Nontypical/ Rising/Duration of High Temperature Phase Less Than 12 Days/ Can Not Rise Stably/ BBT Rise and Drop/ BBT Rise but Do Not Drop
Chief Complaint	
History of Present Illness	

Table 2. Cardinal symptoms part in the personalized template of Cai's Gynecology

Status of Patient	Pre and Post the Menstrual Phase/ Latecycle/ Midcycle (Near/ Exact/ Late); Near the Menstrual Phase; Late Periods; Period Stops for More Than 2 Months; Amenorrhea; Distended Pain (Acid Swells) in Right/Left Lower Quadrant; Sharp Pain in Lower Quadrant; Aching Pain in the Two Sides of Lower Quadrant
Menstrual Period	Regular Periods; Irregular Periods Recently; Early Periods; Late Periods; Irregular Periods; Amenorrhea; Regular Period Last time; Just Begin the Menstrual Phase; Near the Menstrual Phase; Over Due
Amount of Menstrual Bleeding	Pour; Collapse; Heavy; Medium; Fluent; Unimpeded; Impeded; Scanty; Drop Off; One Day Off; Few; Increase; Drip Several Days
Color of Menstrual Bleeding	Slight Dark at the Beginning; Bright Red; Dark Red; Black; Coffee Color
Thickness of Menstrual Bleeding	Thick; Thin; Lump; Filmy; Lump and Filmy
Odor of Menstrual Bleeding	Yes/No
Painful in Periods	Severe Pain in the Lower Abdomen; Distended Pain; Slight Pain; Dull Pain; Sharp Pain; Pain in the Lower Back; No Pain in the Abdomen
Methods for Pain Relief	Relief after Silt Disappear; Relief whenWarming; Relief when Pressing
Leucorrhea	Amount Large/ Small; White/ Yellow/ Green/ Yellowgreeen/ Red/Slight Red/ Coffee Color/ Purple Color/ Slight Purple; Fiber Drawing

Table 3. General accompanying symptoms part of the personalized template of Cai's Gynecology

Exhaustion; Debilitation; Aversion to Cold; Thermophilic; Sweat when Week Up in the Morning; Dysthesia; Fever Disappear after Menses and Hot Flush; Feeling Cold before Menses; Dry Mouth; Drowsy; Baking Fever with Sweat; Baking Fever at Night; Endogenous Heat; Bit Inspiring; Aversion to Wind; Sleeping Badly; Dizziness with Pitching; Face without Floursh; Sallow Complexion; Distended Pain in the Vertex; Weight in Rush; Becoming Fat; Normal Sleep

Table 4. Local accompanying symptoms part in the personalized template of Cai's Gynecology

Head	Headache before Menses; Dizzy; Blurred Version; Migraine in the Menstrual Period; Scrofulosis in Face; Alopecia; Dense Hair; Pain in Forehead Sometimes; Blush; Oral Ulceration; Fetid Breath; Throat Staying with Sputum
Chest	Chest Stuffiness; Engorgement of Breast before the Menses; Sharp Pain in Breast; Feeling Tenderness Like Crawling under Breast; Travail in the Heart Area Recently
Stomach	Nausea; Emesis; Distended Abdomen; Tumult in Stomach
Waist	Lumbago; Soreness of Waist (Slight/ Bitter/Broken); Discomfort in Lumbar Vertebra; Ache and Dropping in Waist, Heavy Waist, Waist Suffering which Prefers Heating
Abdomen	Psychroalgia; Dull Pain; Attacked when Being Cold; Pain Sometimes; Abdominal Distention in Right/Left Side; Dull Pain in Both Sides of Abdomen; Distending Pain around Waist and Double Sides of Hypogastrium; Dull Pain in the Lower Abdomen; Sensation of Fullness in Lower Abdomen; Dyspareunia; Burning Pain in Both Sides of Ovarian Area; Tough Pain in Abdominal Wall; Slight Pain in Liver Area; Slight Dropping in Abdomen
Four limbs	Aching Pain in Arm; Thirsty and Loss of Appetite; Limb Feeling Light; Aching Pain in Knee; Calcination in the Foot Center at Night; Calcination in the Hand and Foot Center ; Limb Feeling Soft; Sour in Legs; Sour and Cold in Legs; Talalgia when Laboring
Genitalia and Anus	Nonsolid Stool; NonFluent Stool; Constipation; Stool Every Two Days; Stool Everyday; Diarrhea; Solid Stool; Easy Relieve Oneself; Anus Bearing-down; Dyschizia with Flart; Pollakisurie; Pruritus Vulvae; Dysuria; Micturition Desire Immediately after Drinking; Urinary

Table 5. Examination part in the personalized template of Cai's Gynecology

HCG	Positive;Weak Positive; Negative
B Ultrasonic	
Hysterosalpingography	
Examination of Endocrine Function	

Table 6. Diagnosis part in the personalized template of Cai's Gynecology

Diagnosis of TCM
Dysmenorrheal (Primary/ Secondary); Amenorrhea; Infertility (Primary/ Secondary/ All the time/ Some time); Irregular Periods; Metrorrhagia; Scanty Periods; Abortion; Miscarriage; GynecologicalMass in Abdomen; Postoperation of GynecologicalMass; Migraine in the Menstrual Period; Metrostaxis; Symptoms During Menopause; Stranguria; Fluor; Leukorrhea with Bloody Discharge
Diagnosis of WM
Endometriosis (Adenomyosis/ Chocolate Cyst); Hysteromyoma; Primary Dysmenorrheal; Secondary Dysmenorrheal; Amenorrhea; Abortion; Postoperation of Chocolate Cyst Decollement; Threatened Abortion; Irregular Periods; Ovarian Cyst; Dysfunctional Uterine Bleeding; Climacteric Syndrome; Menopausal Gong Blood; Chronic Pelvic Inflammatory Disease; Pelvic Congestion Syndrome; Colpitis Mycotica

14 items in the basic part, i.e. name, gender, age, record number, first visit data, menstrual history, marriage status, child birth history, contraception, last menstruation period, preparatory menstruation period, basal body temperature, chief complaint and history of present illness, which are summarized in Table 1.

Cardinal symptoms are those main factors which make major effect when the doctor decides the syndrome, therapeutic principle and method.

Table 7. Syndrome part in the personalized template of Cai's Gynecology

1.ChongRenShiTiao; 2.ShenQiBuZu, LuoDaoBuTong; 3.ShenQiBuZu (ShenQiKuiSun); 4.GanShenBuZu, ChongRenShiTiao; 5.XinShenBuzu; 6.SuYuNeiJie, GanShenBuZu; 7.SuYuNeiJie, LuoDaoQianChang (YuZuLuoDao); 8.YuReNeiYun; 9.SuYuNeiJie, ShenQiBuZu; 10.ShenQiBuZu, LuoDaoQianChang, ShiReXiaZhu; 11.WeiQiBuGu, QieYouGanFeng; 12.SuYuNeiJie, JiErChengZheng (YuKuaiNeiJie); 13.YingYinBuZu, ChongRenQianTiao; 14.FeiShiXuanJiang; 15.ShenQiBuZu, ZhiMoYongZhi (ShenQiBuZu, TanZhiYongZhi); 16.GanYuPiXuShiShe; 17.ShenQiBuZu, ShiYuLuoDaoQianChang; 18.ShenXuXueYu, ShiReXiaZhu; 19.TiXuWeiFu, SuYouYuJie; 20.ShenXuChongRenShiTiao; 21.PiShenBuZu, TanXuYongZhi, ChongRenShiTiao; 22.GanShenYuRe, ChongRenShiTiao; 23.PiShenQiXuShiShe; 24.PiXuShiShe; 25.GanShenBuZu, XueBuYangGan, ShenXuBuJu; 26.GanHuoPianWang; 27.GanYuShenXu; 28.GanWangPiXu; 29.GanPiBuTiao; 30.HanNingBaoGong; 31.XinShenBuZuJianGanYu; 32.GanYuPiXu, XuReZhuoLuo; 33.ShiReXiaZhu; 34.ShiReXiaZhu, RiJiuShangYin; 35.YingXueBuZu, QiXuBuShe; 36.XueXuGanWang, ShiReXiaZhu; 37.YinXuXueRe, ChongRenShiTiao; 38.XueXuShenKui, TanZuBaoLuo; 39.QiXuJiaYu, ChongRenBuGu; 40.QiXuBuZu, ChongRenLiangKui; 41.QiXueLiangWang, ChongRenShiTiao

Table 8. Therapeutic principle part in the personalized template of Cai's Gynecology

1.TiaoLiChongRen; 2.YuShenTongLuo; 3.YuShenPeiYuan; 4.YuShenTiaoLi; 5.YangXinYuShen; 6.YuShenTiaoLi; 7.HuaYuTongLuo (QingYuTongLuo); 8.QingReTiaoJing; 9.FuZhengQingYu (FuZhengQuYu); 10.YuShenTongLuo, QingReLiShi; 11.FuZhengPingGanQuFeng; 12.HuaYuSanJie (JieYuXiaoJian, QingYuTiaoLi); 13.ZiYinTiaoChongRen; 14.XuanJiangFeiQi; 15.YuShenHuaZhi, LiQiTongLuo; 16.JianPiShuGanTiaoShe; 17.YuShenTongLuoTiaoJing; 18.TiaoLiChongRen, ZuoXieHuoZhiDai; 19.FuZhengGongGu; 20.JianShenTiaoJing (YuShenTiaoJing); 21.LiQiHuaZhi, TiaoChongRen; 22.ShuGanXieHuo, TiaoLiChongRen; 23.TiaoShePiShen; 24.JianPiYiQiGuShen (YiQiTiaoShe); 25.JianLiGanShen; 26.ShuGanXieHuo; 27.YuShenShuGanTiaoLi; 28.NingShenShuGan, PingGanHuanJi; 29.JianGuGanPi; 30.WenGongTiaoJing; 31.BuShenYangXinJianShuGan; 32.JianPiShuGan, YangYinQingHuo; 33.QingReLiShiZhiDai; 34.QingReLiShi, YiQiYangYin; 35.BuYiYangXueTiaoShe; 36.LiShiXieHuo; 37.QingYingTiaoGu; 38.YanXuePeiYuan, QuYuTongLuo, HuoXueTiaoJing; 39.YiQiTiaoGu, QuYuShengXin; 40.BuQiYangXue, JianGuPiShen; 41.YiQiYangXueTiaoGu

There are 9 items, i.e. patient status, menstrual period, amount of menstrual bleeding, color of menstrual bleeding, thickness of menstrual bleeding, odor of menstrual bleeding, painful in periods, pain relief methods and leucorrhea. The detail description and properties of the above 9 items are listed in Table 2. All the symptoms are direct symbols of gynecological disorder and can be observed by patients themselves.

Besides the basic information and cardinal symptoms, there are accompanying symptoms, which are important but not critical. As in Table 3, we summarize 22 items like exhaustion, debilitation, aversion to cold, thermophilic, sweat when week up in the morning, dysphasia, fever disappear after menses and hot flush, feeling cold before menses, dry mouth, drowsy, baking fever with sweat, baking fever at night, endogenous heat, bit inspiring, aversion to wind, sleeping badly, dizziness with pitching, face without flourish, sallow complexion, distended pain in the vertex, weight in rush, becoming fat and normal sleep as the general accompanying symptoms, since these occurs in the body. While the accompanying symptoms in the local part of our body are summarized as local accompanying part like head, chest, stomach, waist, abdomen, four limbs, genitalia and anus, pulse and tongue. The above 9 large items have many sub items which are described in detail in Table 4.

Since WM is in the major position in China, many patients conduct physical examinations before they visit the TCM physicians. The examination indices may give some information of the patients, so Xiaosun accepts these indices as some symptoms. The commonly used indices are HCG, B ultrasonic, hysterosalpingography and endocrine function, which are summarized in Table 5.

Disease names of TCM and WM used in "Cai's Gynecology" are presented in Table 6, where there are 15 TCM names and 15 WM names. There are major difference between the expression of diseases in TCM and WM.

Table 7 lists 41 TCM syndromes frequently appearing in the clinical records of Xiaosun. At the

Table 9. Basic formula part in the personalized template of Cai's Gynecology

YuShenPeiYuan-Fang	FuLing (YunLing, YunFuLing) 12g; ShuDi 10g; ShengDi (DaShengDi) 10g; XianMo 10g; XianLingPi 10g; BaJiTian 10g; RouCongRong 10g
SiWuTiaoChong-Tang	ChaoDangGui 10g; ChuanXiong 6g; ShengDi 10g; BaiShao 10g; ZhiXiangFu 10g; HuaiNiuXi 10g
YuShenTongLuo-Fang	YunLing 12g; ShengDi 10g; ShuDi 10g; LuLuTong 10g; JiangXiangPian 3g; XianLingPi 12g; ZhiHuangJing 10g
NeiYiYiFang	ChaoDangGui 10g; DanShen 10g; ChuanNiuXi 10g; ZhiXiangFu 10g; ChuanXiong 10g; ChiShao 10g; Zhi-MoYao 6g; YanHuSuo 12g; ShengPuHuang (BaoJian) 12g; WuLingZhi 10g; XueJie 3g
NeiYiErFang	DangGui 10g; ShengDi 10g; DanShen 10g; BaiShao 10g; XiangFu 10g; ShengPuHuang 30g; HuaRuiShi 20g; ShuJunTan 10g; SanQiMo (Tun) 2g
NeiYiSanFang	YunFuLing 12g; GuiZhi 3g; ChiShao 10g; DanPi 10g; TaoRen 10g; ZaoJiaoCi 30g; ZhiJiaPian 9g; ShiJianChuan 20g; EShu 10g; ShuiZhi 6g
HuaYuXiaoJian-Fang	YunLing 12g; GuiZhi 3g; ChiShao 10g; DanPi 10g; EShu 10g; ShanJiaPian 10g; ZaoJiaoCi 30g; PuGongYing 14g; ShiJianChuan 20g; HaiZao 12g; ShuiZhi 6g; QingPi 5g; ChenPi 5g

same time, 41 therapeutic principles are proposed, each of which is to treat the TCM syndrome with the same serial number as in Table 8. Here the TCM terms used by Xiaosun are unique and most of them are not used by other physicians. So the translation job is hard, we have to use Pinyin in Chinese to express them. The therapeutic principles are combinations of basic TCM therapeutic ones, while most of them have one CMF listed below. We will further introduce them by using one case of womb tumor later.

Seven basic TCM formulas are summarized in Table 9. One of the basic formulas is selected for a patient based on her TCM syndrome diagnosis. The selected basic formula is then modified according to the patient's individual symptoms. Modifications include adding/deleting one of the herbs, or increasing/decreasing the doses.

Implementation of TCM-PEMS

Using the personalized template of diagnosis process of Cai's Gynecology, a unique system for preserving the clinical records is set up. Detailed illustration is presented in this section.

Process of Setting up a Personalized System

With TCM-PEMS, we set up a personalized system for Cai's Gynecology as the following steps.

- **Step 1.** Select the entry *Inquiry and Diagnosis Template Maintenance* in the module tree to go to its main page as in Figure 3. Click button *Add* to pop up the New Template dialog.
- **Step 2.** Input the template number and name.
- **Step 3.** Input the standard or nonstandard TCM diseases and syndromes.
  - **Step 3.1.** Standard TCM diseases Check the option *Use standard TCM disease*. Select a disease from the TCM diseases database as the default value. If there is no corresponding record for certain standard disease and syndrome in the database or the information isn't correct, you can add or modify them in the *TCM Disease Maintenance* and *TCM syndrome Maintenance* modules 3.
  - **Step 3.2.** Nonstandard TCM diseases Uncheck the option *Use standard*

Figure 3. Choose to use standard names of TCM diseases

Figure 4. Input a nonstandard TCM disease

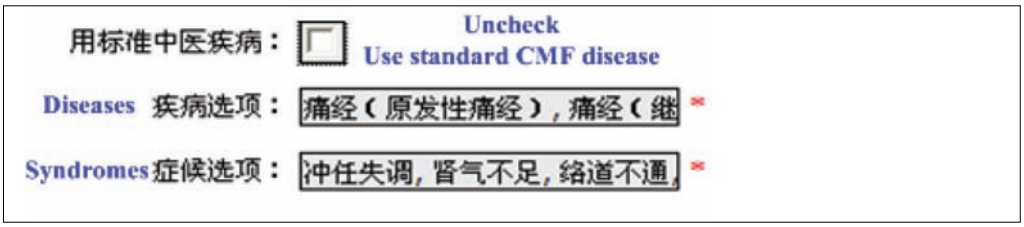
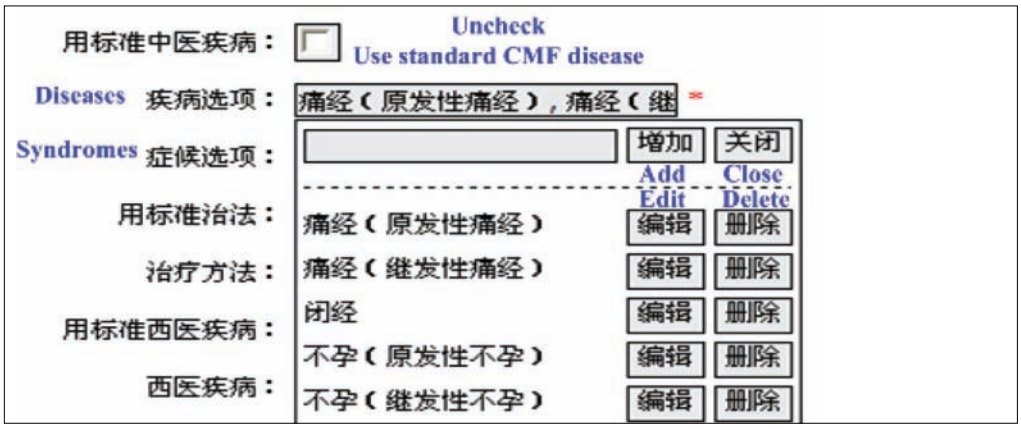


Figure 5. Edit the nonstandard TCM diseases



TCM disease. Input the TCM diseases and syndromes manually. As several diseases may have the same syndromes, users can fill in multiple diseases separated by comma as in Figure 4.

To help users input the correct format, an input dialog will pop up if they click the disease edit box as in Figure 5. Click *Add* to insert a disease. Click *Modify* to modify a disease. Click *Delete* to delete a disease. Similarly, an input dialog will pop up if they click the syndromes edit box as in Figure 6.

- **Step 4.** Input the therapeutic principles.
  - **Step 4.1.** Standard therapeutic principles Check the option *Use standard therapeutic principle*. Select a record from the therapeutic methods database as the default value. If there is no corresponding record for certain standard method in the database or the information isn't correct, you can add or modify it in the *Therapeutic Principle Maintenance* module.
  - **Step 4.2.** Nonstandard therapeutic methods Uncheck the option *Use standard therapeutic principle.* Input

Figure 6. Edit the syndromes of a nonstandard TCM disease

Figure 7. Edit personalized therapeutic principles

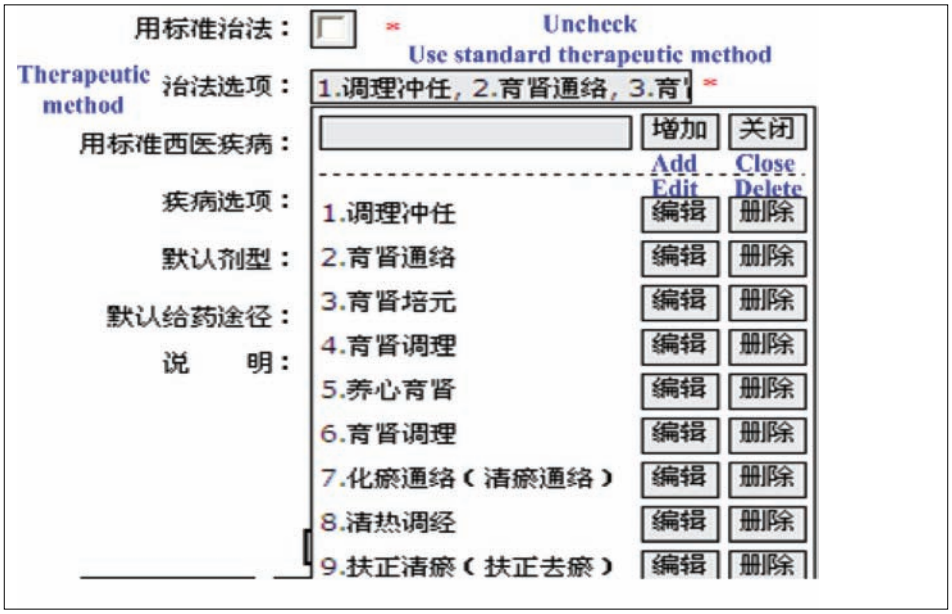


Figure 8. Select the type of WM diseases

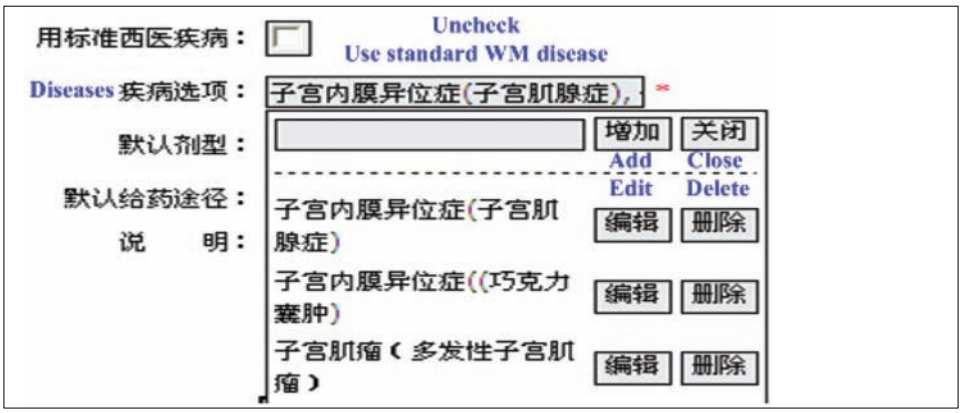
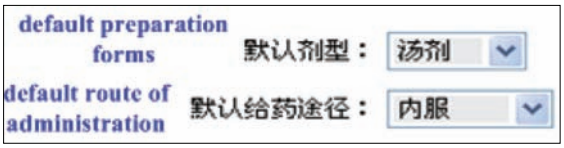


Figure 9. Select preparation form and administration route



the therapeutic principle manually as in Figure 7.

- **Step 5.** Input the standard or nonstandard WM diseases.
  - **Step 5.1.** Standard WM diseases Check the option *Use standard WM disease*. Select a record from the WM diseases database as the default value. If there is no corresponding record for certain standard disease in the database or the information isn't correct, you can add or modify it in the *WM disease Maintenance* module.
  - **Step 5.2.** Nonstandard WM diseases Uncheck the option *Use standard WM disease*. Input the WM diseases manually. As several diseases may have the same syndromes, users can fill in multiple diseases separated by comma as in Figure 8.
- **Step 6.** Select the default preparation forms and default route of administration. The preparation forms might be injection, enema, decoction, etc. The administration route could be by intravenous injection, by enema, by mouse, etc as in Figure 9.
- **Step 7.** Update the first-visit symptoms and return-visit symptoms. Click the button *First visit symptoms* or *Return visit symptoms* to switch the visit mode. Click the button *Add* to pop up the symptoms selection dialog. If there is no corresponding record in the database or the information isn't correct, you can add or modify it in the *Symptom Maintenance* module as in Figure 10. Users can resort the symptom list by dragging and dropping the symptom items in the list as in Figure 11. The Symptom Maintenance module may be modified sometimes. Steps of modification are further illustrated.
  - **Step A.** Select the entry *Symptom Maintenance* in the module tree to go to its main page. Click button *Add* to pop up the New CMF dialog.

Figure 10. Select the symptoms

Figure 11. Resort the personalized symptom list

- **Step B.** Select the root symptom. If you are adding/editing a nonstandard symptom, select the corresponding specialist under *other symptoms* (e.g. Others → Xiaosun Cai) as in Figure 12.
- **Step C.** To describe how serious this symptom is, you can set a question for users to select. The question could be a checkbox, single choice question, multiple choices question, or freeform text question.

- **Step 8.** Input the examination items. Click the button *Examination items* and click *Add* to pop up the examination item selection dialog. If there is no corresponding record in the database or the information isn't correct, you can add or modify it in the *Examination Item Maintenance* module.
- **Step 9.** Contact the administrator after adding a new template. The administrator will update the backend system. Besides symptoms, syndromes and diseases, CMF is

Figure 12. Add the personalized symptom

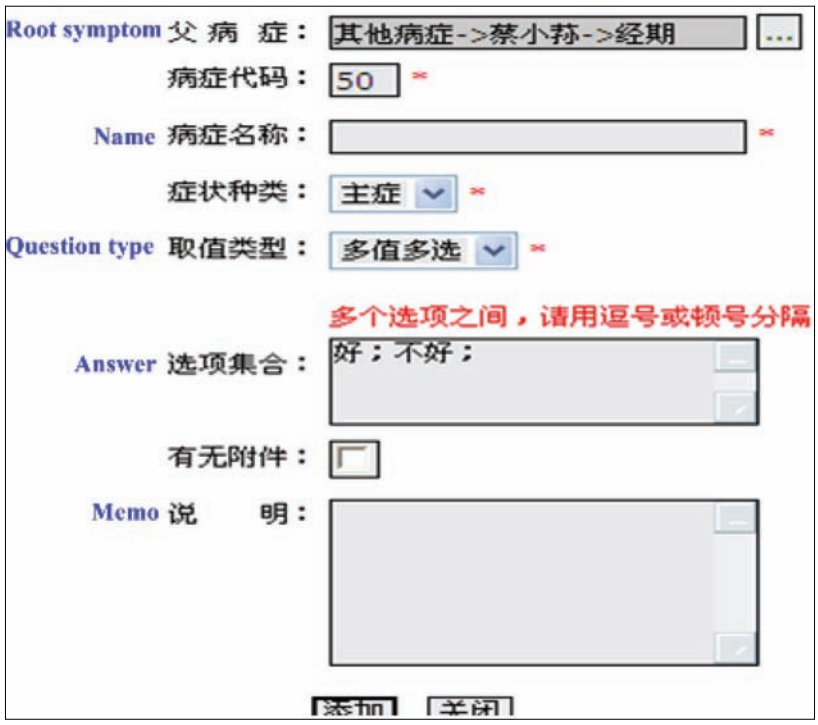
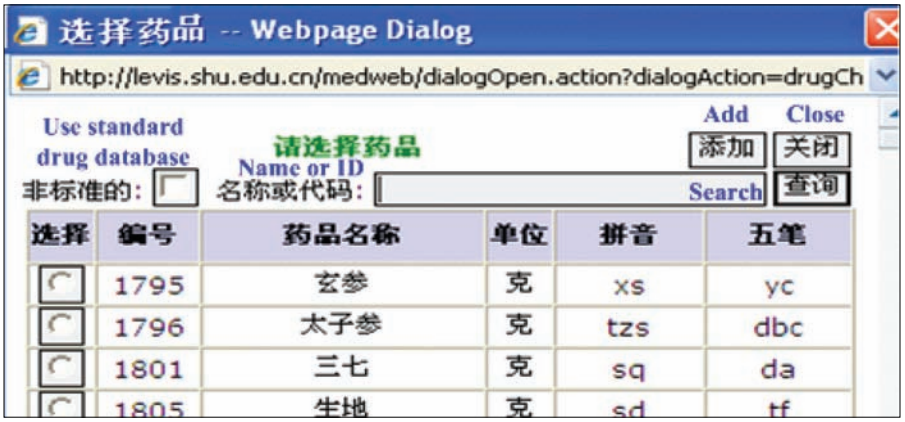


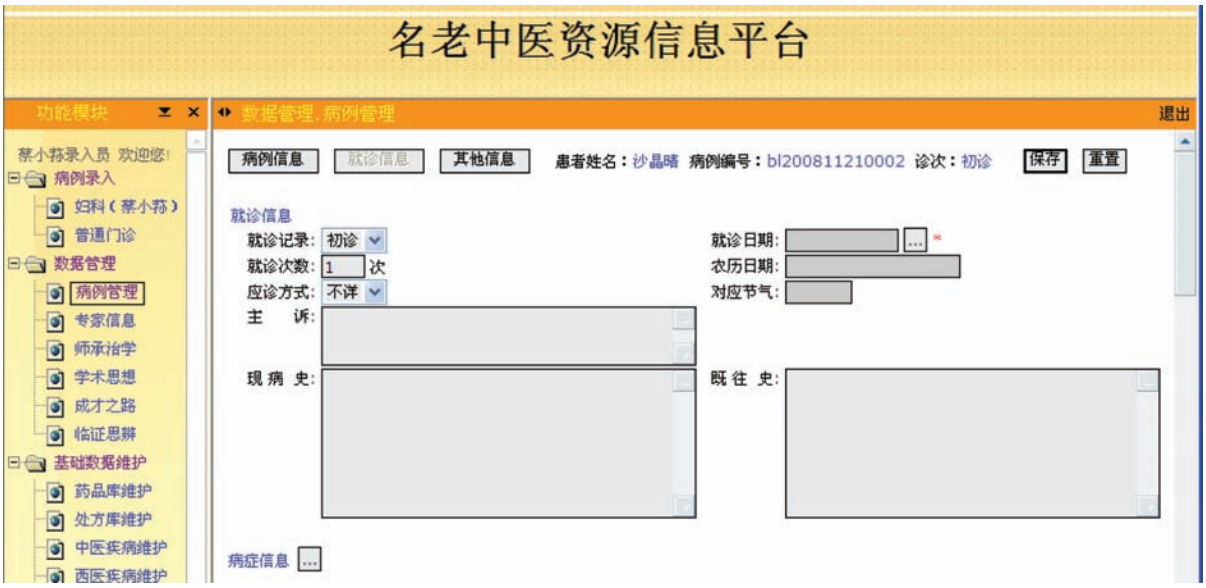
Figure 13. Select or edit the herbs



also designed in a personalized way. There are three steps for CMF maintenance.

- **Step A.** Select the entry *CMF Maintenance* in the module tree to go to its main page. Click button *Add* to pop up the New CMF dialog.
- **Step B.** Input the CMF number and name.
- **Step C.** Edit drugs. Click the button *Add drug*. The drug selection dialog will pop up. Check/uncheck the option *Standard drug database* to

Figure 14. Input the basic information of a patient



switch between standard and nonstandard drug database. Users can search the drug by name, ID or name abbreviation. Double click certain drug or click the button *Add* to append it into the drug list. If certain drug isn't in this database, you can add it in the *Drug Maintenance* module as in Figure 13. Users can modify an existing CMF. Resorting the drug list is quite easy by dragging and dropping the drug item in the list.

Demonstration of the Personalized System

As the size of the computer screen is limited, the interface of the personalized system for Cai's Gynecology is shown from Figure 14, figure 15, figure 16, and Figure 17. Main elements in the interface are marked in the four figures.

The setup platform for TCM can be visited by the homepage [1], with login name ys and password ys123. The interface is demonstrated in Figure 14. The model tree in the left of the figure lists three main functions of the platform, clinical records input, data management and basic data maintenance. When entering a clinical record, basic information of the patient, cardinal symptoms, accompanied symptoms, results of needed examinations, syndrome differentiator, therapeutic principles, TCM and WM diagnosis, CMF and some attachments should be all included. Nine kinds of basic information maintenance, such as medicine, CMF, syndrome, and so on, are supported in the platform.

A Case: Womb Tumor

Tumor is a great challenge to the current medical world. Womb tumor is usually benign and located in the womb, which -–frequently appears in female reproduction system. Womb tumor is classified, according to its location, as intramurals tumor, subserosals tumor, submucosals tumor and cervices tumor caused by the cell growth of smooth muscle in uterus. If there is more than one location, it is multiple-womb tumor. Womb tumor is stony uterine mass in TCM, which takes place in

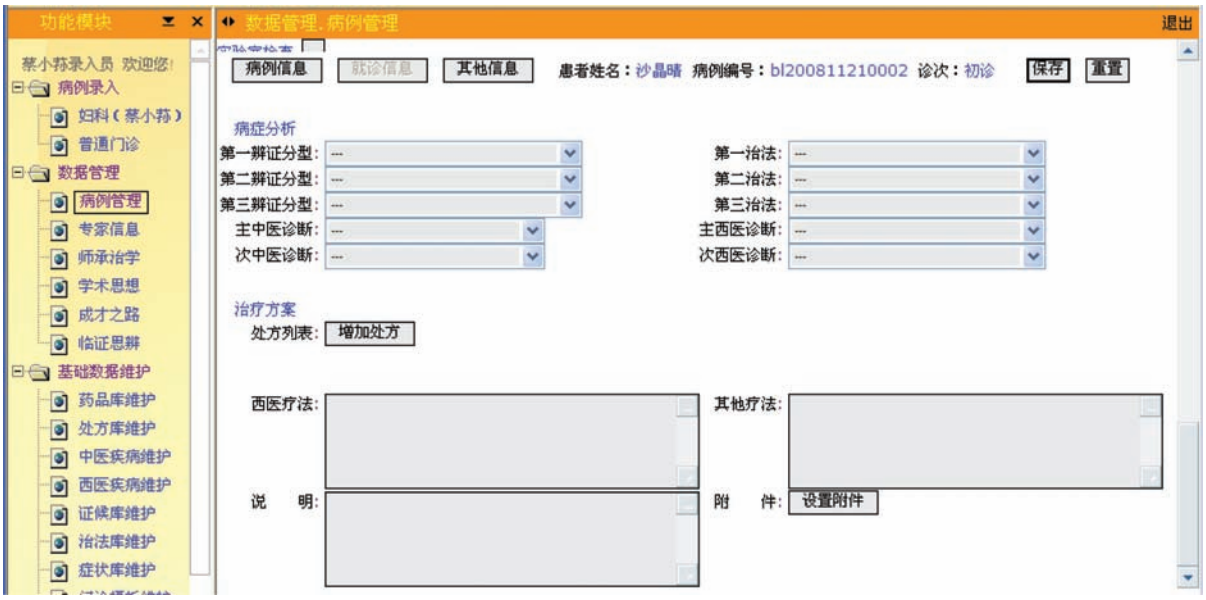
Figure 15. Select the cardinal symptoms

Figure 16. Select the accompanied symptoms

women whose ages are from 30 to 50, and it will wither after menopause.

The symptoms are different depending on the location, size, number and growth speed. Some women feel well, but find it when they conduct physical examination. Many people are with symptoms, e.g. large quantity of menstrual flow and even incessant extramenstral vaginal bleeding. It may reduce or expand the menstrual period leading to anemia. When the diameter of womb is over 10cm, it will constrict other organs and produce other unexpected symptoms, e.g. frequent maturation, urgent urination, even hard urination and defecation. Most women with womb tumor may be infertility, which may be caused by constricting on oviduct of larger tumor.

Figure 17. Select syndrome differentiator and therapeutic principle as well as WM diagnosis



Womb tumor may be related to or caused by estrogen. In TCM, it is caused by qi stagnation and/or blood stasis. In the following conditions, womb tumor may take place: 1) cold assailing the womb after production or menstruation; 2) anger hurting the liver system during menstruation leading to qi stagnation; 3) thinking hurting the spleen leading to blood stasis.

The therapeutic principle of womb tumor is HuoXueHuaYu and XiaoJianSanJie by Xiaosun (Cai, Huang and Mo, 2000). In the medical records, we also mine that Xiaosun has a so called period therapy technique, i.e. separating one month to be during menstruation and between menstruations.

In the period of between menstruations, Xiaosun often uses the therapeutic principle of HuoXueHuaYuXiaoJian. The fundamental prescription is YunFuLing 12g, GuiZhi 3g, ChiShao 10g, DanPi 10g, Taoren 10g, ZaoJiaoCi 30g, JiuJiaPian 9g, ShiJianChuan 10g, GuiJianYu 20g, HaiZao 12g, EShu 10g. There are 14 -21 days in the between menstruation, one day one dose. If the patient is strong, DaHuang, MangXiao may be added to LiangXueHuaYu and RuanJianSanJie, BaiShu is also added to restrict the violent of the above two herbs. If the patient is weak, DangShen is added to make her strong. HuangYaoZhi, YanDanZi, ShuiZhi, DiBieZhong may be added to strengthen the ability of reducing tumor.

During the period of menstruation, the therapeutic principle is HuaYuTiaoJing, where the formula of SiWuTaiChong is employed. It composes of ChaoDangGui 10g, DaShengDi 10g, ChuanQiong 5g, BaiShao 10g, ChaiHu 5g, ZhiXiangFu 10g, and HuaiNiuXi 10g. If there is profuse menstruation, Xiaosun insists on HuaYu, not GuSeZhiXue according to the therapeutic principle of treating the unstopped by unstopping. The formula is that ChaoDangGui 10g, DanShen 6g, ChiShao 10g, BaiShao 10g, ShengPuHuang 30g, XueJie 3g, HuaRuiShi 15g, ShuChuanJun 10g, YiMuCao 10g, XianHeCao 20g, ZhenLingDan 12g. If blooding, add SanQi; else if Qi stagnation, add XianFu; else if abdominal pain, add YanHuSuo; else if congealing cold, add AiYe; else if Qi deficiency, add DangShen, ShengHuangQi.

Womb tumor is a frequent disease in current clinical gynecology. Basic idea of Xiaosun is add-

ing/deleting the herbs according to the symptoms combining the physique, illness of the patients. If the patient is young and earlier diagnosed, the therapeutic principle is to fight with the disease. While if the patient is older or deficiency of Qi and blood after long term blooding, the therapeutic principle should be FuZhengHuaYu, not fight. If the patient is menopause, then the therapeutic principle is to push amenorrhea and make the tumor shrink itself. If necessary, KuShen, HanShuiShi, XiaKuCao are employed to purge the liver and clear heat, preventing tumor to be malign.

A typical medical record is from a woman whose surname is Wang, 35 years old and married. During physical examination, she found the tumor, it is 4.3cm * 7.8cm * 6.4cm. The patient was afraid of operation and looking for TCM therapy. Her menstruation was usually advanced and profuse. The color of menstruation was gray and mixed with blocks. She was distending breast pain before menstruation. Her waist was soreness. Her tongue fur is thin with purple points in margins of tongue. Her pulse is thin and string-like. She often felt tired. Xiaosun judged this patient be SuYuNeiJie in disease pattern, and decided to use the therapeutic principle of HuoXueHuaYu, RuanJianSanJie. The prescription is GuiZhi 3g, ChiShao 9g, DanPi 9g, YunFuLing 12, TaoRenNi 9g, SanLing 9g, EShu 9g, GuiJianYu 20g, ShuiZhi 4.5g, XiaKuCao 12g, HaiZao 9g. 14 doses. The patient did not feel uncomfortable after the therapy. She was in the period of menstruation, quantity is less. She still felt soreness in waist, with thin slimy tongue fur and thin string-like pulse. Therefore, Xiaosun made the prescription according to the previous formula and asked the patient to use after menstruation. After 6 months, the patient performed physical examination by ultrasonic wave and found that the light points were well-distributed, without blocks or dark liquid area. Her menstruation was regular. She was still healthy after one year.

CONCLUSION AND FUTURE WORK

This study proposes a novel platform for setting up personalized systems for TCM masters in China. It helps to preserve the specialists' thoughts and experience in unfiltered ways. The collected materials are invaluable data source for further research. In order to illustrate the idea of the proposed personalized system, clinical records from Mr Xiaosun Cai, a famous national TCM master in gynecologist, are introduced as an example. A complete specialized template for diagnosis is summarized and a personalized system is set up. Data mining methods are also implemented for case analysis. Future work mainly focus on more clinical cases and more types of machine learning methods for data mining. Relationships between symptom and syndrome, symptom and formula will be the focal points.

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KEY TERMS AND DEFINITIONS

Traditional Chinese Medicine: Traditional medicine in China, which is subject to study human physiology and pathology as well as diagnosis and preventing of disease. It is the accumulation of the theory and experience to fight with disease of the Chinese, whose principle is to view the body in total. A well known idea is to prevent the disease from sprouting by observing their fore gleam.

Gynecology: Refers to a subject to study the physiology and pathology of the women reproductive system outside the pregnant period and their diagnosis and therapy. Gynecology usually consists of fundamental knowledge, inflammation, tumor, endocrine, damage, abnormal growth of the reproductive system and other related disease.

Cai's TCM Gynecology: Famous in China, which started 250 years ago in Jiangwan Shanghai. Now the seventh successor is Prof. Xiaosun Cai, who is now 87 years old. Cai's gynecology takes care with all the symptoms of the patients, employs comprehensive consideration of four diagnostic methods. Their prescription is light and effective. Their successors are asked to be careful and amenable with the patients.

Womb Tumor: Takes place frequently in the female reproduction system, which is classified according to its location as intramurals tumor, subserosals tumor, submucosals tumor and cervices tumor caused by the cell growth of smooth muscle in uterus. If there are more than one location, it is multiple womb tumor. Womb tumor is stony uterine mass in Traditional Chinese Medicine, which takes place in the women whose ages are between 30-50, and withers after menopause.

Experience Sharing: A special topic in traditional Chinese Medicine (TCM). Famous TCM practitioners accumulate ample experience integrating the basic theory, the previous experience and their clinical practice. They have the ability to effectively solve doubtful, hard disease like tumor, so they represents the current academic and development of the highest level, which are shown in their medical records and clinical experience. Their experience is hard to be explained in the book, or readers feel hard to understand them even in books. So experience sharing is important in TCM.

ENDNOTE

[1] http://levis.tongji.edu.cn/medweb/

Section 2

Basic Research:
A Bridge to Modern Medicine

Chapter 3
Stem Cell–Based Personalized Medicine:
From Disease Modeling to Clinical Applications

Alessandro Prigione
Max Planck Institute for Molecular Genetics, Germany

ABSTRACT

Regenerative medicine is a rapidly evolving research field whose main aims are to provide new therapeutic approaches and to repair or replace injured tissues with functional cells derived from stem cells. In the past few years, research breakthroughs have revolutionized the field by showing that all somatic cells have the potential to re-acquire stem cell-like properties. Thus, it appears possible to generate relevant cell types starting from cells easily obtained from affected individuals. The obtained differentiated cells could eventually serve as in vitro tools for the study of disease-associated mechanisms and for performing customized drug screenings. Moreover, in the context of cellular transplantation, these cells represent the ideal cell source given that they posses the same genetic code and thus will avoid the occurrence of unwanted immune reactions. Overall, this revolutionary technique called cellular reprogramming might provide substantial support for the future development of personalized medicine. In this chapter, I describe the recent advances in the field of stem cell-based regenerative medicine applications. Parkinson's disease is chosen as a paradigmatic example in which the use of stem cells for study and therapy could have a relevant impact and potentially represent a future cure for this debilitating disorder.

INTRODUCTION

Regenerative medicine aims at repairing or replacing lost or injured tissues and organs due diseases, aging, or congenital defects with living and functional cells derived from stem cells. Stem cells are a special cell type that can be found in every multi-cellular organism. Their unique features include two key properties, i.e. indefinite propagation (self-renew) and the capability to generate a diverse range of specialized cell types through differentiation (potency). Stem cells are classified according to their degree of potency. Embryonic stem cells (ESCs), derived from early-

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stage embryos are pluripotent, i.e. they retain the ability to differentiate into virtually any cell type of the body derived from any of the three germ layers. On the other hand, somatic stem cells, which are found in developed organisms, are usually multipotent, since their differentiation is restricted to one specific lineage.

Regenerative-based applications are historically divided into two branches. The first utilizes cellular transplantation of *ex vivo*-cultured stem cells, while the second seek to activate the endogenous stem cell population. The latter route appear less achievable in the near future, as it requires complex chain of events capable of selectively stimulate the resident stem cell population without inducing unwanted unspecific responses, which might lead to cancerogenic transformations. Thus, most of research studies are currently focused on *in vitro* stem cell cultures and on the optimization of distinct protocols for the generation of specialized cell types, which could be then used for cellular transplantation. To this end, ESCs represent the most plastic type of stem cells.

ESCs were first discovered in mice by Martin Evans in 1981 (Evans & Kaufman, 1981), who has been awarded the Nobel Prize for his landmark discovery in 2007. The derivation of human ESCs was reported by James Thomson in 1998 (Thomson, et al., 1998). Since then, the field has rapidly evolved and considerable advances have been made to define specific differentiation protocols able to efficiently generate functional relevant cell types. These include cardiomyocytes and hepatocytes, which might prove to be useful for drug-screening studies, and dopamine-producing neurons, which could be used in cell-based therapeutic applications aiming at replacing the loss of this specific neuronal population in patients affected by Parkinson's disease.

Despite all these promising results, numerous drawbacks are associated with ESC-mediated regenerative medicine. Indeed, the derivation of human ESCs requires the use of embryos, which can raise ethical concern. Moreover, in a similar fashion to organ transplants, stem cell transplants can give rise to immune rejections, since they are derived from a different individual with a different genetic background (allogenic). Patients should then be subject to immuno-suppressive therapy, which may in turn produce several clinical complications.

Generation of Patient-Derived Pluripotent Stem Cells

One way to circumvent the issues related to the use of human ESCs for disease treatment is the generation of genetically equivalent stem cells, directly obtained from the own cells of the patients (isogenic). All the cells of multi-cellular organisms possess the same genetic code but are functionally heterogeneous, due to their distinct epigenetic status. Through the modulating of their epigenetic profile, differentiated somatic cells can be reverted to stem cell-like cells capable of acquiring the two key properties of ESCs, i.e. self-renewal and pluripotency.

Somatic Cell Nuclear Transfer (SCNT)

The first demonstration of this reversion was obtained by somatic cell nuclear transfer (SCNT), which removes the nucleus of a somatic cell and transplants it into an enucleated oocyte. In the late 1950s, John Gurdon transferred nuclei from adult frog cells into frog eggs and showed for the first time that the resulting cells took on embryonic characteristics (Gurdon, 1962). This established that, although the body's cell types retain their genomes as they specialize, it may be possible to re-activate genes that have become functionally inactive during development. The technique was then used by Ian Wilmut in 1997 to generate dolly the sheep. Finally, the derivation of cloned primates has been recently reported.

The SCNT approach has been reduced to practice in mouse models to treat genetic immunodeficiency and Parkinson's disease (Tabar, et

al., 2008). Nonetheless, the derivation of genetically identical "customized" ESC-like cells by SNCT using donor cells directly obtained from patients is rather complicated. Furthermore, it may encounter similar ethical difficulties as human ESC-based medicine, as it also requires the use of human oocytes. Perhaps most importantly, SNCT has yet to be proven successful in the context of human cells.

Induced Pluripotent Stem Cells (iPSCs)

Recently, a second route to revert somatic cells to pluripotency has been discovered. This groundbreaking technique, called cellular reprogramming, was first demonstrated by Shinya Yamanka in 2006. Cellular reprogramming consists in introducing ESC-specific transcription factors into somatic cells, which subsequently acquire ESC properties and turn into induced pluripotent stem cells (iPSCs). iPSCs and ESCs share the same cardinal features of self-renewal and pluripotency. iPSCs and ESCs appear almost undistinguishable in terms of gene expression, morphology, cell-cycle structure, epigenetic signature, mitochondrial properties, and, most importantly, developmental potential (Prigione, et al., 2010; Takahashi, et al., 2007; Takahashi & Yamanaka, 2006).

The somatic cell source used for cellular reprogramming was first characterized by fibroblasts, but recent studies showed similar results also using different cell types. The procedure was first demonstrated in mouse and later on in humans and other mammals. The methods by witch the factors are introduced into the cells have been also improved. Initially, the technique was dependent on viral transduction, which can result in unwanted genome integration. However, non-integrative methods for iPSC derivation have been recently demonstrated, including the use of plasmids, proteins, RNAs, or microRNAs. Moreover, chemical compounds enhancing the procedure have been identified. Thus, it may be possible to envision that iPSCs might be obtained in the future using only a cocktail of different drugs.

Overall, cellular reprogramming appears as a procedure that could work for virtually any cell type of the body and does not require the use of human embryos. These features put forward patient-derived iPSCs as the most promising tool for regenerative medicine applications (Kiskinis & Eggan, 2010).

iPSCs for Cell Replacement Therapy

Shortly after the first report describing the methodology of cellular reprogramming, therapeutic potentials of iPSC-based regenerative medicine have been shown by a proof-of-principle study in a humanized mouse model of sickle cell anemia (Hanna, et al., 2007). In the study, fibroblasts derived from an anemic mouse were first reprogrammed to iPSCs and then subjected the cells to gene therapy-based correction of the defective mutant gene. The iPSCs were subsequently differentiated into hematopoietic progenitor cells, which have been transplanted back into the same mouse. As a result of the treatment, pathologic features of the diseases were substantially improved.

A similar approach was taken with human fibroblasts obtained from an individual affected by Fanconi Anemia, a blood disorder characterized by genetic instability (Raya, et al., 2009). In this case, the mutant gene was replaced prior to cellular reprogramming. iPSCs could be generated and afterward differentiated into hematopoietic progenitors which maintained a disease-free phenotype.

Mouse iPSCs have been also successfully differentiated into functional dopaminergic neurons. These neurons, once transplanted into a rat model of Parkinson's disease, were able to functionally integrate into the adult brain and in turn lead to a significant amelioration of the disease phenotype (Wernig, et al., 2008).

These studies illustrated the potential of iPSC-based regenerative medicine. By inducing somatic cells to re-acquire ESC-like features, cellular reprogramming appears as the holy grail of cellular replacement therapy. It does not involve any ethical problems and, since the transplanted cells can be obtained from the same individual, it avoids the occurrence of unwanted immunological reactions. Thus, iPSC technology offers the unique opportunity to repair damaged cells with genetically equivalent cells.

Nevertheless, substantial challenges still remain to be overcome before translating the technique to clinical practice. Factors used for cellular reprogramming are known oncogenes, the non-integrative delivery methods display very low efficiency, and iPSC-derived cells may differentiate less efficiently to relevant cell types when compared to original ESCs. For safe iPSC applications, it will be crucial avoid the presence of undifferentiated cells within the cells prepared for transplantation, as they may give rise to tumors. Finally, significant improvement has to be achieved for the correct delivery of cells into affected individuals and selective functional engraftment of these cells into the corresponding tissue. Overall, further work is warranted toward generating and fully characterizing "clinical grade" iPSCs before attempting their use in human cell replacement therapy.

Finally, iPSCs have been recently found to harbor nuclear and mitochondrial genomic alterations, which suggests that reprogramming may be associated with genomic instability (Mayshar, et al., 2010; Gore, et al., 2011; Prigione, et al., 2011). Hence, it will be essential to develop reprogramming protocols able to safeguard the genome integrity of somatic cells, before employing iPSC-based cellular therapy.

iPSCs for Disease Modelling

Although safe and efficient iPSC-based cell replacement therapy may not be immediately achievable, the use of reprogrammed cells for *in vitro* modeling of complex disorders appears as a highly useful short-term application of cellular reprogramming.

The derivation of specific cell types from iPSCs obtained from patients harbouring genetic mutations or with idiopathic disorders represents indeed an invaluable tool for the study of specific disease-related mechanisms. The concept at the base of utilizing iPSCs to model human disease in a dish is that these cells exhibit the unique capacity to continuously replicate without senescence. Thus, they could represent a limitless reservoir of cells that can be differentiated into virtually any given cell type.

This is particularly relevant in the context of human disorders in which the affected cell type can not be obtained and used for biological studies. In neurodegenerative diseases, for example, selective neuronal populations are targeted and lost during time. However, it is technically challenging and ethically impossible to take these neurons from the patients and bring them to a culture dish in order to analyze the mechanistic reasons underlying their selective cell death.

While animal models have been crucial for our understanding of disease-related mechanisms, fundamental biological differences exist between mice and humans. A large number of failed clinical trials may indeed be due to these specie-specific differences. In order to better understand the disease pathogenesis and consequently discover and test new disease-modifying drugs, *in vitro* disease models conducted on human cells are thus necessary. At present culture models are limited to tumor cell lines or transformed derivatives of native tissues, both differing from a "normal" cell due

to immortalization. On the other hand, the novel reprogramming strategy holds the potentiality to generate patient or disease-specific pluripotent cells without the need for human ESCs.

Generation of iPS cells from patients with a variety of diseases has been already reported (Park, et al., 2008). These include neurodegenerative disorders, such as Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and Huntington's disease (HD), and inherited genetic diseases such as adenosine deaminase deficiency-related severe combined immune deficiency (ADA-SCID), Shwachman-Bodian-Diamond syndrome (SBDS), Gaucher disease (GD) type III, Duchenne (DMD), Becker muscular dystrophy (BMD), juvenile-onset, type 1 diabetes mellitus (JDM), Down syndrome (DS)/ trisomy 21, the carrier state of Lesch-Nyhan syndrome, and Fanconi anaemia.

Effective disease modeling has been successfully demonstrated in two cases. In the first study, iPSCs were generated from a genetic form of spinal muscular atrophy (SMA) and thereafter differentiated into motor neurons, which are the cell type affected by this disorder (Ebert, et al., 2009). The derived neurons were initially similar to those obtained from wild-type iPSCs. However, in depth analysis revealed that their number and size declined with time at faster pace in comparison to the control cells. Most importantly, the cells exhibited protein aggregates which are a characteristic phenotype of SMA. In the second study, iPSCs were derived from patients affected by Familial Dysautonomia (FD), a neurological disease caused by a genetic mutation. Neurons differentiated from the disease-derived iPSCs recapitulate key FD features, which were meliorated by using a specific drug treatment (Lee, et al., 2009).

Overall, these examples show the potential of iPSC-based *in vitro* disease modeling. However, it may be more challenging to in *vitro* recapitulate diseases with a long latency, such as Alzheimer's and Parkinson's disease. In these cases, the dynamics of disease progression in the patient may be very different form phenotypes developing *in vitro* in cells differentiated from patient-specific iPSCs. It will thus be necessary to attempt to accelerate the appearance of pathological phenotypes by exposing the cells to certain environmental stressors known to play a role in the development of the disease.

iPSCs for Drug Discovery

The revolutionary iPSC technology could immediately be of tremendous help not only for disease modeling but also for another clinically relevant application, i.e. drug discovery. Indeed, by generating hepatocytes and cardiomyocytes from patient-derived iPSCs, it could be possible to perform personalized drug screenings and develop patient-specific drugs. Human iPSCs could then be used to test mechanisms of action and toxicity of potential new drugs as well as drug metabolism and clearance.

At present, cardio-toxicity is one of the major forms of drug toxicity and accounts for most recalls and delays experienced in drug development and approvals. A big challenge is how to predict cardiac risks early and accurately in the drug development process. Besides cardio-toxicity, hepatic toxicity is another important complication: distinct patients have different levels and tolerance to the clearance of drugs by their liver, which results in distinct effects of a specified drug on these individuals. Overall, hepato-toxicity and cardio-toxicity are two principle causes of drug failure during pre-clinical testing. Moreover, an additional major problem is represented by the occurring of high variability among individual responses to potential therapeutic agents.

Functional cardiomyocytes and hepatocytes have been already efficiently differentiated from mouse and human iPSCs. These cells will soon provide an important *in vitro* model for screening toxicity of drugs and the development of pharmaceuticals. Currently, cell lines and animal models are utilized for toxicity screening and determining

cardiac safety. By using iPSC-derived cells, it will be possible to generate a library of cell lines that may be used to represent a broad spectrum of the population harbouring distinct genetic and epigenetic backgrounds. High-throughput screening might then be used to allow a better prediction of the possible toxicological responses to newly developed drugs. Furthermore, iPSC-derived cells may provide insight into likely mechanisms at the base of the identified toxicity. Overall, this approach might eventually reduce the risks and costs commonly associated with early-stage clinical trials and lead toward a more personalized approach to drug administration.

The Example of Parkinson's Disease

Parkinson's disease (PD) is the most common movement disorders and second most common neurodegenerative disorder after Alzheimer's disease, affecting at least 1% of people by age 70 in the western world. The core clinical features, described for the first time by James Parkinson in his classic 1817 monograph and subsequently refined by Jean-Martin Carchot in the last part of the nineteenth century, include tremor at rest, slowness of movement and muscular rigidity (Savitt, Dawson, & Dawson, 2006).

Pathological studies determined that the central defect underlying the triad of motor systems resides in the degeneration of dopamine (DA) producing neurons in the substantia nigra pars compacta (SNpc). Affected SNpc neurons typically develop intracytoplasmic inclusions, known as Lewy bodies, which are mainly composed by alpha-synuclein (aSN). Subsequent reduction in the nigrostriatal DA innervation to the striatum deregulates the striatal neuron activity leading to locomotor dysfunctions. Nigral neurons physiologically stimulate the direct pathway and inhibit the indirect pathway of the basal ganglia circuitry, overall facilitating motor movements through the stimulation of the motor cortex. Thus, the loss of SNpc cells results in a hypokinetic movement disorder.

Current Therapeutic Strategies of PD

Parkinson's disease is a chronic disorder that requires broad-based management including patient and family education, general wellness maintenance and nutrition. Current medical and surgical therapies for PD are only symptomatic and lack significant disease-modifying effects. Indeed, after more than thirty years from its introduction, the most effective medical treatment is still based on the DA precursor levodopa, which is mixed with a peripheral decarboxylase inhibitor in order to increase the amount of the drug able to reach the central nervous system (CNS).

Additional therapeutic strategies aimed at limiting the breakdown of endogenous DA, through the inhibition of monoamine oxydase type B (MAOB), and at stimulating directly postsynaptic DA receptors, bypassing the need for viable dopaminergic cells to synthesize DA from its precursor. Surgical Deep Brain Stimulation (DBS) appeared as an influential development in symptomatic PD therapy. The results can in fact be quite marked, but the procedure is currently reserved for advanced cases of PD in which motor complications or medication intolerance have led to an unacceptable decline in quality of life. The search for disease-modifying compounds is also an active area of clinical research. Possible neuroprotective strategies include MAOB inhibitors, the antioxidant and mitochondrial component coenzyme CoQ10, and glial-derived neurotrophic factor (GDNF).

Finally, one step beyond neuroprotection is cell replacement therapy, which aims to selectively replace the cells lost upon the development of PD.

Cellular Replacement Therapy for PD

Although medical or surgical treatments can provide long-term symptomatic relief, the procedures do not slow down disease progression and are haunted by the rise of increasing side effects over time. Hence, there is urgent need for therapeutic strategies that could be reparative in nature and be able to finally provide a cure for this debilitating disorder. Among the most promising is cell transplantation, which holds the hope of repairing the actual neuronal damage in PD rather then merely alleviating the symptoms.

The first clinical experiences with cellular therapeutics for PD have been based on fetus-derived cells obtained from post-mortem human embryos. The trials were carried out in Sweden in the early nineties by transplanting aborted fetal brain tissues into the brains of PD patients (Lindvall, et al., 1990). The transferred tissues harbored immature DA neurons that were able to successfully integrate into the striatum and produce DA. However, the initial optimism faded when subsequent clinical trials demonstrated decreased efficiency and the occurrence of dyskinesias, or abnormal involuntary movements.

Furthermore, recent reports of long-term trials of fetal dopamine grafts revealed the presence of Lewy bodies and phosphorylated aSN within grafted neurons (Li, et al., 2008). Thus, the surviving DA neurons can develop the same pathological features associated with PD, suggesting the spreading of PD to some of the transplanted cells in a similar fashion to host DA neurons within SNpc. The mechanism underlying this spreading remains obscure. However, it appears likely that the unfavorable environment and the immune rejection, commonly associated with allogenic transplantation, may play a critical role.

Overall, the use of fetal tissue as a cell source for clinical transplantation appears not very practical. From the start, abortion foes objected the use of fetal-derived tissues. In addition, the number of functional DA neurons obtained from these tissues was very low. The need to generate neuronal cells to be transplanted in large quantities and in a reproducible, steady and safe manner brought the field to consider whether stem cells could offer a feasible alternative.

Unlike their fetal counterpart, ESCs are able to produce a large number of functionally active DA neurons in a more reliable and reproducible way. In addition somatic neural stem cells (NSCs), which are multipotent and can give rise to both glial and neuronal cells, could also be used. Both stem cell types, with their capacity for long-term expansion *in vitro* and their extensive functional stability and plasticity, allowed the establishment of cultures of mature neuronal cells and emerged as amenable cell sources for neural transplantation. Accordingly, transplanted DA neurons derived from murine ESCs were able to restore function in rodent models of PD (Kim, et al., 2002).

More recently, the SCNT technique has been used to generate DA neurons using murine fibroblasts as donor cells (Tabar, et al., 2008). The derived DA neurons were transplanted back in the original donor mice which showed significant functional recovery. These encouraging results imply that autologous transplants composed by genetically matched cells could bring fundamental improvement and reduce the problems related to immune rejection. However, as SCNT has not proven successfully for humans, alternative methods able to generate isogenic cell source for transplantation have to be used. To this aim, cellular reprogramming and iPSCs appear as the most promising source for cellular therapy of PD.

iPSCs for In Vitro Modeling and Cellular Therapy of PD

The generation of iPSCs cells directly from patient fibroblasts and the consequent derivation of DA neurons might represent a breakthrough in PD research. In the context of *in vitro* disease modeling, being the closest mimic of *in vivo* conditions available today, iPSC technology would allow

for a more reliable definition of PD neuronal cell biology and cell reactivity. Moreover, it would open the field of regenerative medicine to new exciting venues as the use of DA neurons derived directly from patients may provide a solution to the problem of immune rejection taunting current cell-replacement therapies.

Nevertheless, many road-blocks still lay ahead for both iPSC-based applications in PD. First, efficient differentiation protocols generating homogeneous DA neurons have to be established. At present, although several protocols exist, the final population appears composed of mixed cell types. Cellular-sorting technique may provide to be useful for improving the selection of DA neurons. This is important for the study of pathophysiological mechanisms of the disease, which occurs specifically in DA neurons within SNpc. In addition, efficient differentiation and elimination of unwanted undifferentiated cells is highly relevant also for the application of iPSC-mediated cellular therapy. Indeed, the formation of tumors represents as a major problem of cell replacement studies. The cause may be due to the persistence of undifferentiated, proliferating cells among the cells used for the transplantation. Thus, reliable methods for obtaining a pure population of DA neurons have to be applied.

The second obstacle lays in the occurrence or lack of occurrence of disease-associated phenotype within iPSC-derived DA neurons. In order to apply iPSC technology to *in vitro* modeling, DA neurons will have to display typical PD phenotypes in the culture dish. iPSCs have been already generated from PD patients fibroblasts and consequently efficiently differentiated into DA neurons (Park, et al., 2008; Soldner, et al., 2009). However, the differentiated cells did not show any difference when compared to DA neurons derived from healthy control iPSCs. Thus, in order to study PD–related mechanisms, certain cell-stressors might have to be used to facilitate disease progression *in vitro*. For example, cultured cells may be exposed to environmental effects known to play a role in PD, such as oxidative stress. Indeed, recent studies showed that iPSC-derived neurons from PD patients exhibited higher sensitivity to cellular stressors (Nguyen, et al., 2011).

On the other hand, the opposite goal is wanted for the purpose of utilizing differentiated DA neurons for cell transplantation, as these cells have to be healthy in order to contribute to clinical improvement of PD. Thus, if iPSC-derived DA neurons would immediately display clear PD phenotypes, it could represent a complication for future cellular therapies. Indeed, as fetal grafts showed disease features ten years after transplantation, it may be possible to propagate the disease from the host environment into the transplanted cells. Thus, it would be auspicial and necessary to use as a cell source for transplants cells that are as healthy and not disease-compromised as possible. Promising results showed that the course of PD might be indeed modified by iPSC-mediated transplants in rodent models of PD. In this study, mouse iPSCs were generated from murine fibroblasts, differentiated into DA neurons and transplanted into Parkinsonian rats, which showed restoration of DA function and disease improvement (Wernig, et al., 2008).

The final hurdle facing iPSC-based strategies is that PD pathology is not restricted to the loss of DA neurons. Indeed, over the years, it has become evident that the disease also implicates several non-motor symptoms, including neuropsychiatric, autonomic and sensory complications, thus involving multiple parts of central and peripheral nervous systems beside the extra-pyramidal motor system. Thus, different differentiated cells will have to be analyzed. Moreover, it might be warranted to study co-cultures composed by DA neurons and glial cells in order to better dissect the influence of glial modulation and environmental milieu on disease progression. In the context of cell therapy, it could be possible to experiment different strategies. For example, astrocytes may be used for transplants and the effects on synaptic transmission and glutamate modulation may be

monitored. Alternatively, other cell types might be used, in order to eventually restore the original physiologic circuit not only of DA cells but also of other neuronal populations that may be affected by the disorder.

Overall, despite all possible pitfalls, the iPSC technology is expected to open new important avenues in PD research both in the context of *in vitro* disease modeling and cellular therapeutics.

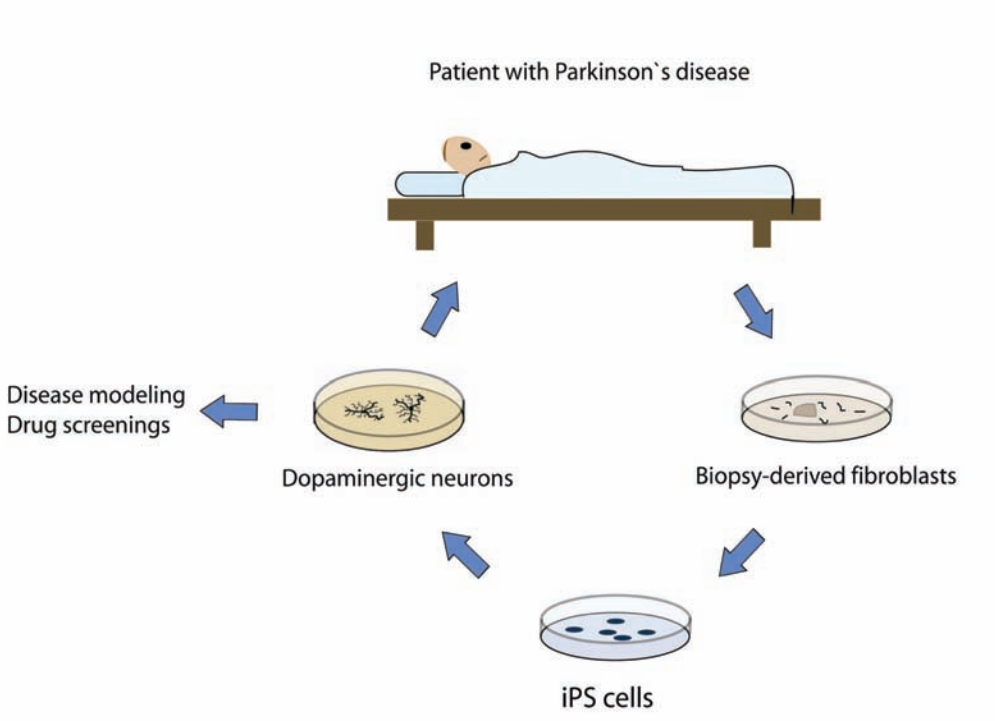
CONCLUSION

The present time is exciting for regenerative medicine research. In particular, high hopes reside in the soon-to-be possible personalized medical applications.

A novel strategy, called direct reprogramming, recently demonstrated the possibility to generate pluripotent cells, which can in turn be differentiated into almost any cell type, starting from a simple skin biopsy. Thus, iPSCs generated from fibroblasts from patients with complex diseases can be differentiated into relevant cell types. This will allow the production of large numbers of differentiated cells with the exact genotype of the diseased ones, which can be used for disease modelling and personalized drug discoveries. Finally, iPSCs may represent a remarkable step forward toward for stem cell therapeutics aimed at curing disorders for which no current therapies significantly modify the disease progression.

A paradigmatic example is Parkinson's disease (Figure 1), a chronic progressive neurodegenerative disorder for which no cure still exists, as current medications or surgical procedures can only provide symptomatic relief. Despite all hurdles that need to be overcome, given the scientific efforts

Figure 1. Schematic description of cellular reprogramming-based regenerative medicine applications in the context of Parkinson's disease



and the significant achievements of the past few year, iPSCs technology have the potential to offer successful and feasible approaches for the study of PD, for its drug intervention, and for cellular treatment strategies that could ultimately provide unprecedented relief to the affected individuals.

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder characterized by the selective loss of nigrostriatal dopaminergic (DA) neurons. Induced pluripotent stem (iPS) cells can be efficiently derived from fibroblast samples obtained through skin biopsy of affected PD individuals. iPSCs can then differentiate into DA neurons, which can in turn be used for several distinct purposes. *In vitro* applications include modeling of disease-associated mechanisms and personalized toxicology drug screenings. Finally, iPSC-derived DA neurons could be applied to cellular therapeutic strategy as an autologous source for cell transplantation.

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KEY TERMS AND DEFINITIONS

Stem Cells: Specialized cell types able to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types.

Embryonic Stem Cells (ESCs): Stem cells that are isolated from the inner cell mass of blastocysts and retain the ability to differentiate into cells of all the three germ layers (ectoderm, mesoderm, and endoderm).

Induced Pluripotent Stem Cells (iPSCs): A novel population obtained from somatic cells through cellular reprogramming and are believed to display features similar to ESCs.

Adult Stem Cells: Found in adult tissues and have limited differentiation abilities; Neural Stem Cells (NSCs) for example can differentiate only into neuronal cells and glial cells.

Self-Renewal: Ability to multiply indefinitely and undergo numerous cycles of cell division while maintaining an undifferentiated state with unchanged differentiation potential.

Potency: Capacity to differentiate into specialized cell types. Different degrees of potency exist.

Totipotency*:* Ability to give rise to embryonic and extra-embryonic cell types (retained only by spores and zygotes).

Pluripotency: Ability to differentiate into virtually any cells derived from all the three germ layers (ESCs, iPSCs).

Multipotency*:* Ability to differentiate into a related family of cells (adult stem cells).

Unipotency*:* Ability to produce only one cell type (germline stem cells can only give rise to either sperm or oocytes).

Differentiation: Process by which less specialized (undifferentiated) cells become more specialized cell types. Differentiation normally occurs during the development of a multi-cellular organism. Through differentiation, stem cells are able to generate distinct cell types *in vitro* and *in vivo*.

Cellular Reprogramming: Procedure of generating pluripotent stem cell artificially derived from non-pluripotent somatic cells. It is obtained by inducing the forced expression of certain key genes, associated with self-renewal features and commonly expressed in undifferentiated ESCs. It may allow researchers to obtain pluripotent stem cells, which are important in research and potentially have therapeutic uses, without the controversial use of embryos.

In Vitro Disease Modeling: Cellular platform to dissect the biochemical mechanisms and molecular processes which are altered upon disease development. iPSCs may represent the ideal candidates since they can be directly derived from affected individuals and retain the ability to differentiate into virtually any cell type of the body.

Cellular Replacement Therapy: Transplantation of stem cell-derived cells into damaged tissue in order to replace or repair diseased or injured host cells. The type of cell source used for transplantation can have an impact on the final outcome.

Allogenic Transplantation*:* Use of a cell source harboring a different genetic background. The major complication of this procedure is the occurrence of immune rejection due to the body response to cells that are not recognized as self.

Isogenic Transplantation (Autologous)*:* Use of a cell source harboring the same genetic background as the target individual in which the cells will be transplanted. This procedure is believed to overcome the problems related to immune rejection. iPSC technology may provide to be useful since it can generate patient-specific genetically identical cells.

Chapter 4
Bifurcation Analysis of a Model Accounting for the 14–3–3σ Signalling Compartmentalisation

Svetoslav Nikolov
Institute of Mechanics and Biomechanics, Bulgaria

Julio Vera
University of Rostock, Germany

Olaf Wolkenhauer
University of Rostock, Germany

ABSTRACT

Bifurcation theory studies the qualitative changes in the phase portrait when we vary the parameters of the system. In this book chapter we adapt and extend a mathematical model accounting for the subcellular localisation of 14-3-3σ, a protein involved in cell cycle arrest and the regulation of apoptosis. The model is analysed with analytical tools coming from Lyapunov-Andronov theory, and our analytical calculations predict that soft (reversible) loss of stability takes place.

INTRODUCTION

14-3-3 proteins have crucial roles in a variety of cellular responses including signal transduction, cell cycle progression, metabolic regulation and apoptosis (Yang, Wen, Chen, Lozano, & Lee, 2003; Wilker, van Vugt, Artim, et al., 2007). Firstly identified through their high level of expression in the mammalian brain, it is currently well-known that mammals have eight different protein forms of 14-3-3 that are encoded by seven distinct genes (β, ε, γ, η, τ, ξ, σ). One particular 14-3-3 isoform, σ, is a p53-responsive gene and was characterized as a human mammary epithelium-specific marker (that is the reason why 14-3-3σ is also called stratifin). 14-3-3σ has a variety of cell functions. Regarding cancer and tumour progression, 14-3-3σ plays a role like regulator of the cell cycle, probably via cytosolic sequestration of critical cell cycle proteins like cyclin B1 and

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cdc2 in response to DNA damage and other stress signals (Hermeking, 2006). Interesting enough, 14-3-3σ can delay the initiation of apoptosis by sequestering mitochondrial p53 in the cytosol and preventing activation of Bax and other initiators of the apoptotic cascade. This assigns to 14-3-3σ a dual role in the regulation of the cell fate after DNA damage (Samuel, Weber, Rauch, Verdoodt, Eppel, McShea, Hermeking & Funk, 2001). The function of 14-3-3σ is frequently lost in human tumours including melanoma, breast and prostate cancers, which suggests that the protein may act as a tumour suppressor. However, the molecular basis for the tumour suppressor function of 14-3-3σ remains unknown (Wilker, van Vugt, Artim, et al., 2007). Moreover, 14-3-3σ is silenced in many cancers via gene methylation (Ferguson, Evron, Umbrich, & et al., 2000; Iwada, Yamamoto, Sasaki, & et al., 2000; Lodygin, Diebold, & Hermeking, 2004, Schultz et al. 2009). Remarkably, 14-3-3σ is involved in a positive feedback loop with its own activator p53 via stabilization and inhibition of ubiquitination (Yang, Wen, Chen, Lozano & Lee, 2003).

With respect to dynamic systems theory (Glass & Mackey, 1988; Shilnikov, Shilnikov, Turaev, & Chua, 2001; Nikolov, 2004), the first Lyapunov value is one of the basic analytical tools to investigate the transition between different dynamical states in biochemical systems. The (un) stability of these transitions may have important consequences on the dynamics of the system, pointing towards changes where parameters are critical for the emergence of pathological configurations. In addition, qualitative knowledge emerging from this analysis could be used in the development of diagnostic methods and in drug discovery (Nikolov, Vera, Kotev, Wolkenhauer, & Petrov, 2008; Nikolov, Vera, Rath, Kolch, & Wolkenhauer, 2009).

The present chapter extends our previous results in the mathematical modelling of 14-3-3σ (Vera, Schultz, Ibrahim, Wolkenhauer, & Kunz, 2009). We here adapt and extend the model to consider the subcellular localisation of the protein (cytosol, nucleus and mitochondria). The model is analysed with analytical tools coming from Lyapunov-Andronov theory to investigate the system and perform analytical (qualitative) predictions, which are complemented by numerical simulation.

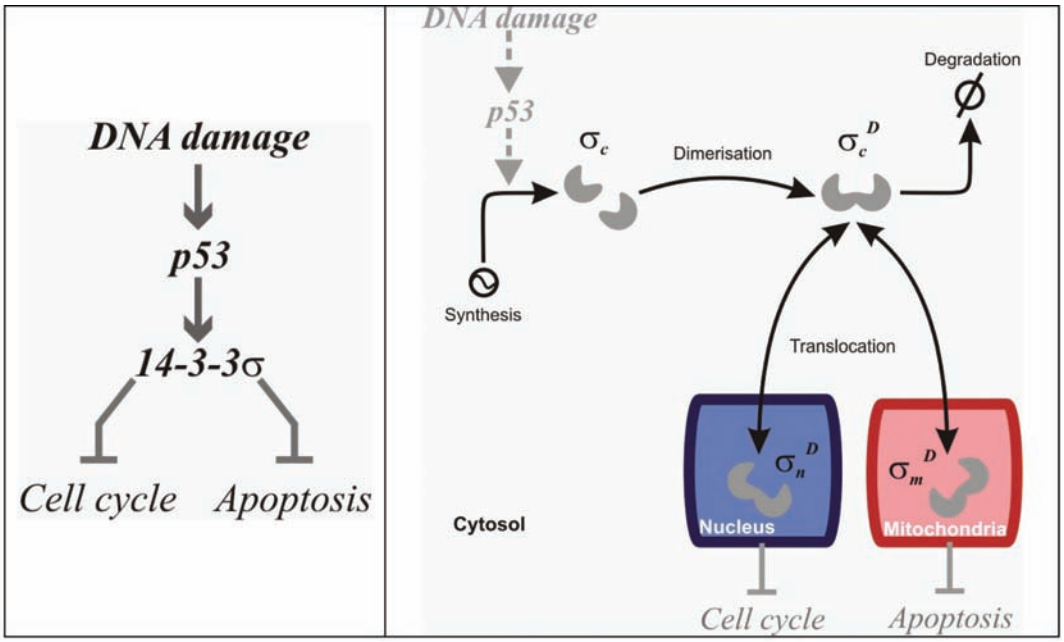
MATHEMATICAL MODEL

Qualitative Analysis

For our investigation, we modified and expanded our previously published model (Vera, Schultz, Ibrahim, Wolkenhauer, & Kunz, 2009). The version of the model discussed here considers the synthesis, dimerisation, degradation and translocation of 14-3-3σ, but also the compartmentalisation of the signaling molecule in the cytosol, nucleus and mitochondria. The model is depicted in Figure 1. As we previously indicated, 14-3-3σ expression is mediated by p53 in response to DNA damage signalling and is able to block the progression of the cell cycle in both G1/S and G2/M transitions, but it also may delay/block the activation of apoptosis (Figure 1, left-hand side). In our model we consider the following critical events in the dynamics of 14-3-3σ: i) p53 mediated synthesis; ii) 14-3-3σ homodimerisation/activation in the cytosol; iii) translocation between cytosol and nucleus; iv) translocation between cytosol and mitochondria; and v) cytosolic degradation of 14-3-3σ (Figure 1, right-hand side).

Based on these hypotheses, we derived a model in ordinary differential Equations (ODE), which describes the dynamics of 14-3-3σ. The model considers the following variables accounting for the subcellular distribution of the protein: monomeric cytosolic (σ_c), dimeric cytosolic (σ_c^D),

Figure 1. Scheme of our mathematical model describing the compartmentalisation of 14-3-3σ signalling. The left-hand side figure described the p53-mediated expression of 14-3-3σ in response to DNA damage and the regulation of cell cycle progression and apoptosis by the protein. The right-hand side is the scheme depicting the processes included in the mathematical model.



dimeric nuclear (σ_n^D) and dimeric mitochondrial (σ_m^D). The ODE model derived has the following mathematical structure:

$$\begin{aligned}
\frac{d\sigma_c}{dt} &= k_S(t) - 2k_D\sigma_C^2,\\
\frac{d\sigma_c^D}{dt} &= k_D\sigma_C^2 - \left(k_{cn} + k_{cm} + k_{\deg}\right)\sigma_c^D + k_{nc}\sigma_n^D + k_{mc}\sigma_m^D,\\
\frac{d\sigma_n^D}{dt} &= k_{cn}\sigma_c^D - k_{nc}\sigma_n^D,\\
\frac{d\sigma_m^D}{dt} &= k_{cm}\sigma_c^D - k_{mc}\sigma_m^D,
\end{aligned} \tag{1}$$

where σ_c, σ_c^D, σ_n^D and σ_m^D are the already defined time-dependent variables describing the subcellular dynamics of 14-3-3σ and k_s, k_D, k_{deg}, k_{cn}, k_{nc}, k_{cm}, k_{mc} are the rate constants of the model (1), whose values were either extracted from (Vera, Schultz, Ibrahim, Wolkenhauer, & Kunz, 2009) or assigned to qualitatively describe the dynamics of the system. For instance, k_{deg} and k_{cn} were directly extracted from our previous model, while for the parameters k_{nc}, k_{cm}, k_{mc} we considered an interval of feasible values around the values described in our previous model to investigate how the dynamics of the system is affected by the modulation of the related processes. Finally, dimerisation of 14-3-3σ is assumed to be a fast, efficient, irreversible process and a high value is assigned to the parameter describing it. The chosen values for the parameters are included in Table 1.

For our analysis, let

$$\begin{aligned}
&k_0 = k_s, \quad k_1 = 2k_D, \quad k_2 = k_D, \quad k_3 = k_{cn} + k_{cm} + k_{\deg},\\
&k_4 = k_{nc}, \quad k_5 = k_{cn}, \quad k_6 = k_{cm}, \quad k_7 = k_{mc},\\
&y_1 = \sigma_c, \quad y_2 = \sigma_c^D, \quad y_3 = \sigma_n^D, \quad y_4 = \sigma_m^D.
\end{aligned} \tag{2}$$

Table 1. Model parameter values

$k_s = 1, \quad k_D = 2, \quad k_{\deg} = 0.0069, \quad k_{cn} = 0.2336, \quad k_{nc} = X.k_{cn},$ $k_{cm} = k_{cn}, \quad k_{mc} = X.k_{cn}, \quad X \in [0.1, 3].$

Then substituting (2) into (1), we get

$$\begin{aligned} \frac{dy_1}{dt} &= k_0 - k_1 y_1^2, \\ \frac{dy_2}{dt} &= -k_3 y_2 + k_4 y_3 + k_7 y_4 + k_2 y_1^2, \\ \frac{dy_3}{dt} &= k_5 y_2 - k_4 y_3, \\ \frac{dy_4}{dt} &= k_6 y_2 - k_7 y_4. \end{aligned} \tag{3}$$

The steady (fixed point in the phase space) state of the system (1) is found by equating the right-hand sides of (3) to zero. It is easy to see that the steady state is only one with coordinates

$$\bar{y}_1 = \sqrt{\frac{k_0}{k_1}}, \quad \bar{y}_2 = \frac{k_4}{k_5}\bar{y}_3, \quad \bar{y}_4 = \frac{k_4 k_6}{k_5 k_7}\bar{y}_3, \quad \bar{y}_3 = \frac{k_0 k_2 k_5}{k_1 k_4 (k_3 - k_5 - k_6)}. \tag{4}$$

The divergence of the flow (3) is

$$D_4 = \sum_{i=1}^{n} \frac{\partial \dot{y}_i}{\partial y_i} = -2k_1 y_1 - k_3 - k_4 - k_7 < 0, \tag{5}$$

and the system (3) has an attractor. Generally, in order to determine the character of fixed point (Equation (4)) we linearise (3) near equilibrium solution, i.e.

$$y_i = x_i + \bar{y}_i, \quad (i = 1, ..., 4) \tag{6}$$

Hence, after some transformations the system (3) has the form

$$\begin{aligned} \frac{dx_1}{dt} &= -c_1 x_1 - k_1 x_1^2, \\ \frac{dx_2}{dt} &= c_2 x_1 - k_3 x_2 + k_4 x_3 + k_5 x_4 + k_2 x_1^2, \\ \frac{dx_3}{dt} &= k_5 x_2 - k_4 x_3, \\ \frac{dx_4}{dt} &= k_6 x_2 - k_7 x_4, \end{aligned} \tag{7}$$

where

$$c_1 = 2k_1 \bar{y}_1, \quad c_2 = 2k_2 \bar{y}_1. \tag{8}$$

According to (Bautin, 1984), the Routh-Hurwitz conditions for stability of (4) can be written in the form: (see Box 1)

The notations *p, q, r, s* and *R* are taken from (Bautin, 1984). When the conditions (12) or (13) are not valid, the steady state (4) becomes unstable. Here we note that conditions (9)-(11) and (13) are always valid in this case. The characteristic Equation of the system (7) (which is equivalent of system (3)) can be written as

$$\chi^4 + p\chi^3 + q\chi^2 + r\chi + s = 0. \tag{14}$$

The stability of a steady state (4) depends on the real part of the roots of the characteristic Equation (14). If all roots are negative then the equilibrium state is stable. If at least one root is positive, then the steady state is unstable. Ac-

Box 1.

$$p = c_1 + k_3 + k_4 + k_7 > 0, \tag{9}$$

$$q = c_1\left(k_3 + k_4 + k_7\right) + k_3\left(k_4 + k_7\right) + k_4k_7 - k_5\left(k_4 + k_6\right) > 0, \tag{10}$$

$$r = c_1\left[k_4\left(k_3 - k_5\right) + k_3k_7 - k_5k_6 + k_4k_7\right] + k_4\left(k_3k_7 - k_5k_6 - k_5k_7\right) > 0, \tag{11}$$

$$s = c_1k_4\left[k_3k_7 - k_5\left(k_6 + k_7\right)\right] > 0, \tag{12}$$

$$R = pqr - sp^2 - r^2 > 0\,. \tag{13}$$

cording to (Andronov, Witt, & Chaikin, 1966; Bautin, 1984), the conditions R=0 and s=0 are the boundaries of stability. In the boundary of stability s=0, the characteristic Equation (14) has one root equal to zero, and the type of the other roots is determined by the expression

$$\Omega = 27r^2 - 18pqr + 4q^3 + 4p^3r - p^2q^2. \tag{15}$$

Thus, we have two cases:

1. If $\Omega < 0, \quad p > 0, \quad q > 0, \quad r > 0, \quad R > 0$ and s=0, then the Equation (14) has one root equal to zero and three negative real roots;
2. $\Omega > 0, \quad p > 0, \quad q > 0, \quad r > 0, \quad R > 0$ and s=0, then the Equation (14) besides one zero root also has a negative root and two complex conjugate roots with negative real parts.

For systems with structurally unstable equilibrium states, the stability theory considers various aspects of stability critical cases. Note that here is included also the bifurcation phenomena. Here, we mention only the two most common and simple cases (Shilnikov, Shilnikov, Turaev, & Chua, 2001; Nikolov, Stoytchev, & Bozhov, 2006), where the characteristic Equation (14) (1) has one zero root and (2) has a pair of complex-conjugated roots on the imaginary axis.

The first case is determined by the condition

$$s = 0, \quad \Delta_k > 0 \quad \left(k = 1, 2, 3\right), \tag{16}$$

where Δ_k is the Routh-Hurwitz determinant. Recall that s=(-1)^{4}det$A$, where A is the matrix of the linearised system at the equilibrium state. In view of this condition, the equilibrium states associated with the first critical case are also called degenerate. Since the implicit function theorem may not longer be applied here, the persistence of such equilibrium state in a neighbouring system is not necessary guaranteed. Thus, a transition through the stability boundary in the first critical case may result in disappearance of the equilibrium state. In this case the system is structurally unstable (un-robust) and through bifurcation it will lose its stability non-reversely. Generally, the stability of cell signalling pathways could, from biological point of view, be connected to homeostasis, i.e. process of keeping an internal environment stable by making adjustments to changes in the external environment. This is achieved by a system of feedback control loops. In other words, for the stability of cell signalling process it is essential that the cell maintains a stable condition where in

fact a constant flux of molecules occurs. However, in this case (p53-sigma) pathway, the homeostasis is disturbed. Studies have shown that such interaction has been observed in cancer disease classifies this type of interaction as disruptive and causing disease.

The second critical case corresponds to

$$s > 0, \quad \Delta_{n-1} = 0, \quad \Delta_k > 0, \quad (n = 4, k = 1, 2) \tag{17}$$

In this case on the contrary of the first critical case, the equilibrium state is preserved in all nearby systems and cal loss its stability. From a biological point of view, this means that the homeostasis can be disturbed but after a certain period of time it will restore. If cancer disease appears, it can be healed with medical assistance.

Further, we focus our considerations on the problem of the transition over the stability boundary s=0, i.e. $k_{\deg} = \frac{1-X}{X} k_{cm}$. Here we note that for $X \in (0,1)$, the fourth Routh-Hurwitz condition for stability (Equation (12)) can be negative. This question has an immediate significance for the subject of nonlinear dynamics. For stationary regimes, the corresponding problem was solved in (Bautin, 1984). There the boundaries of stability are classified as safe or dangerous- safe boundaries (soft loss of stability) are such that crossing them leads to only small quantitative changes of the system's state; dangerous boundaries (hard loss of stability) are such that arbitrarily small perturbations of the system beyond them cause significant and irreversible changes in the system's behaviour. Generally in accordance with Lyapunov-Andronov theory, the so-called first Lyapunov value $l_1(\lambda_0)$ determines the character (safe or dangerous) of the boundary of stability s=0, when bifurcation parameter λ_0 is slowly changed. Thus, in order to define the type of stability loss of steady state (4) it is necessary to calculate $l_1(\lambda_0)$ on the boundary of stability s=0. In the case of fourth first order differential Equations, this value can be determined analytically by the formula in (Bautin, 1984):

$$l_1(\lambda_0) = \alpha \left\{ a_{11}^{(1)}\sigma_1^2 + a_{22}^{(1)}\sigma_2^2 + a_{33}^{(1)}\sigma_3^2 + \frac{1}{\delta^2} a_{44}^{(1)} \left(1 - \alpha\sigma_1 - \beta\sigma_2 - \gamma\sigma_3\right)^2 + \right.$$
$$+ \frac{2}{\delta}\left(a_{14}^{(1)}\sigma_1 + a_{24}^{(1)}\sigma_2 + a_{34}^{(1)}\sigma_3\right)\left(1 - \alpha\sigma_1 - \beta\sigma_2 - \gamma\sigma_3\right) +$$
$$\left. + 2\left(a_{12}^{(1)}\sigma_1\sigma_2 + a_{13}^{(1)}\sigma_1\sigma_3 + a_{23}^{(1)}\sigma_2\sigma_3\right)\right\}_1 + \beta\left\{...\right\}_2 + \gamma\left\{...\right\}_3 + \delta\left\{...\right\}_4. \tag{18}$$

where λ_0 is defined as a value of X or $k_{\deg}$ for which the relation s=0 takes place. Here $a_{11}^{(1)} = -k_1$, $a_{11}^{(2)} = k_2$ and all other a_{ij}^{l} *(i,j,l=1,2,3,4)* are equal to zero for the system (7). The coefficients α, β, γ, δ, σ_1, σ_2 and σ_3 are defined by corresponding formulas presented in (Bautin, 1984).

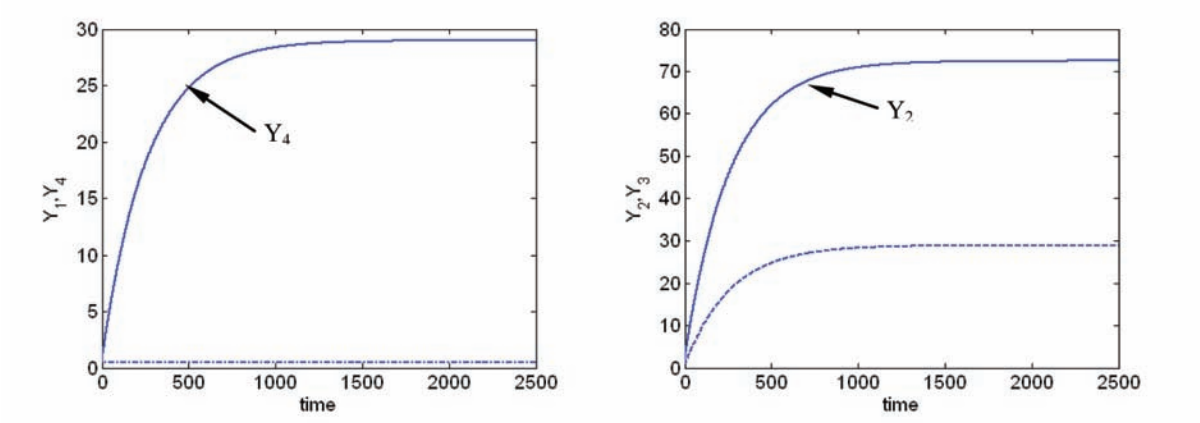
After accomplishing some transformations and algebraic operations for the first Lyapunov value $l_1(\lambda_0)$ (for system (8)) we obtain the main result in this article, i.e. $l_1(\lambda_0)$=0. In other words, the boundary of stability s=0 is safe and soft loss of stability take place.

Numerical Analysis

In previous section, we introduced the analytical tools proposed and used them for a qualitative analysis of the system obtaining some predictions about the dynamics of the system. The values chosen for the parameters and used in numerical analysis are those in Table 1.

In order to compare the predictions with numerical results, the governing Equations of the model, represented by (1), were solved numerically using MATLAB (Mathwork, 2009). In Figures 2 and 3 we illustrate the dependence of the model behaviour on the value for X–. The parameter X accounts for the relation between the two fluxes of subcellular translocation for a given compartment (nucleus or mitochondria). For instance, a value of X smaller than one accounts for conditions in which the translocation cytosol→nucleus is more efficient than the re-

Figure 2. Stable solutions of system (1), when parameter X is equal to 2.5. In this case all Routh-Hurwitz conditions are positive.



verse process nucleus→cytosol; thus, under this biological setup the protein may accumulate in the nucleus. In opposite case, a value for *X* bigger than one would indicate that the translocation nucleus→cytosol is more efficient and therefore most of the protein will remain in the cytosol. The same notion applies to the processes between cytosol and mitochondria. Since both processes (translocation in and out the given compartment) may be regulated by different proteins and mechanisms, is it meaningful to consider how changes in their interplay may affect the dynamics of the system.

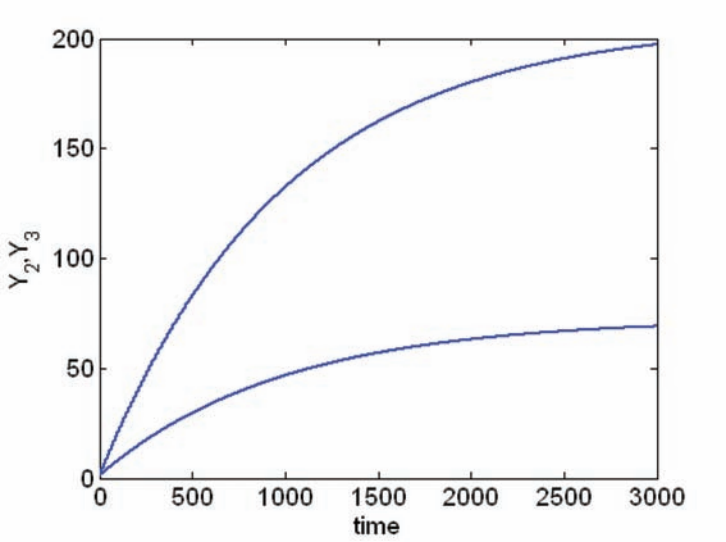
In our numerical simulations it is seen that for *X* equal to *2.5* the system has stable solutions. Note that in this case all Routh-Hurwitz conditions are valid. On the other hand for *X=0.35* (see Figure 3) the system is in unstable zone of parameters space and has unstable solutions. According to analytical results from previous chapter, loss of stability is reversible and after drug therapy the system can be again stable.

CONCLUSION

In this book chapter we present a study of the dynamical features of a 4D model describing the compartmentalisation dynamics of 14-3-3σ using the Lyapunov-Andronov bifurcation theory. During the last decade, the robustness has been considered as a key property of the biological (biochemical) systems (Kitano, 2004; Nikolov, Yankulova, Wolkenhauer, & Petrov, 2007). Under this assumption, small changes in the internal or external conditions of a biochemical system are neutralised by the system which is able to return to the vicinity of the preliminary attractor, while intense changes on the values of parameters characterising the system can provoke the transition to a new state, potentially a different attractor with its own interval of robustness (Kitano, 2004). By knowing the sign of the first Lyapunov value at the bifurcation boundary s=0 as we showed in this work, we are able to define the structural stability (robustness) of the steady state. Here we obtain that first Lyapunov value $l_1(\lambda_0)$ for 14-3-3σ system is always equal to zero and soft (reversible) loss of stability take place.

From a biological perspective, here we investigate the dynamics of subcellular translocation for 14-3-3σ. This protein is synthesised and degraded in the cytosol and has experimentally verified biological activity in the cytosol itself, the nucleus and the mitochondria. Since the translocation in and out a given compartment (in

Figure 3. Unstable solutions of system (1), when parameter X is equal to 0.35. In this case only Routh-Hurwitz condition s is negative, i.e. s=-0.0114.



our case, the nucleus and mitochondria) may be regulated by different proteins and mechanisms, is it important to consider whether the interplay between both translocation processes may affect the dynamics of the system and induce anomalous accumulation of the protein in any compartment. In the normal physiological configuration of the system, experimental evidences suggest that translocation cytosol→nucleus is less efficient than the reverse process nucleus→cytosol (*X* bigger than one), most of existing amount of 14-3-3σ remains in the cytosol, where it is degraded, and only a reduced fraction of the protein translocates to the nucleus and mitochondria (Hemert, Niemantsverdriet, Schmidt, Backendorf & Spaink, 2004). Our analysis suggest that under this normal conditions the solutions of the system are always stable, which means that there is a dynamical equilibrium between protein synthesis, degradation and translocation. On the other hand, under pathological conditions in which the translocation cytosol→compartment is more efficient that the reverse one (*X* smaller than one), our analysis suggest that the systems becomes unstable and big amounts of the protein accumulate on time in both compartments, leading potentially to an abnormal modulation of the cell cycle progression and apoptosis. We hypothesised that this abnormal accumulation of the protein may be related to the fact that degradation of the protein in our models occurs only in the cytosol.

In the coming future, we will improve the characterisation of our model using specifically designed experiments and consider the effect of further regulatory structures in the system, like the positive feedback loop p53-MDM2-14-3-3σ.

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KEY TERMS AND DEFINITIONS

Bifurcation Theory: This theory describes qualitative changes in phase spaces that occur as parameters are varied in the definition of a dynamical system.

Safe Boundaries: They are such that crossing over them leads to only small quantitative changes of the system state.

Dangerous Boundaries: They are such that arbitrary small perturbations of the system beyond them cause significant and irreversible changes in the systems behaviour.

Chapter 5
Engineering Gene Control Circuits with Allosteric Ribozymes in Human Cells as a Medicine of the Future

Robert Penchovsky
Sofia University "St. Kliment Ohridski", Bulgaria

ABSTRACT

Systems and synthetic biology promise to develop new approaches for analysis and design of complex gene expression regulatory networks in living cells with many practical applications to the pharmaceutical and biotech industries. In this chapter the development of novel universal strategies for exogenous control of gene expression is discussed. They are based on designer allosteric ribozymes that can function in the cell. The synthetic riboswitches are obtained by a patented computational procedure that provides fast and accurate modular designs with various Boolean logic functions. The riboswitches can be designed to sense in the cell either the presence or the absence of disease indicative RNA(s) or small molecules, and to switch on or off the gene expression of any exogenous protein. In addition, the riboswitches can be engineered to induce RNA interference or microRNA pathways that can conditionally down regulate the expression of key proteins in the cell. That can prevent a disease's development. Therefore, the presented synthetic riboswitches can be used as truly universal cellular biosensors. Nowadays, disease indicative RNA(s) can be precisely identified by employing next-generation sequencing technologies with high accuracy. The methods can be employed not only for exogenous control of gene expression but also for re-programming the cell fate, anticancer, and antiviral gene therapies. Such approaches may be employed as potent molecular medicines of the future.

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INTRODUCTION

In the last several years sequencing technology has been improving dramatically (Reis-Filho, 2009). Nowadays, 200 million bases can be sequenced per day in the Human Genome Project (HGP) using the latest generation sequencing technology such as the Genome Analyzer IIe from Illumina and the Solid System IV from Applied Biosystems (Oetting, 2010). These machines offer ultra-throughput whole genome sequencing (Bentley et al., 2009), targeted re-sequencing, SNP analysis (Chan, 2009; Ramos et al., 2009), gene expression and small RNA analyses (Jung, Hansen, Makunin, Korbie, & Mattick, 2010), chromatin immune-precipitation and DNA methylation arrays. The next generation sequencing (NGS) technology promises to advance genomic research in the next years by producing whole genome and gene expression data in combination with epigenetic data (Liang et al., 2009). Such complex approach was not available only a few years ago. The huge quantity of experiential data produced by the NGS technology can be fully analyzed by parallel computing (Bateman & Quackenbush, 2009). The combination of NGS technology with high-performance computing (HPC) is set to make a breakthrough in genomics, and many have far-reaching applications in cancer research. This integrated approach promises to bring to light the complexity of factors that make one cell become cancerous (Morrissy et al., 2009; Wyman et al., 2009). Understanding the molecular mechanisms of cancerogenesis is an important step for finding a cure. However, knowing the molecular mechanisms of one disease doesn't lead immediately to its cure. In this chapter we describe two general approaches that can tackle various disorders, which are associated with expression of disease indicative RNA(s). (Teng & Xiao, 2009; Willenbrock et al., 2009).

The methods described in this chapter are based on allosteric hammerhead ribozymes (Porta & Lizardi, 1995), which can work as biosensors *in vitro* and as synthetic riboswitches and gene regulatory elements *in vivo* (R. Breaker & Penchovsky, 2008; Penchovsky & Breaker, 2005). Ribozymes are naturally occurring RNA molecules that possess a catalytic function similar to that of protein enzymes (Muller, 2009). Allosteric enzymes are biopolymers that can change their conformation on binding a specific effector molecule(s) to their allosteric domains (Penchovsky & Breaker, 2005). Effector binding domains are distant from the catalytic center in an allosteric enzyme. Allosteric protein enzymes are discovered to play an important role in biochemical pathways of many organisms. Unlike allosteric protein enzymes all allosteric ribozymes are synthetic molecules. The only naturally occurring ribozyme discovered up to now to sense the presence of an effector molecule, is known as the glmS ribozyme (Blount, Puskarz, Penchovsky, & Breaker, 2006). However, the glmS RNA is not an allosteric ribozyme. Its activator glucosamine-6-phosphate serves as a co-factor in the catalytic center of the ribozyme. The allosteric ribozymes are designed to sense the presence of small molecules or oligonucleotides and to catalyze certain chemical reactions by various engineering methods. Such methods include *in vitro* selection (Guryev & Cuppen, 2009), rational design (Tang & Breaker, 1997), and computational design procedures (Penchovsky & Breaker, 2005).

All allosteric ribozymes described in the current chapter are designed by a published computational procedure (Penchovsky & Breaker, 2005), which is also a subject of a pending patent application (R. Breaker & Penchovsky, 2008). The procedure yields a ribozyme sequence within minutes on an average personal computer with over 90% accuracy. It is advantageous in comparison to *in vitro* selection and rational design methods in terms of design accuracy and time spent. All presented ribozymes have a modular design that allows the allosteric domains to be easily altered. As a result, the ribozymes can be designed to sense any DNA and/or RNA oligonucleotide from 16 up to 22 nucleotides (nt) long. The ribozymes can be engineered to perform essential Boolean logic

functions such as AND, OR, NOT, and YES (Penchovsky & Breaker, 2005). Moreover, allosteric ribozymes can be engineered to execute integrated logic circuits, which will be published elsewhere. The designer ribozyme can be used as biosensors both *in vitro* and *in vivo*. When used as biosensors *in vitro* (in a solution) all allosteric ribozymes are based on the minimal version of the hammerhead ribozyme. These ribozymes can be immobilized in microfluidic devices to build automated microarrays (Penchovsky, Birch-Hirschfeld, & McCaskill, 2000). The designer ribozymes intended to be used *in vivo* are based on the wild-type ribozyme from the human parasite Schistosoma mansoni. Wild-type *schistosoma* hammerhead ribozyme has been used for exogenous control of gene expression both in human cell lines and in mouse (Martick, Horan, Noller, & Scott, 2008; Yen et al., 2004). In contrast to the minimal version, the *schistosoma* hammerhead ribozyme can function under physiologically relevant conditions due to its specific tertiary structure as described in the next section. Therefore, designer constructs based on the *schistosoma* hammerhead ribozyme are to be employed in all *in vivo* applications.

The oligonucleotide-sensing ribozymes can be embedded in 5' or 3' untranslated region (UTR) of exogenous mRNA expressed in the cell via a viral vector. The presence or the absence of predefined RNA(s) in the cell can induce or suppress the self-cleavage of the ribozyme embedded in the UTR of the exogenous mRNA. As a result, the lifetime of the exogenous mRNA will be reduced or prolonged. That will switch OFF, or turn ON the expression of the exogenous protein encoded into the mRNA. Therefore, oligonucleotide-sensing ribozymes can be used as synthetic riboswitches in the cell *in vitro* or *in vivo* for exogenous control of gene expression. The synthetic riboswitches can be designed to sense the presence or absence of disease indicative RNA(s) in the cell and to control the expression of key exogenous or endogenous proteins. Such approach can alter the cell fate, for instance, by inducing apoptosis in the sick cells only. The approach is universal since the expression of any exogenous protein can be controlled by the presence or absence of any significantly expressed RNA(s) in the cell. Different cancer forms have different RNA expression profiles. Therefore, they have to be treated specifically. However due to its steady proliferation, some cancer cells over-expressed common RNAs. For instance, mRNA of telomerase is over-expressed in many tumor cells. Such RNAs can be used as common effectors for the proposed anticancer treatment in tissues with few normally dividing cells.

The synthetic riboswitches reassemble the function of the natural ones found to control gene expression in bacteria, plants, and fungus (Barrick & Breaker, 2007; Winkler & Breaker, 2005). Natural riboswitches are structured RNA domains usually residing in the 5' UTR of mRNAs. They control gene expression by directly binding a specific metabolite. Bacterial riboswitches execute control of gene expression via self-cleavage, prevention of translation, or termination of transcription. Riboswitches in plants and fungus are found to control gene expression via regulation of alternative splicing (Barrick & Breaker, 2007). Bacterial riboswitches are considered to be novel and very promising targets for antibacterial drug discovery (R. R. Breaker, 2009).

The idea of applying ribozymes as a medicine is an old one. Ribozymes have been seen as a tool for gene therapy for a long time (Britton, Larsson, & Ahrlund-Richter, 1994; Freelove & Zheng, 2002; Shaw et al., 2001). The allosteric ribozymes discussed here have the potential to serve as synthetic riboswitches in a human cell, since they have an advance design as discussed in the next sections. They may be used to develop novel therapeutic strategies, which can address many forms of cancer associated with aberrant expression of disease indicative RNA(s). That can be achieved by re-programming the fate of malignant cells through over-expression of exogenous p53 or other key cell factors under the

control of synthetic riboswitches. In addition, allosteric ribozymes can be designed to produce conditionally short hairpin (sh) RNAs in the cell inducing microRNAs or RNA interfering(RNAi) pathways (Li, Li, & Rossi, 2006). Such approaches can be used for conditional down regulation of key cellular proteins. These new synthetic biology approaches have the potential to be employed as novel potent therapeutics as described below.

Identifying Cancer Indicative RNAs

There are some frequently occurring forms of cancer that are associated with over-expression of certain mRNAs such as mantle cell lymphoma (MCL) and hepatocellular carcinoma (HCC). Lymphoma is a sort of cancer that starts in a lymphocyte, usually in a lymph node or occasionally in other organs. It is divided into two main classes known as Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). MCL is one of the most severe subtypes of NHL. There are circa sixty thousands new cases of NHL in the United States alone every year. MCL patients make about six percent of all cases of NHL per annum, which means about three and a half thousands new patients in the United States alone. MCL is associated with a genetic mutation that involves chromosomes 11 and 14, called reciprocal translocation t(11;14) in about 85% of patients (Salaverria, Perez-Galan, Colomer, & Campo, 2006). This mutation makes the transfected B lymphocyte (lymphoma cell) to overproduce a cyclin D1 protein. It is known that the cyclin D1 protein directs cell division and growth (Deboer, Vankrieken, Kluinnelemans, Kluin, & Schuuring, 1995). The resulting accumulation of mutant B lymphocytes leads to the formation of MCL tumors. In a very small number of patients t(11;14) mutation is not present. In most of those patients, other genetic mutations cause excess production of cyclin D1. Rarely, MCL can arise from over-expression of other cyclin genes such as cyclin D2 and cyclin D3. Note that the overproduction of cyclin D1 protein is due to the over-transcription of cyclin D1 mRNA that has been used as a clinical marker for MCL development (Deboer, et al., 1995).

Although the notable progress was achieved in the treatment of MCL with an almost doubling of overall survival over the last three decades, relapses are still very common (Witzig, 2005). Most patients are observed to respond well to initial chemotherapy. Recently, chemotherapy is often combined with stem cell transplantation for patients with MCL. Unfortunately, for most patients, the disease eventually progresses or relapses, despite the therapies employed. In addition, resistance to the chemotherapy frequently develops. Because all these factors, the average progression-free period for patients with MCL is 20 months and the median overall survival prognosis is about four years. Therefore, many researchers continue to look for novel therapies that will prolong remissions and extend survival in patients with MCL, and will have fewer side effects (Goy, 2006).

Liver cancer is also a widely spread disorder as the rates of hepatocellular carcinoma (HCC) have been rising by more than 70% in the last 3 decades in the United States alone. Late diagnosis, due to the lack of clinical symptoms, and decreased hepatic function, caused by underlying hepatic disease, have led to the extremely high mortality rates inflicted by HCC (Graveel, et al., 2003). It has been found that over-expression of CRG-L2 mRNA (Cancer related gene-Liver 2) is associated with HCC (Graveel, et al., 2003). The development of novel gene therapy approaches for curing HCC is the main goal of many clinical trials (Hernandez-Alcoceba, et al., 2007). Despite the progress achieved in the life prolongation of patients with terminal phase of HCC, there is still nocure for such patients (Hernandez-Alcoceba, et al., 2007).

Normal and malignant cells from a same tissue are isolated from one cancer patient. Total RNA extracts are obtained separately from the isolated normal and malignant cells. The RNA pools are

converted into cDNA molecules by reverse transcription. The DNA molecules can be sequenced using next generation sequencing machines like Solid System IV from Applied Biosystems. Apart from this, the cDNA pools can be fluorescently labeled during the reverse transcription and analyzed on DNA hybridization microarrays such as GeneAtlas System from Affymetrix. The obtained experimental results can be analyzed on parallel computing platforms such as Blade Servers from Dell. These methods can be applied for identifying cancer indicative RNAs in many different disorders as a first step in a comprehensive anticancer therapy as described in the next sections.

All these findings strongly suggest that an aberrant expression of normal mRNAs, non-coding RNAs, and the presence of mutant RNAs in tumors can be widely used as reliable molecular markers for diagnostic of many different cancer disorders. Applications of high-throughput RNA expression microarrays and ultra-throughput NGS technologies will certainly increase the number of cancer indicative RNAs known to us. A general scheme for discovery of aberrant expression of RNAs in cancer disorders using NGS or RNA microarray technologies is depicted in Figure 1. Total RNA extracts from one patient can be isolated separately from normal and tumor cells. The extracted RNAs from normal and malignant cells can be transcribed into cDNA libraries using the enzyme reverse transcriptase (RT). NGS machines, such as the Solid System IV from Applied Biosystems or Genome Analyzer from Illuminer, can be employed for ultra-throughput DNA sequencing (Figure 1). Apart from this, DNA hybridization microarrays, such as GeneAtlas System from Affymetrix, can be used for the same goal (Figure 1). In this case, the cDNA molecules have to be fluorescently labeled during the reverse transcription. Both approaches are equally accurate. High-performing computer platforms, such as Blade servers from Dell, can be employed for data manning of the experimental results obtained (Figure 1). Various bioinformatical methods have to be employed to sort out the primary mutations that cause the cancer from many more secondary mutations during cancer progression. The discovery of cancer indicative RNAs can be of significant importance not only for early molecular diagnostics but also for developing novel comprehensive anticancer therapies. General approaches for anticancer and antiviral therapies based on computationally designed allosteric ribozymes will be discussed in the next sections.

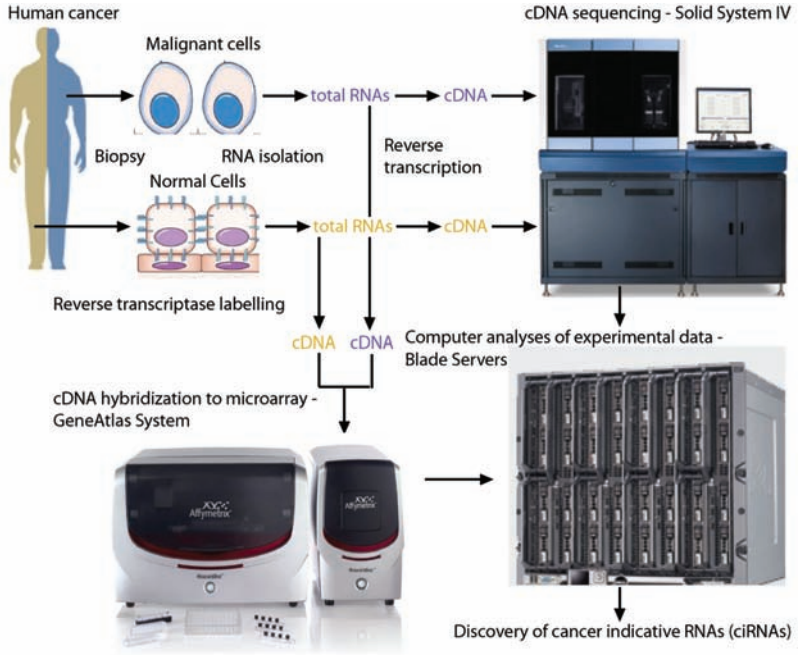
Computational Design of High-Speed Oligonucleotide-Sensing Allosteric Ribozymes with NOT or YES Logic Functions

The hammerhead ribozymes are naturally occurring RNA molecules that possess an autocatalytic function to cleave itself. They are named hammerhead ribozymes because their conserved secondary structure that reassembles the form of a carpenter's hammerhead (Figure 2).

The hammerhead ribozyme catalyzes a transesterification reaction in which the 3', 5'-phosphodiester is cleaved (Figure 3A and 3B). The reaction yields a cyclic 2',3'- phosphodiester on nucleotide 17 and a free 5'-hydroxyl on nucleotide 1.1 (Figure 3C). The chemical reaction requires an in-line conformation and divalent cations such as Mg^{2+} to have an in-line attack from 2'-hydroxyl of the nucleotide 17 to the scissile phosphate. Therefore, the free 2'-OH on nucleotide 17 and divalent cations are essential for the catalytic function of the hammerhead ribozyme (Figure 2).

The hammerhead ribozyme in nature is involved in the replication of viroids and some satellite RNAs. It is found both in animals and plants. Note that the conserved secondary structure of the hammerhead ribozyme defines a certain conserved tertiary (3D) structure that is essential for the catalytic function of the ribozyme (Martick & Scott, 2006). There are two main variants of the hammerhead ribozyme, which differ in their

Figure 1. General strategies for identifying cancer indicative RNAs in humans applying either ultra-throughput next generation sequencing technology or DNA hybridization microarrays

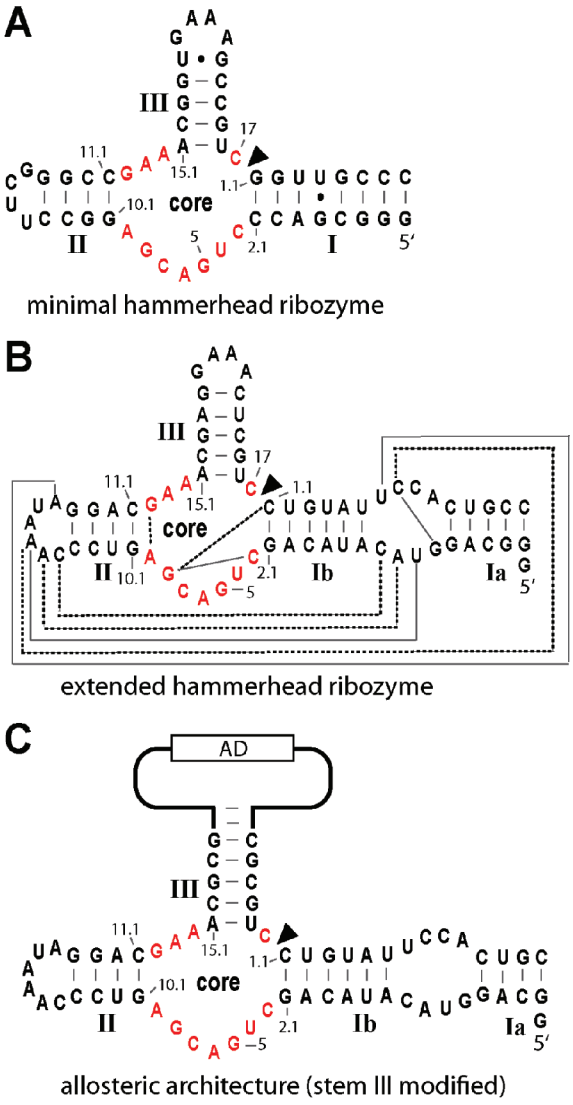


kinetics and concentration of divalent cations needed for the catalytic function. One variant of the hammerhead ribozyme is called minimal (Figure 2A) and the other is called extended (Figure 2B).

As suggested by its name the minimal variant of the hammerhead ribozyme is the shortest possible sequence of the ribozyme that has all essential structures including stem I, II, and III, and the catalytic core (Figure 2A). In contrast, the extended variant of the ribozyme has an additional bulge loop in stem I (Figure 2B). The nucleotides in this loop make contacts with the nucleotides from the loop in stem II in the extended hammerhead ribozyme (Figure 2B). It is found that stems I and II have to get close together in the 3D conformation of the hammerhead ribozyme for its efficient catalysis. It is believed that divalent cations (such as Mg^{2+}) bridge stems I and II. Because of its tertiary contacts between stems I and II, the extended variant of the hammerhead ribozyme requires only 1 mM Mg^{2+} to reach its maximal speed of cleavage. In contrast, the minimal variant of the hammerhead ribozyme requires 10 mM Mg^{2+} to reach its maximal speed of cleavage. In addition, maximal speed of the extended variant of the hammerhead ribozyme is 10 fold higher than that of the minimal variant. Note that the physiological concentration of Mg^{2+} in the cell is 1 mM. That makes the extended variant of the hammerhead ribozyme (Figure 2B) suitable to serve as a pattern construct for designer biosensors that have a cellular function.

Therefore, the extended hammerhead ribozyme from the human parasite Schistosoma *mansoni* is used as a pattern construct for all designer hammerhead ribozymes that are intended to have a cellular function. The wild-type *schistosoma* hammerhead ribozyme possesses high-speed kinetics of cleavage that can reach a speed of circa 10 per min. Moreover, *schistosoma* hammerhead ribozyme has been employed as a regulatory element for exogenous control of gene expression in human cell line (HEK293T) as well as in mouse.

Figure 2. Three variants of the hammerhead ribozyme



However, these experiments are not very useful since the wild-type *schistosoma* hammerhead ribozyme is not an allosteric molecule and cannot sense any specific effector in the cell apart from general RNA inhibitors that are toxic for the whole cell (Yen, et al., 2004).

Many promising applications of computationally designed allosteric ribozymes that can sense specific RNA or small molecules are described in the next sections.

The allosteric domain for all designer ribozymes is introduced in stem III (Figure 2C) to preserve all tertiary contacts between stem I and II existing in the wild-type *schistosoma* hammerhead ribozyme. This maintains the high-speed kinetics of the designer ribozymes. The allosteric domain can sense the presence of either a RNA (or DNA) sequence (Figures 4 and 6) or a small molecule (Figure 5). If the effector is a small molecule, the allosteric domain contains a RNA aptamer (Figure 5). Aptamers are RNA or DNA molecules that specifically bind a predefined molecule. They are obtained by a procedure called *in vitro* selection of nucleic acids. Up to now, there are only two synthetic RNA aptamers, which are found to be able to work in the cells. These aptamers sense theophylline (Figure 5A and 5B) or tetracycline (Figure 5C). The natural riboswitches also have aptamer domains that can bind a metabolite with a great affinity and specificity. For the oligonucleotide-sensing ribozyme, the allosteric domain is complementary to the effector molecule (Figures 4 and 6).

All ribozymes are designed by a published and patented computational procedure that has two main parts. In the first part, the algorithm computes two different states (conformations) of an allosteric ribozyme in the presence or absence of effector. The different conformations in the presence or absence of effector are computed for their thermodynamic stability and kinetics of folding. The thermodynamic stability is computed by a partition function for RNA folding that gives to all possible RNA secondary structures a probability for a formation based on their thermodynamic stability. This produces very accurate designer ribozymes (Penchovsky & Breaker, 2005). One of the states has an active conformation, in which all three stems I, II, and III are formed as in the wild-type *schistosoma* hammerhead ribozyme (Figures 2C, 4A, 5A, and 6B). In an active conformation, the ribozyme cleaves its substrate.

Figure 3. The catalytic mechanism of the hammerhead ribozyme

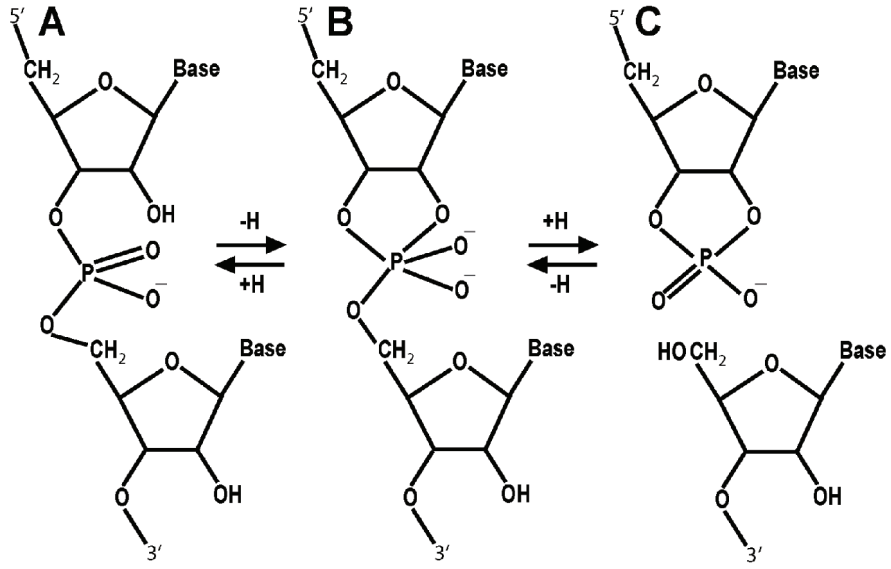
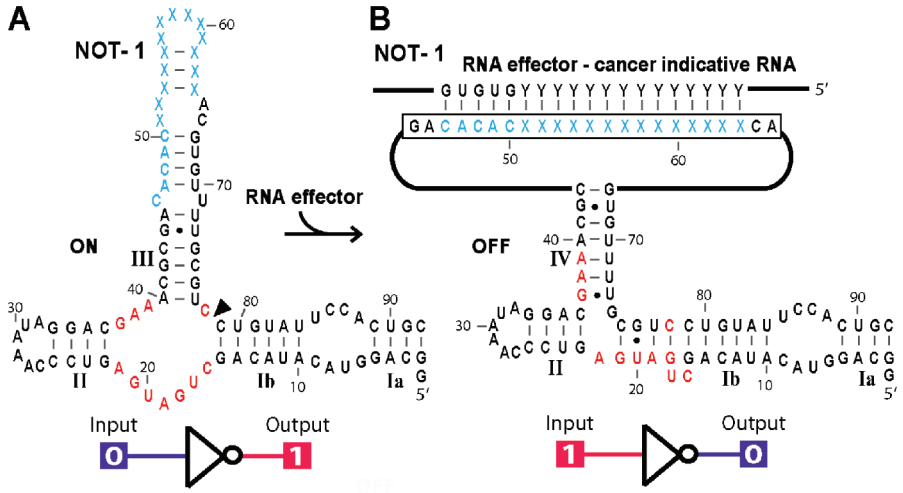


Figure 4. A high-speed oligonucleotide-sensing ribozyme construct with NOT Boolean logic function



In an inactive conformation, the ribozyme forms stem IV instead of stem III (Figures 4B, 5B, and 6A). As a result, the catalytic core of the hammerhead ribozyme is not formed and the allosteric molecule cannot cleave itself or its target molecule.

In a case of an allosteric ribozyme with a NOT logic function, the active state is without the effector while the ribozyme is inactive in the presence of effector (Figures 4A and 5A).In contrast, an allosteric ribozyme with YES Boolean logic function is active in the presence of effector (Figure 6B) and inactive in its absence (Figure 6A). Note that the accuracy of the algorithm does not suffer from any non-computed tertiary interactions because there are kept constant for both computed

Figure 5. A high-speed small molecule-sensing ribozyme with NOT Boolean logic function

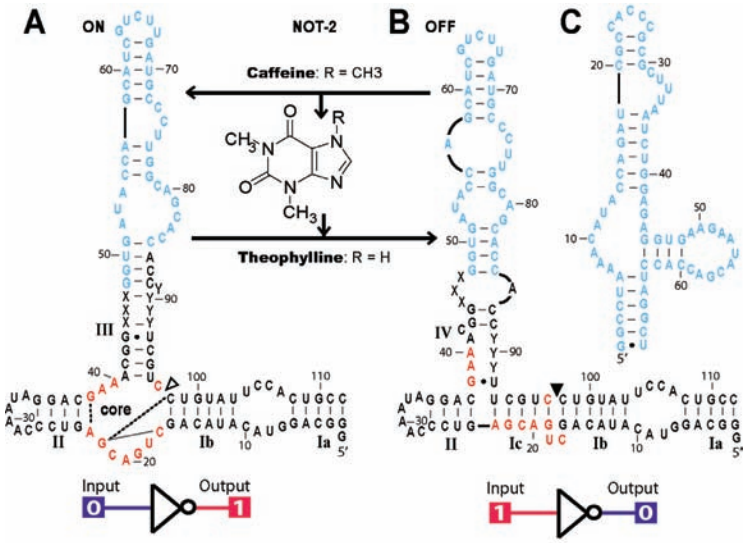
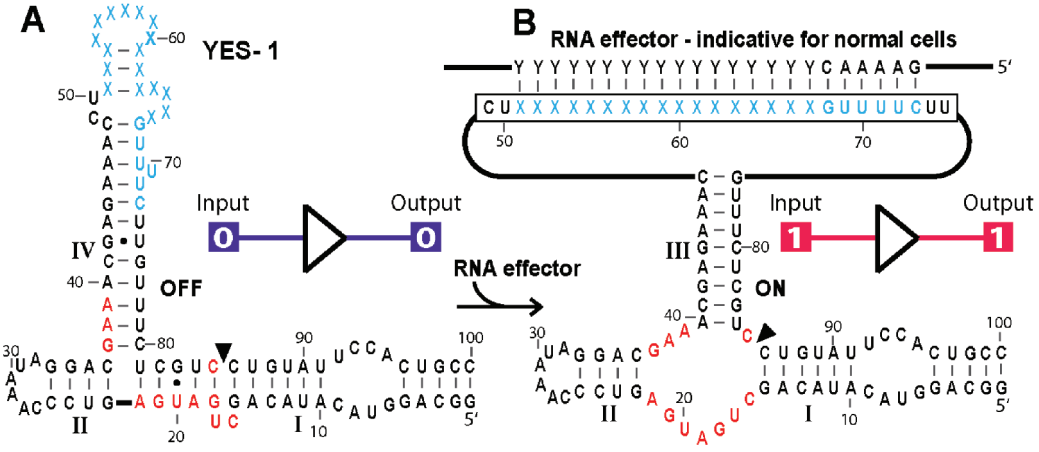


Figure 6. A high-speed oligonucleotide-sensing ribozyme with YES Boolean logic function



states. The properties of the allosteric ribozyme can be tuned by changing the thermodynamic gap between the ON and the OFF states. The applied algorithm exhibits more than 90% accuracy (Penchovsky & Breaker, 2005). It can produce RNA sequences for several allosteric ribozymes in a timescale of several min. All ribozymes are designed in a modular form so that the specificity of the allosteric domain can be easily altered applying reverse folding, which is implemented in the second part of the computational procedure.

The computational procedure discussed here is more efficient than any *in vitro* selection or rational design method employed for production of allosteric ribozymes. It avoids many limitations of *in vitro* selection procedures such as a limited

initial pool, arising "mini monster" molecules that replicate faster than all other molecules, evolving of opportunistic strategies for bypassing of the selection step, and the long time needed for performing all biochemical procedures in many cycles. Rational design methods do not explore many possible variants, since they are not computerized. The computational procedure discussed here is very time-efficient because it can test hundred thousand different sequences for a few hours on an average personal computer employing a random search algorithm. In addition, this computational procedure avoids all handicaps of *in vitro* selection and rational design methods accurately producing allosteric ribozymes with desired potteries as experimentally verified (Penchovsky & Breaker, 2005).

The experimental results obtained from the designer ribozymes demonstrate a huge dynamic range (over 1000 fold) in kinetics of cleavage between the presence and absence of effector (Penchovsky & Breaker, 2005). The allosteric ribozymes show high-speed kinetics cleaving to 80% within one min under physiological relevant conditions such as 1 mM $MgCl_2$, 90 mM KCl, 25 mM NaCl, 50 mM Tris-HCl (pH 7.5 at 37ºC) (Penchovsky & Breaker, 2005). The designer ribozymes possess high-specificity of activation by their effector molecules because they can distinguish two mismatching bases out of a 22 nt long oligomer. All this makes the designer ribozymes very suitable as synthetic riboswitches *in vivo* for various gene expression control applications as discussed in the next sections.

Allosteric Ribozymes that Function as Synthetic Riboswitches for Exogenous Control of Gene Expression and Re-programming the Cell Fate

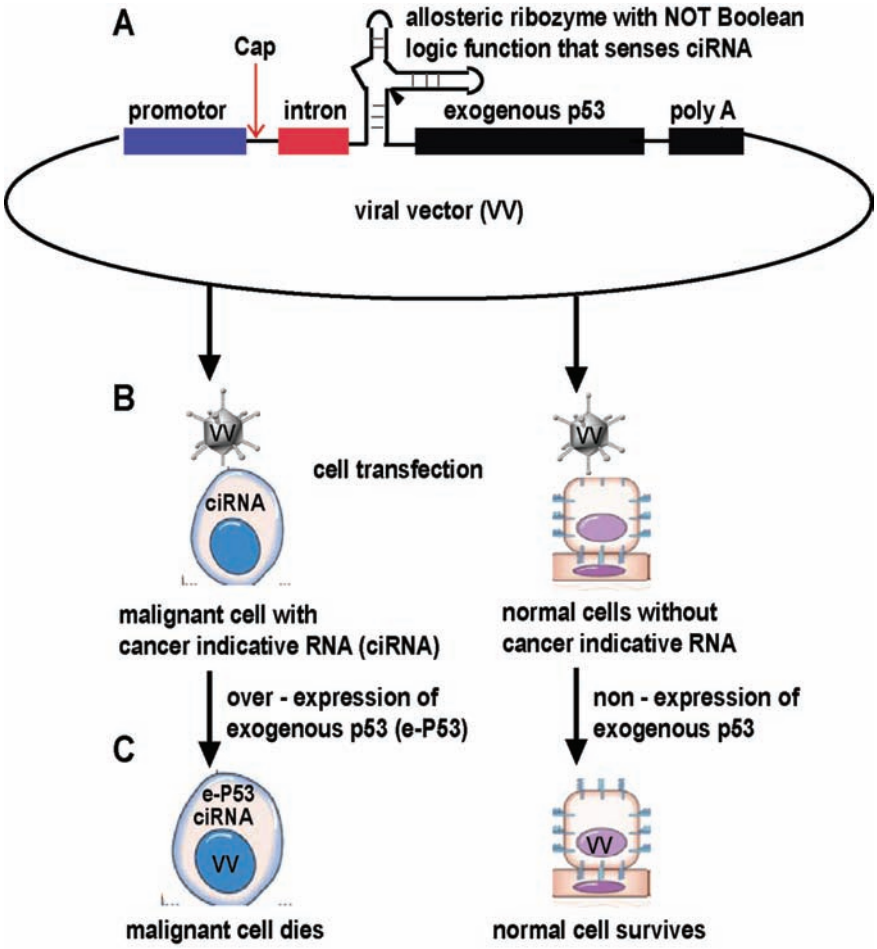
Applications of computationally designed allosteric ribozymes as synthetic riboswitches can open new avenues to the development of universal strategies for exogenous control of gene expression and re-programming the cell fate. These novel molecular bioengineering methods may have many practical applications to nanobiotechnology and pharmaceutical industry in the near future.

At the first place, the oligonucleotide-sensing allosteric ribozymes can be employed as synthetic riboswitches that control gene expression of key exogenous proteins. The approach can be used as a universal tool for reprogramming the cell fate by inducing apoptosis through conditional expression of exogenous proteins such as p53, PUMA, and many others. This general approach may have many far-reaching applications to the pharmaceutical and biotech industries including novel gene therapies of cancer.

A construct containing a p53 gene with a strong promotor under the control of RNA-sensing allosteric ribozymes can be delivered into a tumor tissue by a viral expression vector (Figure 7A). The viral vector should be chosen in regard to the tissue. In the common case, the viral vector should be non-integrating such as adenovirus, adeno-associated virus, herpes virus, and others. All cells, regardless tumor or normal, must be infected by the viral vector (Figure 7B). However, due to the function of the RNA-sensing ribozymes the exogenous p53 gene will be expressed in the tumor cells only because they carry cancer indicative(ci) RNAs (Figure 7C). Normal cells do not have ciRNAs and will not be able to express the exogenous p53 (Figure 7C). As a result, in the tumor cells the apoptosis is going to be induced, and they are going to die (Figure 7C). In contrast, the normal cells are going to survive the treatment (Figure 7C). This is all about the basic idea for curing any type of cancer by finding tools to kill all tumor cells while keeping alive most of the healthy cells. The implementation of this general idea with RNA-sensing allosteric ribozymes that work as synthetic riboswitches is explained in details below.

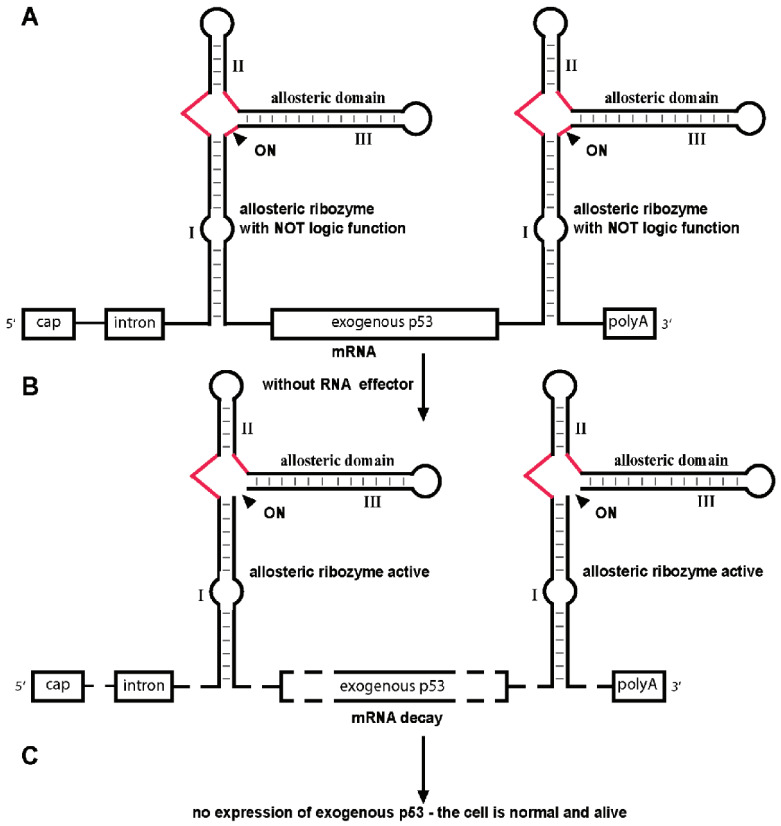
Let us assume that the pathological phenotype of a tumor cell is defined by the co-expression of

Figure 7. A general scheme for anticancer gene therapy based on RNA-sensing allosteric ribozymes



two ci mRNAs. A general anticancer gene therapy, depicted in Figures 8 and 9, can be employed to induce the apoptosis in all cancer cells while sparing the normal ones. The same scheme can be easily simplified for a single ciRNA sequence when the allosteric domains of both ribozymes are identical. A synthetic gene construct expresses a synthetic mRNA molecule that contains exogenous p53 and two allosteric ribozymes with a NOT logic function. The ribozymes are designed to sense the presence of two ciRNAs (Figure 8 and 9). The construct is delivered into the cell via a viral vector. If the cell is normal the two ciRNAs are not going to be expressed. As a result, both NOT riboswitches are going to fold into an active conformation in which all three stems I, II, and II are properly formed (Figure 8A). Therefore, the ribozymes will cleave themselves at both ends (Figure 8B). The exogenous mRNA will decay rapidly due to the absence of a cap structure at the 5'-end and a polyA tail at the 3'-end (Figure 8B). Thus, the exogenous p53 gene is not going to be expressed and a healthy cell will survive the treatment (Figure 8C). The opposite events will be happening in the tumor cells where both ciRNAs are present (Figure 9). The ciRNAs are going to hybridize with the allosteric domains of the ribozymes (Figure 9A). That will result in

Figure 8. Two synthetic riboswitches with a NOT logic function suppress the expression of exogenous p53 in the absence of tumor indicative RNAs



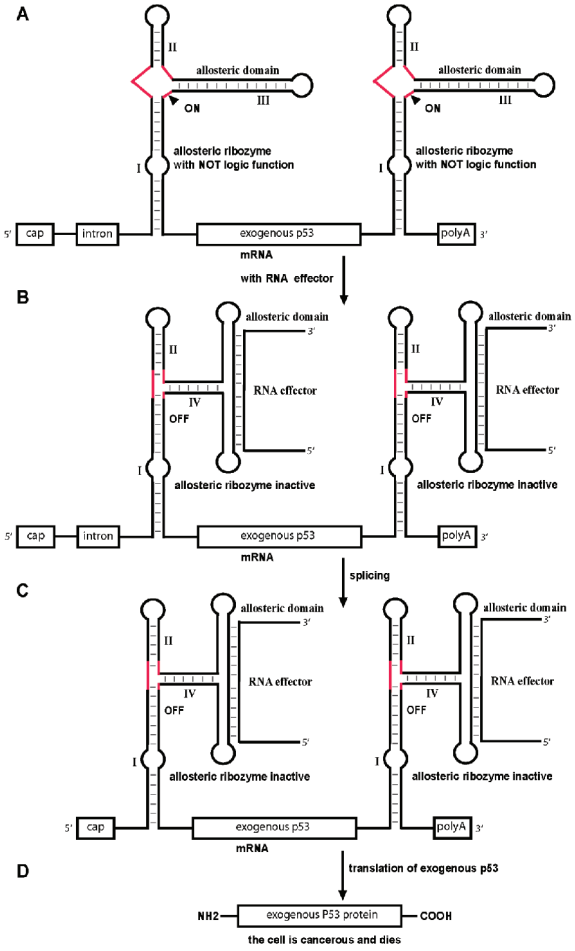
formation of stem IV instead of stem III inactivating the catalytic function of the ribozymes (Figure 9B). The lifetime of the exogenous mRNA will be close to normal. The synthetic p53 gene will be spliced (Figure 9C) and will be over-expressed. All that will result in induction of apoptosis in the tumor cells and their death (Figure 9D).

Note that the allosteric ribozymes can perfectly well distinguish effector oligonucleotides with only two mismatches out of 22 nt. That is sufficient to ensure ribozyme activation only in the presence of a specific effector oligonucleotide avoiding any unspecific activation by other RNAs in the cell. The described anticancer therapy can be potentially applied to many cancer types and other disorders.

The p53 is not the only protein that has to be over-expressed to induce apoptosis. For instance, it is observed selection against PUMA gene expression in Myc-driven B-Cell lymphoma genesis. This means that exogenous over-expression of PUMA in these cells may be more suitable than that of p53 (Garrison et al., 2008). Moreover, selective over-expression of exogenous Arf in some lymphoma cells can lead to suppression of Myc-driven lymphoma genesis (Bouchard et al., 2007).

The proposed therapy is universal since the ribozymes can be designed to sense the presence of any sufficiently expressed RNA(s) and can control the expression of any exogenous gene. This makes the method very adaptive in address-

Figure 9. Two synthetic riboswitches with a NOT logic function induce the expression of exogenous p53 in the presence of tumor indicative RNAs



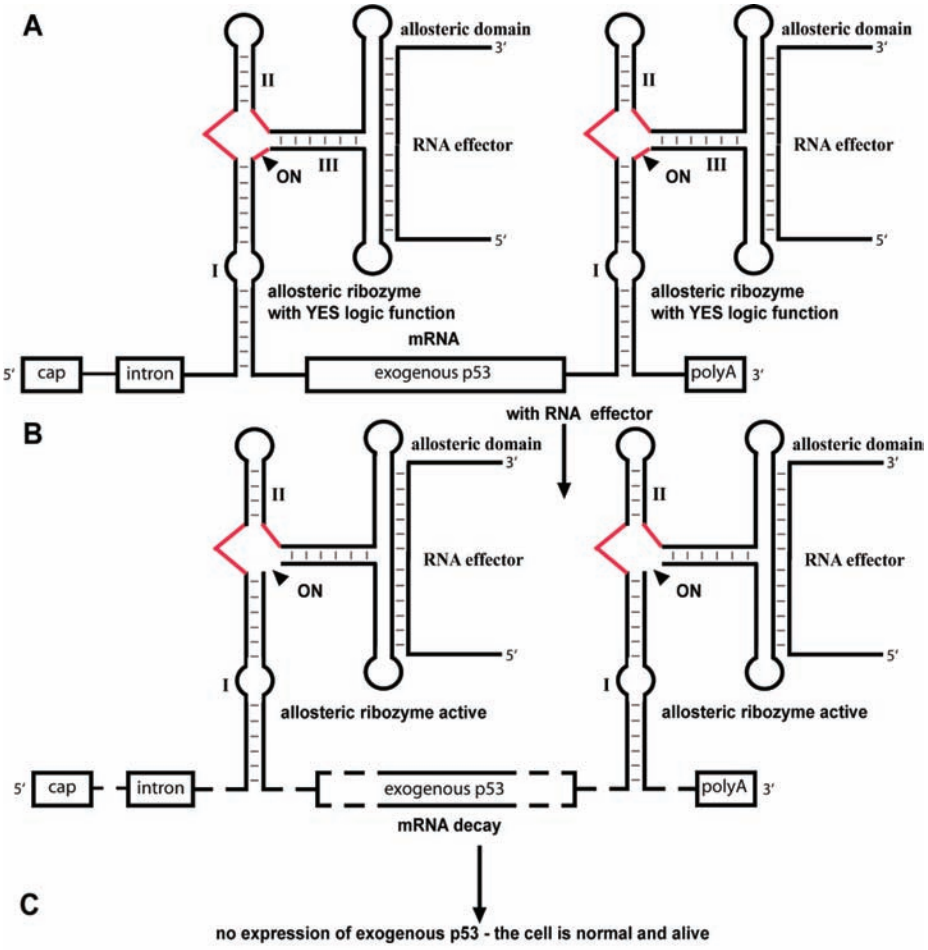
ing various disorders. Thus, the proposed strategy for exogenous control of gene expression can be applied in many regulatory circuits *in vivo*. For instance, one of the promising real-world applications would be addressing some forms of cancer such as HCC and MCL because we can transfect liver and B-cells *in vivo* with high efficiency maintaining constant viral expression for at least two weeks. In addition, there are ciRNAs that are over-expressed during the early stage of HCC and MCL development as detailed in the introduction.

In some types of cancer and other disorders, specific ciRNAs are no expressed. This is the opposite case in which health indicative(hi) RNAs are suppressed. In the general case, cell growth suppressor genes can be inhibited in some tumors. In such cases, a p53 gene (or another synthetic gene) has to be expressed only in the absence of hiRNAs. For this aim, riboswitches with a YES Boolean logic function (Figure 6) have to be employed instead of NOT gates (Figure 4) as depicted in Figures 10 and 11. A synthetic p53 gene under the control of two riboswitches with a YES logic function is delivered into the cell via a viral vector (Figure 7). If the transfected cell is normal hiRNAs are expressed (Figure 10). The hiRNAs hybridize with allosteric domains of the ribozymes. As a result, the YES gates are turned on (Figure 10A). They undergo a self-cleavage (Figure 10B). Therefore, the synthetic mRNA is rapidly decayed and the exogenous p53 is not expressed. The healthy cell survives the treatment. In contrast, the tumor cell does not have the two hiRNAs (Figure 11). As a result, both riboswitches are inactive since stem IV is formed instead of stem III (Figure 11A). Therefore, the exogenous mRNA has a normal lifetime. The p53 mRNA is spliced (Figure 11B) and translated into the exogenous p53 protein. As a result, the tumor cell undergoes apoptosis and dies.

Both methods for exogenous control of gene expression described above can have countless concrete applications to many different disorders, since they are truly universal in terms of effector RNAs and exogenous proteins controlled. The general approaches described in this section have the potential to be adopted for addressing many human disorders that have no efficient treatment up to date. The designer ribozymes discussed here offer new efficient tools for re-programming the cell fate. They are easily to design and to test experimentally and can be widely employed as cellular biosensors for building gene expression control circuits in any species of interest that can be transfected by an expression vector.

Small molecule-sensing allosteric ribozymes (Figure 5) can be also employed as synthetic ribo-

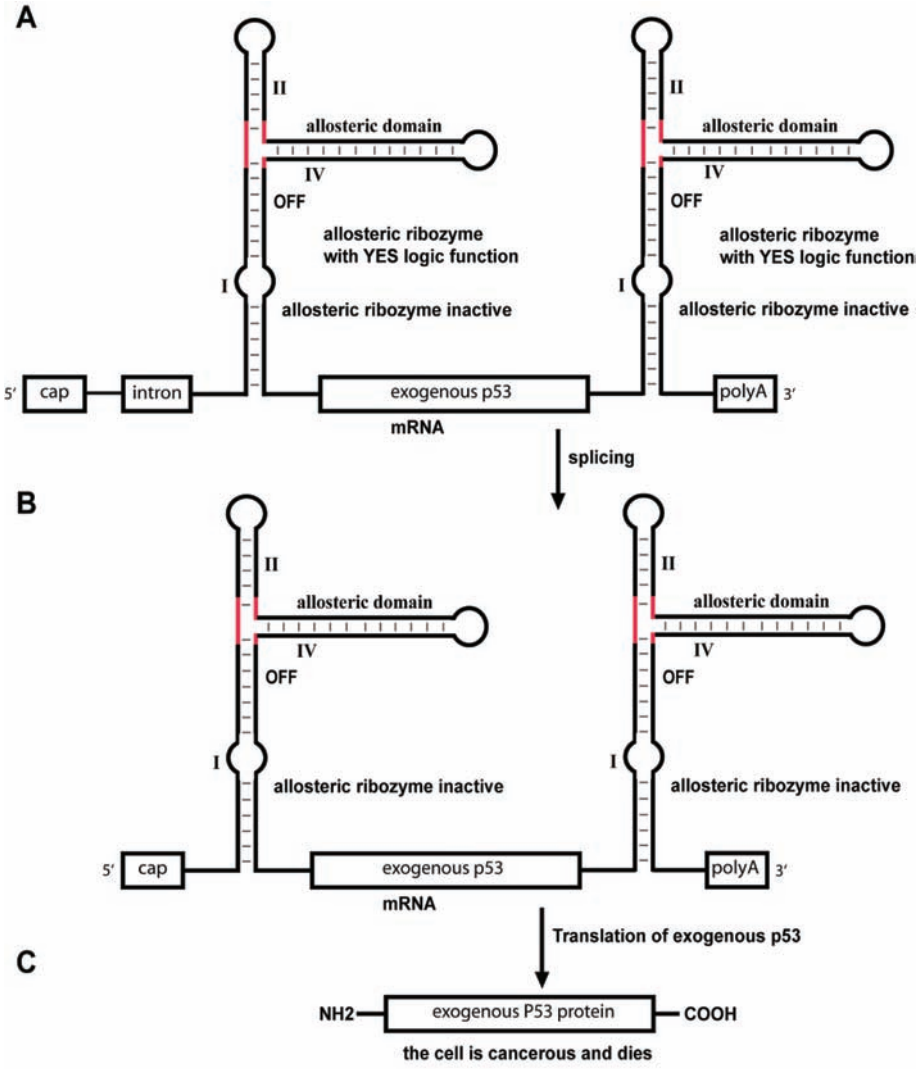
Figure 10. Synthetic riboswitches with a YES logic function suppresses the expression of exogenous p53 in the presence of health indicative RNAs



switches for exogenous control of gene expression as the same methods can be applied like in the cases with oligonucleotide-sensing ribozymes (Figures 8-11). Small molecule-sensing allosteric ribozymes may have useful applications in stem cell research. Instead of controlling expression of exogenous p53, cell growth and differentiation protein factors can be put under the control of synthetic riboswitches. Stem cell differentiation can be triggered by a drug that specifically binds to a synthetic riboswitch in the cell and turn on the expression of key proteins.

Another wide-spread application of small molecule-sensing ribozymes would be for tackling certain chronic diseases, which require a long term recapitulative treatment. For instance, some diabetic patients need to take insulin when their blood sugar's level rises too high. At the same time, they have to be very careful not to take too much insulin because the low level of the blood sugar is a life threatening condition. Therefore,

Figure 11. Synthetic riboswitches with a YES logic function induce the expression of exogenous p53 in the absence of health indicative RNAs



an exogenous insulin gene can be put in the cell under the control of a glucose-sensing synthetic riboswitch with a YES Boolean logic function like shown in Figures 10 and 11. This approach, however, is technically much more complicated than the cases with the oligonucleotide-sensing ribozymes. The main reason for the complication concerns an additional step needed for *in vitro* selection of a glucose-binding RNA aptamer that has to be successfully accomplished previous to the design of a glucose-sensing ribozyme. *In vitro* selection methods have been proven to be efficient for obtaining many RNA aptamers that selectively bind different small molecules. Therefore, despite the additional efforts needed for the *in vitro* selection of a glucose-binding

RNA aptamers that research maybe very helpful for many diabetic patients worldwide.

Conditional Production of Short-Hairpin RNAs by Allosteric Ribozymes with YES Logic Function for Gene Down Regulation through RNA Interfering and microRNA *Pathways*

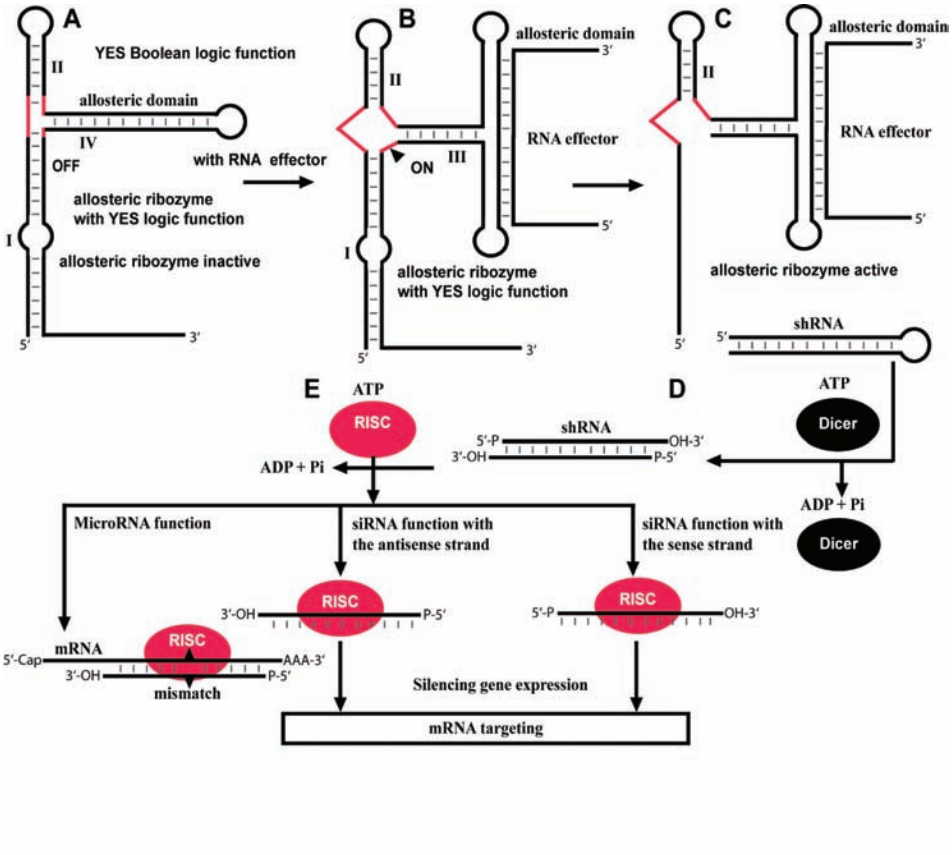
The discovery of RNA interfering (RNAi) and microRNA pathways in mammalians including humans raises many hopes for the deployment of novel and efficient tools for gene therapy of viral infections and other wide-spread diseases such as cancer. That can be achieved by specific targeting of key for the disease' development mRNAs or viral RNAs through microRNA and RNA silencing pathways (Dorsett & Tuschl, 2004). Despite the results achieved in the expression of shRNAs in mammalian cells and the RNA silencing effect demonstrated, there are many side effects that spell significant problems for the application of RNAi technology in the pharmaceutical industry. A major problem for the efficient retroviral RNAs silencing, including HIV, is the fact that viral RNAs tend to mutate very fast under the selection pressure of constantly expressed interfering RNAs. As a result, after certain viral generation the specific interfering RNAs are not anymore effective against the virus RNA. For constantly expressed interfering RNAs strong silencing effect of many non-target mRNAs is often observed.

To avoid these problems, interfering RNAs like shRNAs should be temporarily expressed only when needed. That can be achieved by applying RNA-sensing allosteric ribozymes with a YES logic function as depicted in Figure 12. An allosteric ribozyme with YES logic function can be constantly expressed into the cell using viral expression vectors. Without effector RNAs the YES gate (Figure 4) folds into the inactive state in which stem IV is formed instead of stem III (Figure 12A). In this conformation, the ribozyme's self-cleavage is inhibited. An effector RNA strand with a predefined sequence can bind to the ribozyme's allosteric domain and can activate the ribozyme by making stem III instead of stem IV (Figure 4 & 12B). As a result, the ribozyme's self-cleavage can produce a short-hairpin(sh) RNA Figure 12C. The shRNA can be converted in the cell into double-stranded(ds) RNA with sticky ends by the Dicer enzyme Figure 12D. The dsRNA will be made single-stranded by the RISC complex. The ssRNA in the RISC complex will be used as a template to degrade targeted mRNA or for microRNA to translational repression (Figure 12E). When the effector RNAs, that can be viral and disease indicative RNAs, are not present anymore the ribozyme will form inactive state again (Figure 12A) and shRNA production will cease. The restricted time of shRNA production will not allow the targeted viral RNA to mutate under the selection pressure of the shRNA and to escape the silencing effect. In addition, it will be avoided long term non-specific silencing for many mRNAs. Therefore, the conditional expression of shRNA can reduce the side-effects of RNA interfering technology to a minimum making it more feasible to tackle various disorders such as viral infection diseases including AIDS.

CONCLUSION

The procedure for fully computerized design of allosteric ribozymes offers completely new opportunities for an efficient production of high-precision biosensors with many *in vitro* and *in vivo* applications to nanobiotechnology, pharmaceutical and biotech industries. Perhaps the most promising application of the computationally designed allosteric ribozymes is for exogenous control of gene expression either in cell lines or *in vivo*. The ribozymes can be designed to sense the presence or the absence of any significantly expressed RNA in the cell. In addition, they can be engineered to sense the presence of small molecules. Allosteric ribozymes can be delivered in the cell with a viral vector. They can be used

Figure 12. A scheme for producing short-hairpin (sh) RNAs for siRNA and microRNA gene targeting by allosteric ribozymes with a YES Boolean logic function



as synthetic riboswitches for the control of gene expression of any exogenous protein. This approach can be applied for re-programming the cell fate by over-expression of key regulatory proteins such as p53, PUMA, and many others.

This approach for re-programming the cell fate may be used as a comprehensive therapy of many human disorders including many forms of cancer. There are only two preconditions for the execution of the proposed therapy. The first precondition concerns the necessity for the presence of cancer indicative RNA(s). There are many forms of cancer such as MCL and HCC that are clearly associated with over-expression of certain RNAs. Moreover, application of HGS and DNA microarray technologies enables researchers to discover easily new RNAs that are indicative of a particular type of cancer. The second precondition concerns the existence of viral vectors that can efficiently deliver in the cell a exogenous gene under the control of synthetic riboswitches. There are many human viral vectors such as herpes vectors that can transfect human cells *in vivo* with high-efficiency. At the same time, they are not toxic for humans since all pathogenic viral genes are being excluded from the viral sequence. The field of gene therapy is rapidly improving and offering new more efficient and safe human viral vectors despite some setbacks in the past.

If both preconditions are fulfilled for a particular type of cancer, the synthetic riboswitches can be employed as a cure after preclinical and clinical trails. Note the difference between the method proposed here and many already tested methods

in various clinical trails based on over-expression of exogenous p53 in tumor cells. In all previous methods, the exogenous p53 gene was delivered and over-expressed in any cell of the targeted tissue regardless, cancerous or normal (Lang et al., 2003; Rakozy et al., 1999; Weber & Wenz, 2002). As a result, apoptosis was induced not only in tumor but also in normal cells. To reduce the toxicity for the normal cells the approach was modified by applying viral vectors that transfect tumor cells with higher efficiency than normal cells. However, these approaches are found to be not good enough at distinguishing between tumor and normal cells. When the viral titer was high enough to transfect most of the tumor cells many normal cells were found to be also transfected. Unfortunately, when the viral titer was low enough to transfect a few normal cells; it was not sufficient to transfect most of the tumor cells.

The obvious defect of using exogenous p53 for anticancer gene therapy is resolved by the proposed method based on RNA-sensing synthetic riboswitches. A high-viral titer can be used for transfecting all tumor cells as well as few normal cells. This will not be toxic for the normal cells since exogenous p53 will be over-expressed mainly if not only in the tumor cells.

Allosteric ribozymes can be also designed to produce conditionally shRNAs in the cell inducing RNAi and microRNA pathways. This can be used as an antiviral tool. Besides, the approach can be employed for conditional down regulation of any endogenous gene.

In general, the proposed methods can be employed as universal tools for re-programming the cell fate both *in vitro* and *in vivo*. There are still experiments *in vivo* including clinical trails to be done to cure a particular disorder in humans. However, all essential steps in a comprehensive drug development strategy are clearly laid out and the chances for new efficient drug development must be taken especially in the cases without any cure available.

Small molecule-sensing ribozymes can be also used for exogenous control of gene expression in a similar way like oligonucleotide-sensing ribozyme (Win & Smolke, 2008). The exogenous gene can be induced by small molecule drugs such as theophylline and tetracycline. This approach, however, has to overcome more hurdles than that of the RNA-sensing ribozyme. The small molecule-sensing ribozymes have a much smaller dynamic range of few hundred fold (Link et al., 2007) than that of the RNA-sensing ribozymes with a dynamic range of several thousand fold (Penchovsky & Breaker, 2005) in their activation. This makes the exogenous control of gene expression by RNA-sensing ribozyme much more robust than that of small molecule-sensing ribozymes. This problem may be overcame in some degree by using the computational design procedure for producing small molecule-sensing ribozyme like this for oligonucleotide-sensing ribozymes (Penchovsky & Breaker, 2005). In addition, there are only two aptamers, for theophylline and tetracycline, can be used *in vivo*. Both small molecule drugs have a lot of side effects. In contrast, oligonucleotide-sensing ribozyme can be designed to sense the presence of any RNA sequence, which makes them universal biosensors with a wide range of applications. However, small molecule-sensing ribozymes can be very well suited for stem cell applications. Stem cells can be made to start certain differentiation after exposure to a drug using small molecule-sensing synthetic riboswitches. Such approach may advance the field of stem cell therapy and may have many practical applications to various stem cell therapies.

Apart from being used as synthetic riboswitches in the cell, the ribozymes presented have many applications as biosensors *in vitro*, which will be discussed elsewhere. Engineering gene regulatory circuits in the cell based on computationally designed allosteric ribozymes can have a profound impact to the fields of applied synthetic biology (Isaacs, Dwyer, & Collins, 2006), drug devolve-

ment (R. R. Breaker, 2005; Eisenstein, 2005), and nanobiotechnology (Margolin & M.N., 2005).

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KEY TERMS AND DEFINITIONS

Allosteric Ribozymes: Are engineered functional ribonucleic acid (RNA) molecules. Their catalytic function is modulated by effector molecules. The allosteric domain is distant from the catalytic center of the ribozyme.

Apoptosis: A set of many different biochemical pathways in the cell. When induced, it can lead to cell death.

Computational Design of Allosteric Ribozymes: A computerized approach that employs different algorithms to find RNA sequences of allosteric ribozymes with desired properties by testing a large pool of random sequences.

Gene Therapy: is a set of methods for curing different diseases by employing viral vectors.

Exogenous Control of Gene Expression: An engineering approach for a regulation of synthetic genes.

In Vitro Selection: A set of different biochemical methods used for obtaining RNA, DNA, and polypeptides with desired properties by repeatedly applying amplification, mutation, and selection steps over an initial pool with random sequences.

Riboswitches: RNA molecules that control gene expression by directly binding effector molecules. Natural riboswitches are discovered in bacterial and plant species. They are usually found in the untranslated regions of mRNAs. Synthetic riboswitches can be designed to control expression of synthetic genes.

Ribozymes: are functional RNA molecules that can catalyze specific biochemical reactions such as self-cleavage, self-splicing, and others. They are found in plants and animals. They can accelerate the rate of specific biochemical reactions by 10^{11} fold. They have conserved secondary and tertiary structures.

RNA Aptamers: Are structured molecules that can bind to a specific ligand. RNA aptamers are usually engineered by an *in vitro* selection procedure. There are naturally occurring aptamers in bacterial and plant species as a part of a riboswitch.

Section 3

Medical Treatment and Research:
Ethics and Applications

Chapter 6
Ethical Guidelines for the Quality Assessment of Healthcare

Amit Chattopadhyay
National Institutes of Health (NIDCR), USA

Khushdeep Malhotra
National Institutes of Health (NIDCR), USA

Sharmila Chatterjee
George Washington University, USA

ABSTRACT

One of the keystone concepts of healthcare improvement is the belief that informed choices will lead to enhanced quality of life and healthcare. Medical Ethics, or Bioethics, is the study of moral issues in the fields of medical treatment and research. It is also used to describe ethical issues in the life sciences and the distribution of scarce medical resources. This chapter will review and describe the principles of ethics and discuss how ethical principles can be used as guidelines for the quality assessment of healthcare provision. It will also discuss areas such as: ethical handling of information, patient safety, communication, obligation for impartial quality assessment, private health information protection, ethical committees and supervision authorities, competence of the assessor, supervision of ethical guidelines for health quality assessment, research and publication ethics, and global ethics of healthcare. Another goal of the manuscript will be to serve as a central reference to access of information about resources related to this topic.

INTRODUCTION

As a branch of Philosophy, Ethics guides the leading of a good life. Ethics also guides moral conduct in life. Applied Ethics, on the other hand, is a discipline that guides application of ethical theory to real-life situations. Medical Ethics or Bioethics, studies the role of value judgments and "moral issues in the fields of medical treatment and research. The term is also sometimes used more generally to describe ethical issues in the life sciences and the distribution of scarce medi-

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cal resources. The professional fields that deal with ethical issues in medicine include medicine, nursing, law, sociology, philosophy, and theology, though today medical ethics is also recognized as its own discipline" (McGee & Caplan, 2007). Over time, Bioethics has grown as a discipline and its practitioners play important roles in clinical decision making, research studies, legislature, professional organizations, and community activities; in academia, government, non-governmental organizations and industry. Events that have shaped the development of Bioethics as a discipline over time are outlined in Table 1.

One of the major applications of applied ethics in healthcare is to provide guidelines for assuring quality in healthcare through its assessment. Healthcare quality encompasses a variety of issues, each with ethical implications; therefore ethical assessment issues will as well encompass several issues from a variety of angles. The goal of quality assessment in health care is continuous improvement of the quality of services provided for patients and populations and of the ways and means to produce these services (Chattopadhyay, 2009).

Although several ethical principles have been defined (in addition to the main principles) such as: Trusts in relationships; Veracity; Fidelity; Avoidance of killing; Gratitude; and Reparation, that are very important in healthcare action, decision making, quality assurance and policy making. The four main principles of Bioethics are: Autonomy, Beneficence, Non-maleficence, and Justice. These principles and their applications are outlined in Figure 1.

Table 1. Developments in Bioethics – A timeline

Year	Development	Resource for Further Reading
1947	The Nuremberg Code	http://ohsr.od.nih.gov/guidelines/nuremberg.html
1964	The Declaration of Helsinki	http://ohsr.od.nih.gov/guidelines/helsinki.html
1966	Animal Welfare Act	http://www.aphis.usda.gov/animal_welfare/awa.shtml
1974	National Research Act	http://www.hhs.gov/ohrp/irb/irb_introduction.htm
1979	The Belmont Report	http://ohsr.od.nih.gov/guidelines/belmont.html)
2009 (update)	US Code of Federal Regulations Title 45 Public Welfare Part 46 Protection of Human Subjects	http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm
1995	National Bioethics Advisory Commission- Ethical and Policy Issues in International Research: Clinical Trials in Developing Countries	http://bioethics.georgetown.edu/nbac/human/overvol1.html
1997	International Conference on Harmonization: Guidance Documents	http://www.fda.gov/regulatoryinformation/guidances/ucm122049.htm
2002	Council for International Organizations of Medical Sciences (CIOMS) guidelines	http://www.cioms.ch/publications/layout_guide2002.pdf
2002	Nuffield Council on Bioethics- The Ethics of Research Related to Healthcare in Developing Countries	http://www.nuffieldbioethics.org/fileLibrary/pdf/errhdc_fullreport001.pdf
2003	HIV Trials Prevention Network- Ethics Guidance for Research	http://www.hptn.org/web%20documents/EWG/HPTNEthicsGuidanceFINAL-15April2003.pdf)

CHALLENGES TO QUALITY OF HEALTHCARE

Healthcare systems across the world generally do not provide consistent, high quality medical care to all people in respective nation states. Understanding of disease process, various determinants of disease and advancements in available treatments, medical technology, shifts and drifts in disease patterns along with emerging and re-emerging infections across the world have made the response to health challenges more complex and have modified the needs of public health; especially prevention of diseases which has become a major challenge. The nature of this change was captured well by the Institute Of Medicine's (IOM) report: Crossing The Quality Chasm: A New Health System For The 21st Century in the United

Figure 1. Principles of Bioethics and their application

The Principles of Ethics

- Respect for persons
  - Autonomy
    - Informed consent
  - Protection of vulnerable populations*
  - Respect for communities
- Non-maleficence
- Beneficence
  - Maximization of possible benefits
  - Minimization of possible harms
  - Benefits for communities
- Justice
  - The Impartiality Principle
  - The Well-Being Principle
  - The Equal Chance Principle
  - The Importance Principle
  - The Substantial Difference Principle
  - The Fairness Principle

(Adapted from: Chattopadhyay, Oral Health Epidemiology Principles and Practice)

Applications of the Principles of Ethics

When applying the general principles of ethics to the conduct of research, the following elements need to be considered:

Informed consent

- Elements of informed consent
  - Detailed description of study including (but not limited to)**
    - Study objectives
    - Duration of the study
    - Expected participant responsibilities
    - Description of involved procedures
    - Use of placebos
  - Description of risks involved
    - Nature of risk
    - Impacts of risk
  - Description of benefits
    - Nature of benefits
    - Impacts of benefits
    - Duration of benefits
  - Alternatives

States, a report that is perhaps valid for the whole world. The Healthcare delivery system has fallen far short in its ability to translate knowledge into practice and to apply new technology safely and appropriately. There exists a large gap between the quality of the current healthcare and the quality of healthcare that can be achieved even under the current socio-economic-medical-technological environment.

A number of factors have combined to create this chasm. Medical science and technology have advanced at an unprecedented rate during the past half-century. In tandem has come growing complexity of health care, which today is characterized by more to know, more to do, more to manage, more to watch, and more people involved than ever before. Faced with such rapid changes, the nation's health care delivery system has fallen far short in its ability to translate knowledge into practice and to apply new technology safely and appropriately. And if the system cannot consistently deliver today's science and technology, it is even less prepared to respond to the extraordinary advances that surely will emerge during the coming decades.

The public's health care needs have changed as well. Americans are living longer, due at least in part to advances in medical science and technology, and with this aging population comes an increase in the incidence and prevalence of chronic conditions. Such conditions, including heart disease, diabetes, and asthma, are now the leading cause of illness, disability, and death. But today's health system remains overly devoted to dealing with acute, episodic care needs. There is a dearth of clinical programs with the multidisciplinary infrastructure required to provide the full complement of services needed by people with common chronic conditions.

The health care delivery system also is poorly organized to meet the challenges at hand. The delivery of care often is overly complex and uncoordinated, requiring steps and patient "handoffs" that slow down care and decrease rather than improve safety. These cumbersome processes waste resources; leave unaccountable voids in coverage; lead to loss of information; and fail to build on the strengths of all health professionals involved to ensure that care is appropriate, timely, and safe. Organizational problems are particularly apparent regarding chronic conditions. The fact that more than 40 percent of people with chronic conditions have more than one such condition argues strongly for more sophisticated mechanisms to coordinate care. Yet health care organizations, hospitals, and physician groups typically operate as separate "silos," acting without the benefit of complete information about the patient's condition, medical history, services provided in other settings, or medications provided by other clinicians (IOM, 2001).

GLOBAL HEALTHCARE AND ETHICS

Despite much discussion, biomedical research and healthcare quality, administration and assessment in many developing countries remains poorly regulated. This is attributed to the lack of a well defined and uniform set of ethical regulations/ guidelines (Chattopadhyay, 2009). The purpose of setting up ethical guidelines is to strengthen the pursuit of improvement of quality of healthcare by means of quality assessment practices and to create ethical grounds for audit. The greatest challenge in adopting uniform global ethical standards stems from inequalities in resource and wealth distribution across populations throughout the world. Additionally, the role of social determinants of health plays a larger role in determining the health of populations compared

to individual medical treatments. The inertia associated with social forces is also greater, and so is the potential for large scale impacts, making it necessary to develop strong ethical guidelines to harness these forces aiming to improve health of populations globally. Current guidelines on global ethical standards address ethics as applied to the individual, rather than in the larger social context. Singer & Benatar (2001) argue that unwarranted dependence on international declarations and even revisions to ethical codes like the Declaration of Helsinki will hardly result in research becoming more ethical throughout the world unless ethical capacity is truly strengthened. We believe that the same applies to improvement of healthcare quality too.

Developing global ethical standards that work in impacting healthcare quality, will require defining a reasonable 'standard of care' (SOC) for practice and research in developing countries, redefining concepts such as fair benefits and ancillary care, encouraging community engagement and using these concepts to improve health care and foster social justice through research (Benatar & Singer, 2010). Ultimately, to overcome these challenges, the idea of international research ethics and practical global ethics will need to be broadened "to include the ethics of how institutions operate and interact, the ethics of public health and social and economic rights (based on concern for equity) and the ethics of international relations (based on solidarity) that affect whole populations" (Benatar & Singer, 2010). Furthermore, efforts to achieve the ethical goals of "in an interdependent world, where the poorest suffer most from systemic forces adversely affecting health, will need to be underpinned by promotion and achievement of solidarity with all as global citizens, and political efforts to move towards new paradigms of thinking and action that could begin to narrow widening economic disparities that threaten both our humanity and global security" (Benatar & Singer, 2010).

Distribution of Scarce Health Resources

In general, resources are always scarce compared to the demand for resources. Allocation of scarce resources to address healthcare quality issues can become the source of a major ethical vexation globally. Most health economists agree that some kind of public preferences should play a major role in setting criteria for distributing scarce resources.

A free-market resource allocation strategy would argue in favor of adopting a distribution strategy that maximizes financial efficiency and utilization of resources by "free will" of the users. The quality-adjusted life year (QALY) is used as a preference-based measure for the outcome of health-care activities in health economic evaluative studies (Schwappach, 2002). However, a "social worth" perspective strategy would lean in favor of a distribution of resources based on social worth/ social utility of the end-user; whereas a philanthropic based perspective would endorse charity-based resource allocation. "Social value" may be derived out of the characteristics of patients and/or factors related to the characteristics of the intervention's effect on patients' health.

Sagev (2005) proposed a set of potential guidelines that may be used for resource allocation (see below). These guidelines were based on two premises: 1. That individual well-being is the fundamental value that should guide allocation; and 2. That "interpersonal conflicts affecting well-being should be resolved in light of several conceptions of fairness, reflecting the independent value of persons and the moral significance of responsibility of individuals for the existence of interpersonal conflicts". The general principles outlined by Sagev (2005) are:

1. **The Impartiality Principle:** reasons for action are agent-neutral, rather than agent-relative, apply to all agents equally; particularly, reasons for actions should be evaluated from an impartial perspective, rather from an agent-relative perspective that accords special weight to the personal aspects of agents' life.
2. **The Well-Being Principle:** there is a reason to protect and enhance the well-being of persons.
3. **The Equal Chance Principle:** in resolving interpersonal conflicts of well-being, there is a reason to accord equal weight to the well-being of each person, by following two hierarchical sub-principles (a) there is a reason to distribute benefits or inevitable costs between all persons equally, so that each would get the maximum possible (roughly) equal benefit or bear the minimum possible (roughly) equal loss - provided that the benefit or reduction of cost for each person is significant: or (b) when this is impossible, there is a reason to give each person the highest possible equal chance to be preferred.
4. **The Importance Principle:** the strength of the reason provided by the Well-Being Principle depends on the importance of the interest at stake and the conjectured probabilities concerning the possible effects of the considered action or inaction on it (positively or negatively). Assuming equal probabilities, the more important is the interest, the stronger is the reason to protect it. In resolving interpersonal conflicts, there is, therefore, a reason to prefer the person who would otherwise suffer the most severe harm or the person who could be benefitted most significantly.
5. **The Substantial Difference Principle:** the reason provided by the Importance Principle prevails over the reason provided by the Equal chance Principle if, assuming that all relevant probabilities are equal, there is a substantial gap in the importance of the competing interests.
6. **The Principle of Fairness:** Responsibility: when an interpersonal conflict requires a choice between the well-being of individuals, there is a reason to prefer a person who is not responsible for the existence of the conflict to a person who is and a person who is less responsible to a person who is more responsible."

Distribution of Scarce Medical Interventions

Allocation of very scarce medical interventions such as organs and vaccines is a persistent ethical challenge all over the world and is becoming an important issue as globalization and medical tourism increases in scope. Although several principles and strategies have been developed to allocate medical interventions, for example: treating people equally, favoring the worst-off, maximizing total benefits, and promoting and rewarding social usefulness; "no single principle is sufficient to incorporate all morally relevant considerations and therefore individual principles must be combined into multi-principle allocation systems" (Persad et al., 2009).

Simple principles may include: 1. Treating people equally (random allocation or first come first serve); 2. Favoring the worst-off: prioritarianism (sickest first/ youngest first); 3. Maximizing total benefits: utilitarianism (Number of lives saved/ prognosis or life-years saved); and 4. Promoting and rewarding social usefulness (instrumental value/ reciprocity). Multi-principle systems have also been used such as; 1. UNOS points systems for organ allocation in the USA (includes First-come, first-served; sickest-first; prognosis); 2. QALY allocation (includes Prognosis; excludes save the most lives; 3. DALY allocation (includes Prognosis; instrumental value; excludes save the

most lives); and 4. Complete lives system (includes Youngest-first; prognosis; save the most lives; lottery; instrumental value, but only in public health emergency) (Persad et al., 2009).

ETHICAL HANDLING OF INFORMATION AND PATIENT SAFETY

Ethical handling of health information includes privacy of the information and in order to improve patient safety, it implies that patients should have participatory role in their health information. Such a role requires certain attributes that guide collection and characterization of the information i.e. the patient should have:

- Capacity to understand the information;
- Consented (informed consent) to the information;
- Confidentiality assured about the information;
- Disclosure from the clinicians about use of the information;
- Veracity assured by the clinicians about information, procedures and tests; and
- Voluntariness to provide the information.

Patients should therefore be encouraged to ask questions if they have doubts or concerns; keep and bring a list of all the medicines they take; get the results (or copies) of any test or procedure conducted on them; talk to their contact clinician about which hospital or procedure would be best for their health needs; and to make sure that they understand what will happen if they need invasive tests or invasive treatment procedures such as surgery, chemotherapy etc. Even if patients fail to ask questions, all relevant fundamental and potentially important questions should be addressed by the clinician. For example, for a surgical procedure, the patients must be informed about: the exact nature of the procedure; its duration; post surgical expectations about recovery; how the patients may feel during recovery; and when they should contact the clinician; what they can eat and drink; any restrictions and alarming signs they should look for.

Collecting, processing and assessing information is a critical activity in (healthcare systems -) clinical practice, research and public health activities. Information flow is two-way process where the clinician/ researcher/ public health practitioner conveys information to the patient/ research participant and/or the public, and at the same time, assesses the responses to questions and dialogues that are generated. Several issues such as consent, capacity, disclosure, voluntary action, veracity of statements, and confidentiality of information being sought and disclosed require firm guidance to comply with ethical principles (Chattopadhyay, 2009) appropriate for conduct of research, clinical management and control of healthcare quality.

Private Health Information Protection

In the U.S., The Office for Civil Rights enforces the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule, which protects the privacy of individually identifiable health information; the HIPAA Security Rule, which sets national standards for the security of electronic protected health information; and the confidentiality provisions of the Patient Safety Rule, which protect identifiable information being used to analyze patient safety events and improve patient safety. The goal of ethical handling of information is to ensure participant protection, which is in direct compliance with the principles of ethics. In the United States, the "Privacy Rule," is a Federal regulation under the HIPAA of 1996 that protects certain health information. Most parties subject to the Privacy Rule were required to implement the Rule's standards and requirements by April

14, 2003. The Rule established a Federal floor of privacy protections for most individually identifiable health information by establishing conditions for its use and disclosure by certain health care providers, health plans, and health care clearinghouses. The Rule applies to all HIPAA-defined covered entities, regardless of the source of funding. Guidelines for participant protection are outlined in Figure 2.

HEALTHCARE QUALITY ASSESSMENT

The process of healthcare quality improvement and assessment has involved a host of government agencies and commissions, professional organizations, insurance underwriters, corporations, and more recently, market forces. Traditional approaches to quality control have stressed case-by-case analysis and identifying outliers, whereas newer approaches include creating practice guidelines and profiles of hospitals and physicians (Luce et al., 1994).

One of the keystone concepts of health care improvement in the United States is the belief that informed choices will lead to enhanced quality. Purchasers and the public will get better care if they can choose care that meets their needs and expectations. Health plans, clinicians, and institutions will provide better care to attract more patients, especially by appealing to the public's ability to choose and make decisions based on this information.

Figure 2. Ethical handling of Information

Guidelines for Participant Protection

- Ensuring scientific correctness
- Ensuring confidentiality
- Health Information Portability and Accountability Act (HIPAA) http://www.hhs.gov/ocr/privacy/
    - *Privacy rule of HIPAA*
        - Regulates the use and disclosure of *Protected Health Information (PHI)*by *covered entities*
            - *Covered entities* include
                - Healthcare clearinghouses
                - Health insurers
                - Employer sponsored health plans
                - Medical Service providers
- Ensuring informed consent
- Ensuring disclosure
  Information dispensed to the participant must be scientifically sound and complete. The patient must truthfully be informed about:

- Type of treatment
- Risks and benefits
- Alternative treatments
- Use of medications and placebo
- Standard of care
- Current state of evidence about the procedure
- Expected outcomes
- Ensuring veracity
  The researcher should be forthright with the participants about every aspect of the study. This will encourage confidence, good decision making and compliance on the part of the patient.
- Ensuring voluntariness of patient participation

(Adapted from: Chattopadhyay, 2009; Family Health International www.fhi.org)

Collecting this information on quality so that it is valid and reporting it so that it is useful present many methodologic and practical challenges. In addition, there are lingering doubts about the effect that performance information has on the choices made by the public and those who purchase health insurance on their behalf. Some also question whether quality improvement efforts undertaken by plans and providers will be designed in response to this information.

How, then, can quality measurement in health care transcend the evaluation of technical performance? What are those aspects of health care and health caring that matter to people, and how can they be measured (Eisenberg, 2001)?

Donabedian (1988, 2003) suggested three headings under which assessment of quality of care could be conducted: structure, process, and outcome. Although there is no clear separation between the three domains, they are linked in ways such that structure influences processes that influence outcomes. This linking may involve different probabilities with which they may impact outcomes. The three domains may include several factors such as those outlined in Figure 3. These domains are very helpful in quality assessment, for example, in diabetic care; the following attributes may be used in quality assessment.

Structure Attributes

Patient access to building, parking facilities, location and staffing of information desk, adequate computer resources and access, patient seating arrangement, clinical equipment procurements and maintenance, clinician rooms and their maintenance etc.

Figure 3. Components of Donabedian matrix for healthcare quality assessment (Adapted from Donabedian, 1988, 2003)

Structure

Structure relates to physical resources or organizational structures. These could be the major determinants of the quality of care. It may include factors such as:

- Material resources (infrastructural facilities and equipment);
- Human resources (number, variety, and qualifications of personnel);
- Organizational characteristics (kinds of supervision, performance review and methods of payment for services).

Process

The activities that constitute health care, such as diagnosis and treatment, usually carried out by professional personnel but also by patients and family. Process measures have the ability to identify smaller variations in quality and may relate closely to immediate, indications of qualify and it is easy to obtain information about processes.

Outcome

Outcome refers to the changes in individuals attributable to the care they received. For example:

- Changes in health status of the recipient of care;
- Changes in behavior of patients and family members, and changes in knowledge acquired by them;
- Satisfaction of patients and family members.

Process Attributes

Training for physician, non-physician clinical and non-clinical staff, Expert team visits, adequate computer software utilization, and training for its use, data recording and data management, information given to patients, confirming, scheduling and re-scheduling of appointments, notifying patients and clinicians about any changes in plans, establishing protocols for adequate communication and interaction with laboratory for following up on tests, and their results and their reporting, hygiene and cleanliness of equipment, rooms and other quality control issues etc.

Outcome Attributes

Clinical Outcomes: Effective glycemic control, increased rates of retinal screening, increased rates of foot-care examination and education, increased rates for testing for microalbuminurea, increased testing for HbA1c, and reduced HbA1c levels, Laboratory test follow ups, clinical follow ups, patient re-visit rates, return rates, patient satisfaction through interviews/surveys, physician, non-physician clinical and other staff satisfaction.

Ethical guidelines to assess healthcare quality must also ensure that structure, processes and appropriate outcomes are correctly assessed when evaluating healthcare quality. Figure 4 outlines the possible ethical guidelines check list that can be used by Ethics Boards for the quality assessment of health care

Healthcare Quality Improvement

Healthcare quality improvement has been defined by Batalden & Davidoff (2007) as "the combined and unceasing efforts of everyone—healthcare professionals, patients and their families, researchers, payers, planners and educators— to make the changes that will lead to better patient outcomes (health), better system performance (care) and better professional development (learning)".

The IOM (2001) suggested six goals that healthcare systems should adopt to guide their gains to "far better at meeting patient needs", emphasizing that "the health care system must be responsive at all times, and access to care should be provided over the Internet, by telephone, and by other means in addition to in person visits". These six aims for improvement of healthcare quality are that the healthcare systems should be:

- Safe (i.e. avoid injuries to patients from the care that is intended to help them);
- Effective: (i.e. provide services based on scientific knowledge to all who could ben-

Figure 4. Ethical guidelines for the quality assessment of health care

- Purpose of the guidelines
- Application of the guidelines
- Obligation for quality assessment
- Preconditions for good quality work and its assessment
- Realization of assessment
- Secrecy of patient records
- Confidentiality of assessment
- Establishment ethical committees
- Competence of the assessor
- Impartiality of the assessment
- Assessment and supervision by authorities

efit, and refrain from providing services to those not likely to benefit);
- Patient-centered (i.e. provide care that is respectful of and responsive to individual patient preferences, needs, and values, and ensure that patient values guide all clinical decisions);
- Timely (i.e. reduce waits and sometimes harmful delays for both those who receive and those who give care);
- Efficient (i.e. avoid waste, including waste of equipment, supplies, ideas, and energy); and
- Equitable: (i.e. provide care that does not vary in quality because of personal characteristics such as gender, ethnicity, geographic location, and socioeconomic status).

Although it is not possible to specify a complete set of rules that would be necessarily useful of applicable universally, the IOM (2001) formulated a set of ten simple rules (or general principles) to inform efforts to redesign the health system to help improve healthcare quality. These ten "rules" state that:

1. Care should be based on continuous healing relationships.
2. Care should be customized according to patient needs and values.
3. The patient should be the source of control.
4. Knowledge should be shared and information should flow freely.
5. Decision making should be evidence-based.
6. Safety should be a system property.
7. Transparency should be necessary.
8. Needs should be anticipated.
9. Waste should be continuously decreased.
10. Cooperation among clinicians should be a priority.

Overall, reducing risk and ensuring safety require greater attention to systems that help prevent and mitigate errors. Whereas government funding support would be pivotal to improve healthcare quality, it is critical that leadership from the private sector, both professional and other health care leaders and consumer representatives, be involved in all aspects of this effort. Incorporation of advances in information technology and establishment of fair payment systems for compensating clinicians for good patient management should be the cornerstone for such efforts.

SUPERVISION OF ETHICAL GUIDELINES FOR HEALTHCARE QUALITY ASSESSMENT

Healthcare Quality Supervision

Healthcare quality improvement, at any level, may it be implemented (overall systems level, at a hospital level, or small practice level), should generally be supervised by a knowledgeable team. The supervision team should be cognizant of the knowledge systems involved in improvement, some characteristics of which was suggested by Batalden & Davidoff (2007). The knowledge systems should include:

1. Generalizable scientific evidence (i.e. controls and limits context as a variable; tests hypotheses)
2. Particular context awareness (i.e. characterizes the particular physical, social and cultural identity of local care settings e.g., their processes, habits and traditions)
3. Performance measurement (i.e. assesses the effect of changes by using study methods that preserve time as a variable, use balanced measures [range of perspectives, dimensions], analyze for patterns)

4. Plans for change (i.e. describes the variety of methods available for connecting evidence to particular contexts)
5. Execution of planned changes (i.e. provides insight into the strategic, operational and human resource realities of particular settings (drivers) that will make changes happen).

Supervision of ethical aspects of healthcare quality usually lies with the Ethics Board or institutional Review board of the local institutions. However, as more international tie-ups of hospital chains occur, and more international research gets conducted, the role of responses of different Ethics Boards at different sites has demonstrated major differences between how these Boards conduct their functions. Gold and Dewa (2005) examined the issue of whether the current system for ethics review of multisite health services research protocols is adequate, or whether there exist alternative methods that should be considered. They reported that: (1) Investigators at different sites in a multisite project often have very different experiences with respect to the requirements and requests of the review board. Other reported problems included the waste of time and resources spent on document preparation for review boards, and delays in the commencement of research activities. (2) "There are several possible reasons why there is variability in ethics review. These include the absence of standardized forms, differences in the background and experiences of board members, the influence of institutional or professional culture, and regional thinking. (3) Given the limited benefits derived from the variability in recommendations of multiple boards and the numerous problems encountered in seeking ethics approval from multiple boards suggest that some sort of reform is in order". Solutions to these problems could include: establishment of multisite Ethics Review Boards; standardization of documents and procedures; use of technology to facilitate ethics review; and education and certification for Ethics Review Board members. However, challenges to making these changes could include local Board power politics and increased feeling of competitiveness that could modify the interaction between the researcher and the Review Board.

The increasing number of multisite, health services research studies calls for a centralized system of ethics review. The local review model is simply not conducive to multisite studies, and jeopardizes the integrity of the research process. Centralized multisite review boards, together with standardized documents and procedure, electronic access to documentation, and training for board members are all possible solutions. Changes to the current system are necessary not only to facilitate the conduct of multisite research, but also to preserve the integrity of the ethics approval process in general Gold and Dewa (2005).

Obligation for Impartial Quality Assessment

Ethical guidelines for quality assessment concern all clinicians, institutions providing health care services for patients and producers of audit services. Ethical codes of clinicians include a commitment to an obligation for continuous improvement in their professional abilities and to evaluate the methods used. For example, provision 5 of the Code of Ethics for Nurses With Interpretive Statements (American Nursing Association, 2010) states that "the nurse owes the same duties to self as to others, including the responsibility to preserve integrity and safety, to maintain competence, and to continue personal and professional growth"; and Provision 7 states that "the nurse participates in the advancement of the profession through contributions to practice, education, administration, and knowledge development."

A clinician, therefore has to maintain and increase his/her knowledge and skills, and should recommend only examinations and treatments that are known to be effective and appropriate

based on medical knowledge and experience. Quality aspects are included also included in ethical guidelines, for example, provision 6 of the Code of Ethics for Nurses With Interpretive Statements states that "the nurse participates in establishing, maintaining, and improving health care environments and conditions of employment conducive to the provision of quality health care and consistent with the values of the profession through individual and collective action". This assumes that patients have the right for high quality healthcare. That the clinician is obliged to provide optimal care is also ingrained in the ethics code for example, provision 4 of the of the Code of Ethics for Nurses With Interpretive Statements states that the nurse is responsible and accountable for individual nursing practice and determines the appropriate delegation of tasks consistent with the nurse's obligation to provide optimum patient care.

Because ethical conduct and following the professional ethical guidelines is required for maintaining professional status and employment credentials, employers of health care professionals have an obligation to therefore help in creating opportunities for a health care professional's participation in appropriate further education, the completion of which needs to be followed by assessment. Alternately the employers may facilitate the professionals' access to such programs.

THE ASSESSOR OF HEALTHCARE QUALITY

The healthcare quality assessor should be trained in and conversant with auditing techniques, quality program and operations of healthcare systems, hospitals and associated agencies, and the competitive, legal and ethical issues associated with healthcare quality assessments. Competence of the Assessor must be well-established. A guide to competence of Assessors is outlined in Figure 5.

Schemas for competence assessment of Assessors may include evaluation of the following.

- How are competences Assessors appointed?
- What competence assessment methods are followed?
- The method, accuracy and security of record keeping
- Outcomes of the competence assessment that are carried out
- Consistency of controller competence assessment
- Refresher and unusual/emergency situations training
- Who are subject to competence assessment?
- Have these subjects been assessed?
- Development, testing and adoption of performance objectives

Selection and Initial Qualification

Criteria for selection of assessors include: education, demonstrated working knowledge, working experience, training, and assessment experience, communication/interpersonal skills, auditing skills, flexibility and performance motivation.

Demonstration of Assessor Competence

Assessors should be competent to demonstrate, assess and report assessments. They can be evaluated of their competence by interviews, written examination, demonstration, casual observation, formal evaluation, documentation, attestation, verification, documenting, interviews and review of previous work. Assessors should be aware of and understand the accreditation criteria, reference documents and their application, assessment principles, practices and techniques.

Figure 5. Competence of assessors (Adapted from ILAC, 2006)

Criteria for assessors:

- Open minded, observant, mature, self-reliant, tenacious and decisive; should have sound judgment, analytical skills and be ethical and diplomatic.
- Assessor should apply the attributes to:
- Obtain and assess evidence fairly with full attention and support and without fear, favor or distractions, preserving the natural conventions of the country.
- To constantly evaluate the observations and draw a conclusion which should be strictly adhered to.

Knowledge and skills:

- In applying accreditation criteria, the assessor should understand the criteria, fundamental basis of the standard used and its proper application.
- In matters of quality principles, the assessor should have the knowledge and skills for application of principles, assessment performance techniques, quality systems and adequate knowledge of the organization.
- Additional knowledge required by lead assessors include selecting team members, preparing assessment plan, preventing and resolving conflicts, taking decisions and leading, guiding and representing the assessment team and preparing a report of the assessment.
- Specific knowledge pertaining to technical assessors include proper performance of relevant testing and calibration methods, estimation of measurement uncertainty, analysis and interpretation of assigned value, knowledge of the standards and guidelines of the organization, tests, calibrations and the associated problems and terminologies.
- For the purpose of inspection body assessments, assessors should have knowledge about the inspection requirements, methods, the products/processes inspected, safety issues, sampling methods and assessment techniques.

RESEARCH AND PUBLICATION ETHICS

As emphasis on evidence-based clinical practice increases, clinical research is being conducted increasingly in all parts of the world in institutions and by professionals who may or may not have had experience in conduct of research and communication of its findings. Among these are international clinical trials that also involve large sums of money that may be paid to participating institutions. Ethical conduct of research must be therefore made mandatory and staff should be appropriately trained. However, healthcare quality assessment must also assess such activities. Figure 6 outlines some concepts and resources that may be tapped for more information on research ethics.

A key aspect is informed consent about which some misconceptions perhaps persist: is mere obtaining of informed consent sufficient to make clinical research ethical?

Many believe that informed consent makes clinical research ethical. However, informed consent is neither necessary nor sufficient for ethical clinical research. Drawing on the basic philosophies underlying major codes, declarations, and other documents relevant to research with human subjects, we propose 7 requirements that systematically elucidate a coherent framework for evaluating the ethics of clinical research studies: (1) value-enhancements of health or knowledge must be derived from the research; (2) scientific validity-the research must be methodologically rigorous; (3) fair subject selection-scientific objectives, not vulnerability or privilege, and the potential for and distribution of risks and benefits, should determine communities selected as study sites and the inclusion criteria for individual subjects; (4) favorable risk-benefit ratio-within the context of standard clinical practice and the research protocol, risks must be minimized, potential benefits enhanced, and the potential benefits

Figure 6. Research and publication ethics

Research Ethics
Conflict of interest resolution
Institutional Review Board (IRB) clearance
http://www.hhs.gov/ohrp/irb/irb_guidebook.htm
Council for International Organizations of Medical Sciences (CIOMS) *Ethical Guidelines for Biomedical research involving Human Subjects(2002):*
http://www.cioms.ch/publications/guidelines/guidelines_nov_2002_blurb.htm
Requirements for evaluating ethics of clinical research studies
- *Value of research*
- *Scientific validity*
- *Fair subject selection*
- *Favorable risk-benefit ratio*
- *Independent review*
- *Informed consent*
- *Respect for enrolled subjects*

(Source: http://jama.ama-assn.org/cgi/content/full/283/20/2701)
A comprehensive list of guidelines on the *Ethics of Biomedical Research With Human Subjects can be accessed at*
http://jama.ama-assn.org/cgi/content-nw/full/283/20/2701/TABLEJSC90374T1

Publication Ethics
Resources
Committee On Publication Ethics (COPE)
- COPE Code of Conduct
  http://publicationethics.org/files/u2/New_Code.pdf
- COPE Best Practice Guidelines for Journal Editors
  http://publicationethics.org/files/u2/Best_Practice.pdf
  (More information about publication ethics can be accessed at
  http://publicationethics.org/)

International Committee of Medical Journal Editors (ICMJE) Ethical Considerations
http://www.icmje.org/
Research Reporting Guidelines and Initiatives: By Organization
http://www.nlm.nih.gov/services/research_report_guide.html
Public Library of Science (PLoS)
http://www.plos.org/index.php

to individuals and knowledge gained for society must outweigh the risks; (5) independent review-unaffiliated individuals must review the research and approve, amend, or terminate it; (6) informed consent-individuals should be informed about the research and provide their voluntary consent; and (7) respect for enrolled subjects-subjects should have their privacy protected, the opportunity to withdraw, and their well-being monitored. Fulfilling all 7 requirements is necessary and sufficient to make clinical research ethical. These requirements are universal, although they must be adapted to the health, economic, cultural, and technological conditions in which clinical research is conducted" (Emanuel et al., 2000).

Several issues need to be addressed regarding communication of information such as: "What types of information do people want? Are we giving it to them? What are the characteristics of information that influence peoples' behavior? What effect does the type of decision have on the type of information needed? Do people in different circumstances use the same information in different ways" (Eisenberg, 2001)? These issues and results of inquires, studies, improvement projects and even advertisements have to be communicated by professionals and organizations to their peers, staff, patients and their families. Publications in scientific and lay media are effective and commonly used mechanisms to communicate research findings, and other information relating to healthcare services and quality to others.

The Committee on Publications Ethics (COPE) is a forum for editors and publishers of peer-reviewed journals to discuss issues related to the integrity of work submitted to or published in their journals. COPE supports and encourages editors to report, catalogue and instigate investigations into ethical problems in the publication process. The COPE web site may be accessed at: http:// publicationethics.org/ for gaining further insights into the role of publication ethics and how it may impact ethical communication and conduct of research and other healthcare information. Figure 6 outlines some concepts and resources that may be tapped for more information on publication ethics.

Note: The views expressed in this chapter are those of the authors and do not represent the position of NIH/ NIDCR/ United States Government.

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KEY TERMS AND DEFINITIONS

Ethics: Branch of Philosophy that guides moral conduct life and helps living a good life.

Privacy Rule: Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule, protects the privacy of individually identifiable health information.

Healthcare Quality Improvement: The combined and unceasing efforts of everyone—healthcare professionals, patients and their families, researchers, payers, planners and educators— to make the changes that will lead to better patient outcomes (health), better system performance (care) and better professional development (learning).

Obligation for Impartial Quality Assessment: Ethical codes of clinicians include a commitment to an obligation for continuous improvement in their professional abilities and to evaluate the methods used.

Chapter 7
Systems Biology Methodologies for the Understanding of Common Oncogenetic Mechanisms in Childhood Leukemic and Rhabdomyosarcoma Cells

George I. Lambrou
University of Athens, Greece

Maria Adamaki
University of Athens, Greece

Eleftheria Koultouki
University of Athens, Greece

Maria Moschovi
University of Athens, Greece

ABSTRACT

Human neoplasias are considered lethal in the majority of cases. Cancer does not discriminate between age, gender, or race. Besides the detrimental health effect caused by the disease, it also has a great impact on the quality of life of those affected by it. One of the main characteristics of cancer is that it deprives the patient from their human dignity, at least compared to other diseases. For example, more people die every year from coronary disease than from cancer, but the latter is considered more severe due to the slow, painful, and deteriorating effect it has on the human body. However, in the case of childhood hematologic neoplasias, progress in the field of treatment has taken immense steps forward, and five-year survival reaches 80%. At the same time, several new therapeutic agents, such as glucose analogs or telomerase inhibitors, are currently being tested in clinical trials, and several others, such as kinase or proteasome inhibitors, are already in the market. Yet the most effective therapies are the ones performed with classical chemotherapeutics, which are non-specific and more aggressive.

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One of the main concerns of modern life is the level and quality of health services, and at the same time, there is a great debate on the nature of health services provision, i.e. public or private. One of the main burdens of health services is drug administration. Especially in the case of cancer treatment, drug administration is extremely expensive, both for the patient and the health provider. If we think in terms of the Western world, then this is really a matter of finance, and the majority of the population can afford, either by private or state health insurance, the cost of a cancer treatment. However, if we see things in terms of developing countries or under-developed countries, where finances and health insurance are limited, then cancer treatment can not even be considered as an option for the majority of the population. The etiology for the high cost of cancer treatments comes from the process of drug development, as the latter takes almost 10 years of research and about 800 million dollars for a drug to reach the market. Most of the efforts in drug discovery are based mainly on the method of trial and error. This, in turn, is mainly due to the lack knowledge on the mechanisms underlying the disease.

Therefore, let us imagine a best case scenario where oncogenesis is understood, at least in part, and drug design, effectiveness, and side effects could be resolved by simply modeling the system. This would dramatically reduce drug costs, with positive consequences not only for treatment, but also on the social level. Drugs would become much more affordable, hence curing and improving the lives of more people. This hypothetical, best-case scenario should point scientists to move towards the direction of attempting to make scientific endeavors for the social benefit. Systems biology is a discipline that does indeed move towards that direction. Of course, as most things in life, the use of systems biology could be used in a dual manner, that is, for social benefit or profit.

INTRODUCTION

The Definition and Necessity of Systems Biology in Biological Phenomena

Systems biology can be considered a relatively young discipline. It was in the advent of the 21st century that the first coordinated attempts became reality. However, the term had been previously mentioned both as a necessity and as a discipline. We couldn't phrase it in better words than *Mihajlo Mesarović* in 1968:

…in spite of the considerable interest and efforts, the application of systems theory in biology has not quite lived up to expectations…one of the main reasons for the existing lag is that systems theory has not been directly concerned with some of the problems of vital importance in biology… The real advance in the application of systems theory to biology will come about only when the biologists start asking questions which are based on the system-theoretic concepts rather than using these concepts to represent in still another way the phenomena which are already explained in terms of biophysical or biochemical principles. …then we will not have the application of engineering principles to biological problems but rather a field of systems biology with its own identity and in its own right… (Mesarovic, 1968) (adopted from the book Systems Biology-Dynamic Pathway Modeling by Olaf Wolkenhauer (Wolkenhauer, 2010)).

But in order to further define the field of systems biology we should refer to an older reference from *Henri Poincaré*:

…life is a relationship among molecules and not a property of any molecule…Science is built up of facts, as a house is with stones. But a collection of facts is no more a science than a heap of stones is a house… (Wolkenhauer, 2010).

We think that there is no better way for describing the necessity for a systems approach to biological phenomena. Natural sciences in general have matured with time especially with the integration of mathematics. For example, the disciplines of physics and chemistry have benefited from the mathematical discipline and matured enough to create a common language for describing phenomena. Similarly, biological sciences could greatly benefit by integrating knowledge from the mathematical and engineering disciplines.

Biology deals with some very complicated phenomena such as embryogenesis, neurophysiology, oncogenesis and others. Oncogenesis is of crucial importance not only from the biological point of view but also from the disease perspective. Tumors are considered to be lethal in the majority of cases and can deprive the affected individual from their quality of life. Despite the research efforts in the field most of the mechanisms underlying this disease are largely unknown. Besides the biological effects that are under investigation in active research, there are several very important aspects of social life that are also affected. Cancer does not discriminate between age, gender, or race. Besides the detrimental health effect caused by the disease, it also has a great impact on the quality of life of those affected by it. For example, more people die every year from coronary disease than from cancer but the latter is considered more severe due to the slow, painful and deteriorating effect it has on the human body.

Perhaps the most crucial concern of modern life is the extent and quality of health services while at the same time there is a great debate on the nature of health services provision i.e. public or private. One of the great burdens of health services is drug administration. Up-to-date, chemotherapeutics consist of the main arsenal against the disease. Another important aspect is treatment. From the economics point of view cancer treatments are extremely expensive both for the patient and the health provider. If we think in terms of the western world, then this is really a matter of finance and the majority of the population can afford, either by private or state health insurance, the cost of a cancer treatment. However, if we see things in terms of developing countries or under-developed countries, where finances and health insurance are limited, then cancer treatment can not even be considered as an option in the majority of cases. The etiology for the high cost of cancer treatments comes from the process of drug development, as the latter takes almost 10 years of research and about 800 million dollars for a drug to reach the market. Most of the efforts in drug discovery are mainly based on the method of trial and error. This is due to the lack knowledge on the mechanisms underlying the disease. Yet, classical chemotherapeutics are nowadays affordable since for most of them patent rights have expired and generic forms can be produced. However, the problem with classical therapy is the side effects. Unfortunately, effectiveness is accompanied with non-specificity. Classical chemotherapeutics do not discriminate between healthy and diseased cells. They rely on the fact that cancer cells are more active both metabolically and transcriptionally, especially on the level of cell signaling.

Therefore, let us imagine a best case scenario where oncogenesis is understood, at least in part, and drug design, effectiveness and side effects could be resolved by simply modeling the system. This would dramatically reduce drug costs with positive consequences not only for treatment but also on the social level. New drugs would become much more affordable, hence curing and improving the lives of more people. Therefore, a question arises when it comes to systems biology and cancer. How can the discipline of systems biology help improve treatments and help maintain the patient's quality of life? There is no straightforward answer to this.

Due to the complicated nature of biological systems, it has been impossible until the advent of personal computers to even imagine that one would be able to study biological phenomena on the systems level. Nonetheless, the term systems

biology is not new. As we mentioned, it appeared in the mid 60s' and since then a considerable amount of effort has been made to model biological systems. Nowadays, it has become more evident that maturity in biology would come through its interaction with the disciplines of mathematics and engineering. In other words, a better understanding of biology can be achieved through the progress of computational and systems biology. Systems biology is still in its infancy, since orchestrated and coordinated efforts find their start at the beginning of the 21st century. Until recently, the principle of biology was in a way restricted on the level of studying several factors simultaneously. With technological progress, through the application of high-throughput methodologies such as microarrays and deep sequencing, mass production of data became possible. This gave us the ability to examine multiple factors simultaneously, bringing closer a more systems-oriented analysis of biological phenomena.

As mentioned earlier, in order to improve a certain condition, one needs to first understand the phenomenon underlying it or, in other words, understand the governing rules of the system. Systems biology does exactly that, i.e., it attempts to understand and define the rules governing biological phenomena. In the case of chemotherapeutics, two routes could be followed. The first would be to understand the mechanisms that lead to a disease, i.e. the pathogenetic mechanisms, and intervene in such a way that it would correct "mistakes" in cell function or, even better, eliminate diseased cells in the case of cancer. The second route would be to develop new or improve the existing drugs specific for a disease. In the case of cancer treatment there could be an improvement of classical therapies, since these are already available and would therefore be more feasible to do so financially, as opposed to exclusively concentrating on the discovery of new drugs for treatment, which is undoubtedly an extremely expensive and time-consuming process. In the present chapter we will analyze the workflows that can be followed in order to understand the causes of oncogenesis. We will provide useful information on the computational work flow that could help investigate the process of tumor progression on a small scale, and we will present this approach through an example study of two distinct, but devastating nonetheless, childhood neoplasias. Such an approach could assist in the understanding of the mechanisms underlying malignant transformation and as a consequence have direct effects on human health practitioning and quality of life. More specifically, we will present a computational and systems biology approach to the study of common mechanisms between two childhood neoplasias, acute lymphoblastic leukemia and rhabdomyosarcoma. Childhood leukemia is the most common form of childhood malignancy, whilst on the other hand rhabdomyosarcoma is a rare type of malignancy, belonging to the primitive neuroectodermal family of tumors. We know that tumors possess distinct characteristics not only between different types of malignancy but also between two patients affected by the same type of tumor. Despite all this, there is enough evidence to suggest that tumors might share some common characteristics which, once discovered, would lead to: a) more efficient treatments, b) a better understanding of the origin of oncogenesis and c) the discovery of universal tumor biomarkers.

Theories of Carcinogenesis and Elucidation of Their Mechanisms

In the case of cancer tumorigenesis it is the interconnection of thousands of factors acting simultaneously that bring about the malignant phenotype. Several theories have been suggested for the occurrence of tumors, the most prominent one being the accumulation of mutations throughout an individual's lifetime (Knudson, 2001); yet other equally significant theories have been suggested, such as the multistage oncogenesis theory (Moolgavkar, 1978; Ritter, Wilson, Pompei, & Burmistrov, 2003). However, in the case of child-

hood neoplasias these models/theories cannot be applied directly since the elapsed time of exposure to mutagenesis is not adequate to explain the appearance of cancer. There are several hypotheses regarding childhood neoplasias, as for example in the case of childhood leukemia it has been suggested that cytogenetic aberrations might play a role in the transformation of malignancy. Another interesting theory is that tumors are directly connected to the industrialization of modern life and it has been suggested that cancer is not actually a disease but an evolutionary adaptation of the cells in order to survive certain environmental changes/threats. The origins and etiology of cancer are still being investigated.

It is therefore evident that in order to be able to improve a certain condition, one first needs to understand the phenomenon or, in other words, to understand the governing rules of the system. Systems biology works towards understanding and defining the rules governing biological phenomena. Here we suggest two routes for the accomplishment of this purpose. The first route would be to understand the mechanisms that lead to disease, i.e. the pathogenetic mechanisms, and intervene in such a way that would try to correct "mistakes" in cell function or, even better, eliminate diseased cells in the case of cancer. This route could be further divided into two subcategories: a) the discovery of differences between tumor subtypes or differences in the reactions of tumor cells to specific treatments, one of the principal approaches used so far, and b) the discovery of common traits between different types of tumors, not only between subtypes of the same family of tumors but also between different types, both histologicaly and phenotypicaly, an approach not often referenced in literature. The second route would be to develop new drugs specific for a disease, or improve the existing ones. In the case of cancer treatment there could be an improvement of classical therapies, since these are already available, and would therefore be more feasible to do so financially, as opposed to exclusively concentrating on the discovery of new drugs for treatment, which is undoubtedly an extremely expensive and time-consuming process. We think that the common aspects that characterize tumors irrespectively of type or subtype are of major scientific interest. It is possible that the mechanics leading a cell to become malignant could be similar among certain cancer types. For example, a common characteristic of almost all types of tumors is the known *Warburg effect* described by *Otto Warburg* in 1924. This simple observation remains until today a fundamental trait of tumor biology i.e. that tumors perform a shift from oxidative phosphorylation to aerobic glycolysis for their energy needs (Warburg, Posener, & Negelein, 1924). This is a characteristic that all tumors have in common and pharmaceuticals exploiting this trait are already in clinical trials. We believe that it is possible for a common oncogenetic mechanism to exist and that its elucidation could provide a wide range of weaponry in the fight against cancer. Not only would it be possible to gain a more in-depth understanding of tumor biology, it would also open up new ways for treating cancer.

Childhood Neoplasias and Common Mechanisms

Until recently, scientific approaches concentrated on examining several factors simultaneously, which only revealed part of the picture. Oncogenesis is a highly complicated process and one of the tools that current research has in its arsenal is computational and systems biology. In this respect, we are now able to investigate possible common mechanisms that govern different neoplasias. In the present chapter we will analyze and present workflows that can be followed in order to understand the causes of oncogenesis. We will do so using two childhood neoplasias, both of different cellular origin and of different phenotype, as an example. These are acute lymphoblastic leukemia and rhabdomyosarcoma. The first is the most common cancer of childhood cancer and the second

one of the rarest tumors found in children. ALL is considered to be one of the great successes of the century with cure rates that reach 80% of the cases, whereas survival rates in other neoplasias still remain very low. We use reverse engineering methodologies as an approach of finding common mechanisms of cancer progression.

ACUTE LYMPHOBLASTIC LEUKEMIA AND RHABDOMYOSARCOMA AS CASES

Acute lymphoblastic leukemia (ALL) is the most frequent occurring malignancy among childhood cancers. It originates in the undifferentiated lymphoblast, which abnormally ceases develop into the mature lymphoid cell giving rise to a tumor. Acute leukemia mainly appears during childhood but it also occurs in adolescence manifesting a poor prognosis regardless of age. Progress in childhood leukemia has been immense the last decades with an overall survival rate exceeding 75% (Carroll et al., 2003). The prognostic factors have also been well characterized for childhood leukemias; these include white blood cell count at presentation (diagnosis), age and gender, as well as certain chromosomal aberrations (Carroll, et al., 2003). Treatment of childhood leukemias is successful for the majority of patients, mainly due to the use of classical chemotherapeutics. Recently individualized treatments have also been applied, as in the case of BCR/ABL positive (also known as Philadelphia positive (Ph+)) leukemias, using imatinib mesylate, a new agent specific for the particular gene fusion.. This represents a great example of how childhood leukemia can benefit from individualized therapy.

On the other hand, rhabdomyosarcoma (RMS) is a rare childhood cancer representing 5-8% of all tumors emerging during childhood. However, the sarcomas of head and neck comprise 12% of all childhood neoplasias (Chadha & Forte, 2009) with an incidence rate of 250 cases every year in the United States. Rhabdomyosarcoma is a malignant tumor of mesenchymal origin and it is within the category of small blue cell tumors, including neuroblastoma and primitive neuroectodermal tumors (Chadha & Forte, 2009). The embryonal form is most common at birth whereas the alveolar form peaks in childhood and adolescence (Toro et al., 2006). Embryonal rhabdomyosarcoma, characterized by spindle cells and botryoid form, is associated with better prognosis (Leaphart & Rodeberg, 2007). As in the case of childhood leukemia, many alveolar rhabdomyosarcomas present chromosomal translocations, such as the PAX3-FKHR, which is an indicator of bad prognosis, since it is highly associated with metastasis (Chadha & Forte, 2009; Davicioni et al., 2009; Oda & Tsuneyoshi, 2008; Sorensen et al., 2002). Rhabdomyosarcoma is rarely cured by one method of treatment alone. Therapy protocols include chemotherapy and either surgery or radiotherapy. In general the 5-year survival rates are in the extent of 97% in embryonal non-orbital, non-parameningeal head and neck cases, and 67% in the alveolar cases (Pappo et al., 2003). The cell of origin is considered to be the myoblast or cells that will form the skeletal muscle. These cells are different from the smooth cells that line the intestinal tract. Theoretically, rhabdomyosarcomas can emerge at any part of the body that has skeletal muscle but it originates mainly in the head and neck.

COMMON CHARACTERISTICS BETWEEN ALL AND RMS CELLS

A common aspect between ALL and RMS is that both comprise of cells that are undifferentiated, immortal and with the potential to divide infinitely. This means that proliferation takes place in an uncontrollable pattern. Interestingly, it has been reported that rhabdomyosarcoma can be present in the bone marrow of patients presenting a leukemic image, without the presence of a primary tumor (Morandi, Manna, Sabattini, & Porcellini, 1996;

Parham, Pinto, Tallini, & Novak, 1998; Putti et al., 1991; Sandberg, Stone, Czarnecki, & Cohen, 2001). In addition, it has been reported that there is a possibility for presentation of secondary malignancy after successful treatment of a primary (therapy-related leukemia). For example, following the successful treatment of a paravertebral embryonal rhabdomyosarcoma (ERS), a patient developed acute T-lymphoblastic leukemia (Kaplinsky et al., 1992). The question that could be posed here is whether this development of the secondary tumor originated from cells of the primary tumor, as in the case of therapy-related leukemia, or whether there were "dormant" leukemic cells in the bone marrow which got triggered to proliferate uncontrollably after rhabdomyosarcoma chemotherapy. In the case where cells from one type of cancer develop into another, it probably means that the tumor cell possesses two traits: first it is able to migrate and second it is able to differentiate to another type of cell manifesting stem cell properties. This is in accordance with the stem cell theory of cancer origin, as cancer stem cells keep their ability to differentiate, migrate and even give rise to a new malignancy with almost totally new traits. Furthermore, it has been reported that therapy-related leukemia can occur due to the use of chemotherapeutics (Dedrick & Morrison, 1992; Park & Koeffler, 1996). This phenomenon has not been thoroughly investigated and the mechanisms behind it are still obscure. However, it points out the fact that carcinogenesis is a complicated process, implicating a number of mechanisms and not just single events in a cell's life time. If we were to accept the hypothesis that two different tumor cells can co-exist in different locations as true, then we could accept the notion that stem cells play the main role in carcinogenesis and tumor growth. On the other hand, an interesting report by *Kelly et al.* showed that, at least in part, the presence of cancer stem cells is not necessary for tumor growth to be sustained (Kelly, Dakic, Adams, Nutt, & Strasser, 2007).

By looking into the developmental progress of the aforementioned cell types, both seem to originate from the embryonic mesoderm. Myoblasts originate from the *dorsal (paraxial) mesoderm* whereas blood cells derive from the *lateral mesoderm*, which gives rise to the *splachnic mesoderm* and this, in turn, to the *hemangioblastic tissue*. Further on, *paraxial mesoderm* gives rise to the un-segmented presomitic mesoderm, formed during *gastrulation*, and mesoderm generated from the *primitive streak*. One of the earliest genes expressed in the *paraxial mesoderm* is T (branchyury). This is also an embryonic transcription factor that is expressed in a gradient across developmental sites derived from the primitive streak and continuously expressed in the notochord (Sewell & Kusumi, 2007). The FGF (Fibroblast Growth Factor) and RA (Retinoic Acid) pathways are the main routes followed in early somatic development (Sewell & Kusumi, 2007). Another important signaling pathway that has been found to participate in developmental as well as oncogenic pathways is the NOTCH pathway. Notch genes encode transmembrane receptors. The human genome contains four NOTCH receptor homologues. In somite stage, NOTCH1 is expressed across the presomitic mesoderm, where mutations have been found to cause defects in somitic segmentation and anterior-posterior polarity (Dunwoodie et al., 2002; Kusumi et al., 1998). On the other hand, during embryogenesis, blood cells originate from two sites: the first is thought to be the *ventral mesoderm* near the *yolk sac*, which gives rise to the intra-embryonic hematopoietic precursors, whereas the hematopoietic cells that last throughout the entire life time of an organism are derived from the mesodermal area surrounding the aorta (Godin & Cumano, 2005). From the point of mesoderm differentiation, strictly orchestrated regulation of various genes leads to two similar cell types with different functions and roles in the body. This is the result of a complicated network of gene regulation and expression. It is easy to assume that aberrations in the regulatory

networks underlying development would lead to tumor cells. Developmentally, there are several factors that affect gene regulation in order for differentiation to take place.

Tumors can be as different as the patients that carry the disease. It is these differences that make tumors so hard to cure, since therapies have different results on different patients. To our present knowledge there are not many reports trying to identify common gene expression profiles between the two aforementioned tumors. An interesting reference highlights the fact that stem cells are probably found in different types of tumors, thus suggesting that stem cells are implicated in the etiology of tumor maintenance and growth (Nicolis, 2007). However, there is evidence that even normal, already differentiated cells, can be transformed to tumorigenic ones (Nicolis, 2007). Based on this observation, there was a case reporting a child manifesting 5 different tumor types simultaneously (Perilongo et al., 1993). It could be that stem cells originating from the same organism possess similar defects but enough alterations to be able to give rise to five different types of tumor. The discovery of similar or opposing gene expression profiles may lead to the understanding of a common tumor origin, if such exists.

Another point on which attention should be drawn is the regulation of genes through transcription factors (TFs). Knowledge of gene regulatory networks is considered to be of crucial importance for the understanding of diseases such as cancer, as it may lead to new therapeutic approaches (Chang, Nagarajan, Magee, Milbrandt, & Stormo, 2006). The knowledge of common transcriptional regulatory networks could potentially lead to a universal treatment for diverse diseases such as cancer, and it is through this possibility that the need for computational methods in the study of carcinogenesis becomes apparent.

IN VITRO CELL MODELS FOR THE STUDY OF COMMON MECHANISMS

The study of the mechanisms underlying carcinogenesis or tumor progression or resistance to chemotherapeutics requires biological systems that would resemble these processes *in vivo*. There are several models available both in experimental praxis as well as those reported in the literature. One of the main approaches utilized is the use of immortalized cell lines, taken as biopsies either from human or other species, and transformed to divide indefinitely in laboratory conditions. Cell lines manifest several advantages and disadvantages. Among the advantages, we value the possibility for repeatability in experimental procedures, the ease of use in daily laboratory praxis and the feasibility of use from an economical perspective. On the other hand, cell lines do not accurately represent the cellular systems, the pathogenesis or the phenotype as it is presented *in vivo*. However, as far as the ethical part of experimentation goes, cell lines outreach all other study models. Ethical considerations have been raised due to the uses of human specimens or laboratory animals. There is also a general tendency for an increase in the use of cell lines as models of study and the decrease of other types of biological systems, although patient samples and animal models are irreplaceable in certain cases. Here we use two cell lines as our reference systems. We examine the experimental and analysis steps taken in order to elucidate common patterns of cellular function between these two systems. The two cell lines are the T-cell acute lymphoblastic leukemia CCRF-CEM cell line and the rhabdomyosarcoma TE-671 cell line. Both were obtained from the European Collection of Cell Cultures (ECACC). The CCRF-CEM cell line, a $CD4^+$ (Miranda, Wolf, Pichuantes, Duke, & Franzusoff, 1996) and $CD34^+$ presenting cell line (Naujokat et al., 2000), was initially obtained from the peripheral blood of a 2-year-old Caucasian female diagnosed with lymphosarcoma, which later on progressed

to acute leukemia (Foley et al., 1965). The child underwent irradiation therapy and chemotherapy prior to obtaining the cell line. Although remission was achieved at various stages, the disease progressed rapidly (Foley, et al., 1965). The cell line has been observed to undergo minor changes after long-term culture, except for the presence of dense granules in the nucleoli (Uzman, Foley, Farber, & Lazarus, 1966) and to manifest autocrine catalase activity, an important regulator of the cell line's growth(Sandstrom & Buttke, 1993). The fact that these cells have been therapeutically treated before collection makes them an ideal model for the investigation of drug resistance; as a matter of fact, it has been previously reported that the particular cell line manifests resistance to several chemotherapeutic agents, e.g. glucocorticoids (Lambrou et al., 2009).

The TE-671 was initially reported to be obtained from a cerebellar medulloblastoma, before irradiation therapy, of a six-year old Caucasian female (McAllister et al., 1977) and was characterized later on (Friedman et al., 1983). Nowadays we know that this cell line is parental, if not identical, to the RD (McAllister, Melnyk, Finkelstein, Adams, & Gardner, 1969) rhabdomyosarcoma cell line. However, several reports still refer to this cell line as medulloblastoma (Chu, Wong, & Yow, 2008; Yeung, An, Cheng, Chow, & Leung, 2005).

Based on our positions stated above, these cells share several common characteristics despite their obvious differences. Both cell types are primarily derived from the mesoderm, where cells stopped differentiating, probably at an early stage, before maturing into their final phenotype. Physiologically, these cells would have matured and differentiated into the cells constituting the blood and muscle cells. Also, at some unknown point, normal differentiation ceased and the cells started accumulating uncontrollably, i.e. they became malignant. From that point on, until the first manifestation of symptoms of malignancy, there is a lack of knowledge of the mechanistical steps leading to oncogenesis. In other words, we cannot acquire ant form of knowledge for the stages that involve the emergence of the blasts and their proliferation dynamics up to the point that they are clinically presented. This represents another good reason for studying such systems using *in vitro* models, since *in vitro* models allow the creation of proliferation and growth models in long term cultures, something that is impossible to achieve with *ex vivo* cells or animals.

Our point here is whether two distinct cell types, destined to perform different functions, manifest similar mechanisms of growth and progression due to their malignant character. In other words, is the phenotypic character of the cells originally inherent through their developmental *histoire*, or is it due to the malignant character, acquired at some point of development, that determines cell proliferation, growth, gene expression and regulation. For the present, we will focus on the differential expression profile underlying the two cell lines and on the computational approach of the aspect surrounding a common expression profile. In particular, we will look at groups of genes based on their function and on their regulation. For example, it is expected for two different cell lines to manifest differential gene expression. Yet, what are the genes that are commonly expressed? We mainly focus on the similarities between the two cell types. Based on these observations we could narrow down our search for common mechanisms and focus on groups of genes such as the active developmental genes, the molecules secreted from both cell types, genes that sustain growth and genes involved in the cell cycle.

We will present the use of high throughput and computational approaches and try to elucidate which genes (within a certain group of genes) are typical for each cell type, but at the same time genes specific for one cell type appear in the other. We expect to find some genes active in both cell types. We will attempt to find similarities in tumor progression, cell cycle and secretory extracellular signaling. We will try to elucidate the possible common regulatory mechanisms for the two

cell types based on their expression profile and, investigate whether there are pathways common to both cell types; the latter could be modeled as a means of investigating how gene function is implicated in the networking observed in all biological systems, especially in cancer. Finally, we will attempt to reach some conclusions regarding the origins of tumorigenesis. In other words, from the computational analysis performed we will try to deduce whether stem cells play a role in tumor progression.

Several studies have been occupied with the detection of cancer germ line genes (CGGs) both in pediatric sarcomas (Jacobs et al., 2007) and pediatric brain tumors (Jacobs et al., 2008). The discovery of global antigens for tumor vaccines could provide salvation for childhood malignancies (Jacobs, et al., 2007). Some studies have reported the appearance of common antigens in Ewing sarcoma/primitive neuroectodermal tumor (EWS/PNET) and lymphoblastic lymphoma (Mhawech-Fauceglia et al., 2007). Rhabdomyosarcoma is included in the primitive neuroectodermal tumor types, where embryonic genes are involved in cancer progression (Blandford et al., 2006). Leukemia, and more specifically ALL, on the other hand, originates from the lymphoblast, also known to employ embryonal mechanisms for its differentiation and progression.

This type of approach opens up the road to a more holistic understanding of the tumor/oncogenetic dynamics, since by finding common mechanisms of action it would be possible to better understand the origins of cancer. Different neoplasias with different characteristics are expected to possess different traits and phenotypes. However, an approach that identifies commonalities between such diverse biological systems could lead to the understanding of the common mechanisms that transform physiological cells to malignant. This could have a very positive outcome in drug development and contribute to new, more global, therapeutic approaches.

EXPERIMENTAL PROCEDURES AND COMPUTATIONAL WORKFLOW

The analysis can be divided in two parts: the experimental part and the computational part. The experimental part determines the outcome of the computational analysis to a greater extent. This means that experimental design should be performed in such a way that it should outline, when possible, the main questions that need to be answered. This is not always the case though. There are several experimental set-ups which are mostly discovery-driven and less hypothesis-driven. In the present case we use a discovery driven approach. Below is an outline of the total workflow that can be used for such analyses:

- Cell culture and growth
- Allow cell proliferation to reach the desired extent
- Cell proliferation measurements
- Cell collection, at desired time points, for further processing
- Cell collection for:
  - RNA extractions
  - DNA extractions (optional)
  - Protein extractions (optional)
  - Cell culture supernatants for measurements of extracellular molecules and factors
  - Cells for flow cytometric analyses
- Extractions of the aforementioned molecules
- Flow cytometric analyses for cell death, cell size, granulation and cell cycle distribution
- Computational analysis of flow cytometric data (optional)
- Microarray experimental design
- Microarray experimentation between selected samples
- Computational analysis of microarray data
  - Signal filtering
  - Background correction

- Signal intensities normalization
- Statistical tests for differential gene expression detection
- Hierarchical clustering
- Other clustering methods (e.g. k-means, Principal Component Analysis)
- Transcription Factor Binding Motifs predictions (TFBMs)
- Chromosome distribution analyses
- Gene Ontology (*GO*) analyses
- Pathway participation and mapping analyses
- Pathway simulations (optional)
- Protein-protein interactions prediction (optional)
- Final conclusions

At this point we are able to describe the workflow in a step-by-step manner and to further explain the necessities for each of the steps taken.

Cell Culture and Growth

Both cell lines were seeded at time -24h and left to grow overnight. This is a necessary step for the cells to re-adjust to their environment and avoid the stress effects caused during feeding and seeding in future measurements.

After 24h (time: 0h) a sample was taken from both cell lines in order to perform measurements and every 24h thereafter. Cell lines were grown in culture media, as for example the T-Lymphoblastic Leukemia CCRF-CEM cells were grown in RPMI-1640 medium supplemented with 2mM L-Glutamine and Streptomycin/Penicillin 100 U/ml, 20% FBS at 37 ^{o}C, 5% CO_2 and ~100% humidity. TE-671 cells were grown in DMEM medium supplemented with 2mM L-Glutamine, 10% FBS and 100 U/ml Streptomycin/Penicillin. Cells were allowed to grow to a population of ~1.500X10^3 cells/ul for CCRF-CEM and at ~80% confluence for TE-671. Cells were harvested at confluence using 0,1% trypsin (only for TE-671) and centrifugation at 1000 rpm for 10 min. Supernatant was discarded and cells were washed with pre-warmed 1X PBS, re-centrifuged at 1000 rpm for 10 min and the cell pellet was kept for further processing.

Cell Proliferation

Cell population is an important characteristic of cell type. It is important to mention that the two cell systems used are very different in the way they grow. The CEM cell line grows in suspension i.e. in a 3D-environment very closely resembling the *in vivo* condition of the bone marrow. On the other hand the rhabdomyosarcoma cells grow in an adherent mode, i.e. they attach to the collagenous substrate of the flasks they grow in. This immediately manifests two basic differences. In the case of leukemic cells confluence can not be directly determined since it is a question of nutrition abundance and a question of availability of space. On the other hand, RMS cells, regardless of nutrition abundance, are highly depended on space availability, since the mechanism of contact inhibition can lead them to cell death once confluence is reached. Therefore, cell counts must be regularly performed. In the present case cells were counted at the -24 h time point as well as at 0 h, 4 h, 24 h, 48 h, and 72 h after having been let to grow under normal conditions. For this purpose, 200 μl of cell suspensions were obtained from each flask and counted directly with a hematologic analyzer.

Flow Cytometry

Flow cytometry is a powerful method that allows the simultaneous determination of multiple factors such as cell size, granulation, membrane markers, cell death and viability, DNA content or cell cycle distribution. In the present case we present as an example the determination of three factors: 1) cell size and granulation, 2) apoptosis and 3) cell cycle distribution. Cell cycle distribution and DNA content was determined with standard propidium

iodide (PI) staining, a stain with high affinity for nucleotides. When PI gets excited by an energy source it gives off fluorescence proportional to the DNA content of the cells, thus indicating the G_1, G_2 and S phases. Briefly, 1ml of cell suspension from each flask was centrifuged at 1000 rpm for 10 min. Supernatant was removed and cells were suspended in 1 ml of 75% ethyl alcohol. Cells were incubated at 4° C overnight. Following incubation, cells were centrifuged at 1000 rpm for 10 min. The supernatant was removed and cells were washed with 1 ml ice-cold PBS, pH 7.4. Cells were re-centrifuged and re-diluted in 1 ml PBS pH 7.4. 0.25 μg/ml. RNase A was added and cells were incubated at 37° C for 30 min in order to remove any remaining traces of RNA that could interfere with PI. PI was added to a final concentration of 1 ug/ml. All experiments were performed in triplicate. The reported data constitute the average of three independent experiments.

RNA Isolation

RNA isolation is one of the most crucial steps for the success of subsequent analyses since good RNA quality determines the yield in gene expression later studied with microarrays. In the present case the procedure followed was as follows: RNA was isolated with Trizol. The amount of RNA isolated was measured with a BioRad SmartSpec 3000 spectrophotometer and RNA integrity was estimated by 2% agarose gel electrophoresis. At least 40 μg of RNA from each sample was used. DNase treatment followed. Finally, RNA samples were further purified using a column-based kit and RNA amounts and integrity were determined again as above. Samples with a 1.8 to 2.0 A_{260}/A_{280} ratio were selected. In addition, those RNA samples that empirically showed the 28S band twice as bright as the 18S band on the gel were utilized.

Microarray Experiments

Since their advent, microarrays have been used to discover differences between biological samples. For example, on the level of gene expression, a sample is compared to a reference, in order to discover differences in the gene expression profile. Samples that are treated differently, for example a drug treatment against no treatment or normal cells against tumor cells, are used for RNA extraction and hybridized, either as cDNAs or as cRNAs (*complementaryRNAs* or also referred to as *anti-sense amplified RNAs*).

Similarly, microarrays have been used for the detection of polymorphisms. During the past few years, microarray technologies have attracted a major deal of interest from the scientific community due to their potential in screening thousands of factors simultaneously. The first use of microarray technology was for detecting differences between two samples at the mRNA level. Microarrays consist of glass slides or membranes printed with oligonucleotides or cDNA fragments. Based on this property and the progress of high fidelity robotic technologies, microarrays have proven extremely valuable for the detection of literally tens of thousands of targets within one experiment. For this reason alone, this particular method has found applications in many areas of life sciences.

With time, microarrays expanded to various types of high-throughput experimentations. Examples of these include *methylation studies*, *SNP* (*Single Nucleotide Polymorphism*) detection, *CGH* (*Comparative Genomic Hybridization*) microarrays and *CHIP-chip* (*Chromatin Immunoprecipitation Chip*) microarrays. A very important aspect of microarrays is the experimental design. Another important issue of microarray analysis is the normalization of data. This probably includes the most important aspect of microarray methodology, since it is the component that influences all further decisions and conclusions.

At this point we need to define the types of nucleic acids that are utilized in microarray analyses. The first and most abundantly used molecules were cDNAs. The *in vitro* reversely transcribed total RNA yields the respective cDNAs of all expressed exons at a certain time point under investigation. This method usually requires large amounts of total RNA as starting material, which ranges from 20-50ug per sample. Since this is not always the case, i.e. samples could be rare or difficult to obtain or of low quantity, other alternatives should be considered. A solution to these aforementioned problems came with the methodology of RNA amplification. It was first described by *Van Gelder et al.* in 1990 (Van Gelder et al., 1990). This methodology is based on the linear amplification of dsDNA transcribed to cRNA with use of the *T4 DNA Polymerase, T3 RNA Polymerase* and *T7 RNA Polymerase*. It is a very powerful technique since it can amplify 20ng to 2ug of total RNA yielding approximately a 5000-fold amplification. This means that samples of 200ng could yield 40ug of cRNA or samples of 2ug of total RNA could yield up to 100ug of *cRNA*. *cRNA* (complementary RNA) is also called *aaRNA* (*anti-sense amplified RNA*). Finally, when the desired molecules have been extracted and sample quality has been assured, nucleic acids are labeled with fluorochromes and let to hybridize. The basic and decisive step in microarray methodology is the sample selection and nucleic acid extraction, with RNAs or DNAs. In order to define the expression profile of genes at specific time points, one needs to "capture" the cell's RNA or DNA at that specific time point. DNA and RNA quality defines the experimental output.

The result is the 'snap-shot" of the transcriptome at a given time point. Usually, in order to obtain more accurate information, it is necessary to perform multiple experiments at different time points or conditions, and then one speaks of a *functional genomic assay*.

In the present case, for the assay of mRNA levels two sets of microarray chips were used: cDNA microarray chips (4.8 kgenes) obtained from TAKARA (*IntelliGene*™ II Human CHIP 1) (Chung et al., 2004) and microarray chips (9.6k genes) from the Institut fuer Molekularbiologie und Tumorforschung, Microarray Core Facility of the Philipps-Universitaet, Marburg Germany (IMT9.6k). Hybridization was performed with the CyScribe Post-Labeling kit (RPN5660, Amersham) as described by the manufacturer. The fluorescent dyes used were Cy3 and Cy5. The RNA extracted form the CCRF-CEM cells was stained with *Cy3* (reference/control) and RNA from the TE-671 cell line with *Cy5* (experiment/treatment). cDNAs were purified with QIAGEN PCR product clean-up kit (Cat # 28104). Slides were activated at 55^{o} C for 30 min in 1% BSA. Samples were applied on the slides and let to hybridize overnight at 55^{o} C. The following day, slides were washed in 200 ml 0.1× SSC and 0,1% SDS for 3× 5 min, in 200 ml 0.1× SSC for 2× 5 min and in 200 ml ddH_2O for 30 sec. Slides were dried by centrifugation at 1500 rpm for 3 min and scanned with a microarray scanner (ScanArray 4000XL) (*Perkin Elmer Inc.* MA, former *GSI Lumonics, USA*). Images were generated with ScanArray microarray acquisition software (*Perkin Elmer Inc.* MA, former *GSI Lumonics, USA*).

Flow Cytometry Data Analysis

Flow cytometry data contain a great amount of information that needs specialized software in order to be analyzed. There are several commercially available software packages and also several open-source software packages that are very good for flow cytometric data analysis. For the present analyses we have used the *WinMDI* software version 2.8 (*The Scripps Institute, Flow Cytometry Core Facility* http://facs.scripps.edu/software.html) and *Cylchred* version 1.0.2 (*Cardiff University, Wales*) which is based on the algorithms proposed by Watson et al. and Ormerod et al (Ormerod & Payne, 1987; Ormer-

od, Payne, & Watson, 1987; Watson, Chambers, & Smith, 1987).

Microarray Data Analysis

The availability of software packages for microarray data analysis is really overwhelming. There are several commercially available software packages but there is also a very strong community of programmers and scientists that have created open-source software for microarrays. For the present analyses we have used mainly open-source software, except for the steps of image acquisitions and raw data extractions. Hence, microarray raw image analysis and raw data acquisition was performed with *ImaGene® v.6.0* Software (*BioDiscovery Inc, CA*). ARMADA software (National Hellenic Research Foundation, Athens Greece, http://195.251.6.234/armada/) (Chatziioannou, Moulos, & Kolisis, 2009) was used for further filtering, normalization and clustering analyses. Normalization was performed using six different methods: a) No further processing after background correction, b) $\log_2$ transformation, c) LOWESS normalization, d) division with the Global Median (median of the 50% percentile), e) subtraction of Global Median (the median of the 50% percentile) and f) Rank Invariant with running Median. Finally, the Rank Invariant normalization method was chosen for further processing and analysis. Background correction was performed with global background correction, sub-grid based and negative control spots. Genes were filtered first for their quality of spot. This was done by acquiring a '*0'* flag marking on each spot as assessed by *ImaGene* software (Signal-to-Noise Ratio (SNR). Notice that Signal-to-Noise Ratio is calculated by the software from the equation $SNR = \frac{\mu_{R,G} - \mu_B}{\sigma_B}$, where $\mu_{R,G}$ and μ_B is the mean value intensity for the respective channel (Cy3 or Cy5) and mean background intensity respectively and σ_B is the background mean signal standard deviation. A threshold of *2* has been set as a cut-off value, meaning that spot intensity for at least one channel should be twice as much than that of the background). Furthermore, each gene was tested for its significance in differential expression using a *z*-test. Genes were considered to be significantly differentially expressed if they obtained a *p*-value <0.01. The False Discovery Rate was calculated as described previously (Klipper-Aurbach et al., 1995; Storey & Tibshirani, 2003a, 2003b). There was a FDR of 0.12% for $p<0.01$ for the *IntelliGene* microarray chip and a FDR of 9% for $p<0.01$ for the IMT 9.6k microarray chip. Calculating the FDR for the combination of both platforms gives a FDR of 6% for $p<0.01$.

Chromosome Mapping

Chromosome mapping has also appeared to be a promising method in finding common patterns of expression among genes. The main idea, reported initially by *Cohen et al. 2000*, is to map genes on chromosomal regions and if correlations exist between genes then these would probably appear through the genes' locations on chromosomal regions, since consecutive genes are often similarly expressed (Cohen, Mitra, Hughes, & Church, 2000). For chromosome mapping analyses we have used the *Genesis* software package (*Technische Universitaet-Graz, Austria*) (Cohen, et al., 2000; Reyal et al., 2005; Sturn, Quackenbush, & Trajanoski, 2002) and *WebGestalt* web-tool (Vanderbilt University, The Netherlands, http://bioinfo.vanderbilt.edu/gotm/) (B. Zhang, Schmoyer, Kirov, & Snoddy, 2004).

Clustering Analysis

Clustering analysis and chromosome mapping were performed with ARMADA (Chatziioannou, et al., 2009) *Genesis 1.7.2* (*Technische Universitaet-Graz, Austria*) using *Pearson's* correlation and *Spearman's rank order* correlation (Cohen, et al.,

2000; Reyal, et al., 2005; Sturn, et al., 2002) and *WebGestalt* web-tool (Vanderbilt University, The Netherlands, http://bioinfo.vanderbilt.edu/gotm/) (B. Zhang, et al., 2004). For clustering analysis, hierarchical clustering by Euclidian distance was used (Quackenbush, 2001). In addition, k-means clustering as well as PCA analysis was performed among differentially expressed gene groups.

TFBMs Analysis

One very important aspect of gene expression we have mentioned before is the regulation of gene expression through transcription factors. With the availability of thousands of genes simultaneously, new methods could be developed which would allow the prediction of transcription factors that regulate the differentially expressed genes. Hence, in the present case, Transcription Factor Binding Motifs (TFBMs) were searched in the Transcription Element Listening System Database (TELiS) (www.telis.ucla.edu) (Cole, Yan, Galic, Arevalo, & Zack, 2005). The TRANSFAC transcription factor database was used for the identification of gene transcription factor binding sites (Wingender, Dietze, Karas, & Knuppel, 1996).

Gene Ontology (GO) Analysis

Gene Ontology (GO) analysis is essential in the deduction of conclusions from microarray data. Gene ontology is a database with curated annotations for known genes i.e. gene biological processes, molecular functions and cellular components. *GO* analysis was performed, using the *eGOn* online tool for Gene Ontology (The Norwegian University of Science and Technology, Trondheim, Norway, http://www.genetools.microarray.ntnu.no/egon/) (Beisvag et al., 2006), *Genesis 1.7.2* software (Sturn, et al., 2002) and *WebGestalt* web-tool (B. Zhang, et al., 2004). Relations of the differentially expressed genes and the transcription factor binding motifs were further investigated using the *Pubgene Ontology Database* (www.pubgene.org). Furthermore, we have used on-line tools for literature searches and determination of gene functions based on the available literature. For literature search the MILANO (**Mi**croarray **L**iterature-based **Anno**tation, http://milano.md.huji.ac.il/, Department of Molecular Biology, Hebrew University–Hadassah Medical School, Jerusalem) web-based tool was utilized (Rubinstein & Simon, 2005). In the present analyses gene definitions and functions were based on the *National Institute of Health* databases (http://www.ncbi.nlm.nih.gov/sites/entrez/).

Pathway Analysis

This is the final step in the process of microarray data analysis. This attempts to create a connection between the genotype, as revealed by transcription analysis, and the observed cellular phenotype. This process can be further divided into smaller steps. The first step would be the mapping of the expressed genes on known pathways. This would give an immediate first picture of the participation of known genes in curated pathways. The second step would be the modeling of the mapped genes within the desired pathway. This step is more complicated since it includes the dynamical modeling of the chemical reactions between gene products i.e. proteins and smaller molecules. In the present analysis the differentially expressed genes were mapped on different pathways using the *Pathway Explorer* software (*Technische Universitaet-Graz, Austria*) (Mlecnik et al., 2005),after the percentage of genes present in all known pathways had been determined using the databases available through the *Pathway Explorer* software. The *KEGG* database of pathways was used for our analysis (Kanehisa, 1997, 2002; Kanehisa & Goto, 2000; Nakao et al., 1999; Ogata et al., 1999), *CellDesigner* (Funahashi et al., 2008; Funahashi, Morohashi, Kitano, & Tanimura, 2003) and *MATLAB*® v.7.6.0 computation environment with *SimBiology*® Toolbox were used for simulation of coupled JAK/STAT/MAPK pathways.

For the analysis of merged pathways the *KEGG Converter* Tool was utilized (National Hellenic Research Foundation, Athens Greece, http://www.grissom.gr/keggconverter) (Moutselos, Kanaris, Chatziioannou, Maglogiannis, & Kolisis, 2009).

Physical Protein-Protein Interactions Prediction

This step is optional in any microarray analysis since protein-protein interactions prediction is still in its infancy. Gene expression, however, can predict protein interactions. Nonetheless, it is of great interest to search, even within a noisy dataset, for interacting proteins. Such predictions can be very promising for future investigations as they could majorly contribute to the understanding of genotype to phenotype interactions. In the present analysis we used the methodology proposed by Soong et al. 2008 (Soong, Wrzeszczynski, & Rost, 2008). Briefly, the microarray set of post-normalized genes was entered in the DIP database of protein interactions (Deane, Salwinski, Xenarios, & Eisenberg, 2002; Salwinski & Eisenberg, 2003; Salwinski et al., 2004) and predictions were performed using the *Cytoscape* environment. This step of analysis was performed only for trial reasons. We did not obtain any significant results from our search for protein interaction predictions.

EXPERIMENTAL AND COMPUTATIONAL WORKFLOW: EVALUATION OF DATA

Acute lymphoblastic leukemia is the most frequent type of childhood malignancy. Rhabdomyosarcoma on the other hand is a rare type of sarcoma belonging to the family of primitive neuroectodermal tumors. The two malignancies are of different cell type (lymphoblast and myoblast respectively) although they both are of mesodermal origin. Understanding the origin of tumors on the "*poiesis"* level, such as hemopoiesis/lymphopoiesis (Brown et al., 2007) or myogenesis, may lead to the discovery of new therapeutic targets. Therefore, we hypothesized that the two cell types possess common characteristics, first of all due to their common developmental origin, and second due to their malignant character. We used a microarray and computational approach to examine whether there would be any common gene expression patterns among the two cell types. Also, as mentioned in the introduction of this chapter, such approaches should provide evidence for the elucidation of possible common mechanisms underlying oncogenesis or, in the worst case scenario, they would assist in the improvement of cost-effective drug design.

Cell Proliferation, Morphology, Cell Cycle

These steps were performed in order to gain insight into basic differences between the two cell types. Cells were allowed to grow under normal conditions for a total of 96 (from -24h to 72h) hours until they reached a final population of ~1.5×10^3 cells/μl. Cells were harvested and further processed n for cell cycle distribution as we have described it in the previous sections. As expected, cells manifested different *FS* vs. *SS* distributions (Figure 1A, 1B). CCRF-CEM cells manifested a more homogeneous population compared to TE-671 (Figure 1C, 1D). TE-671 cells manifested a cell population with greater variance both for size and granularity. This was expected since CCRF-CEM cells grow in suspension (Figure 1E) which gives more uniformity to their morphology, whereas TE-671 are adherent cells (Figure 1F) and when trypsinized produce a cell population with different morphology. Cell cycle distribution showed a different pattern of growth. CCRF-CEM cells rapidly entered the S-phase after 24h in culture (from -24h to 0h) and showed a cycling behavior thereafter (Figure 2A, 2B). This indicated that cell cycle for the CEM cells is rapid, since such interchange between cell

cycle phases was observed. No significant differences were observed in the G_2-phase between the two cell lines. Both cell lines, despite entering cell cycle phases in different percentages, follow the same pattern of growth, indicating a common pattern of reaction to environmental stimuli, in this case spatial-temporal growth. This pattern of growth was expected to be reflected on the gene expression profile at 72h. Since cells manifested different morphological types, cell cycle distribution patterns and proliferation dynamics, it was expected that most differences would be observed in their gene expression. This was true in part, since several significant genes were similarly regulated in both cell types, as we will explain further on.

Microarrays, Normalization and Clustering

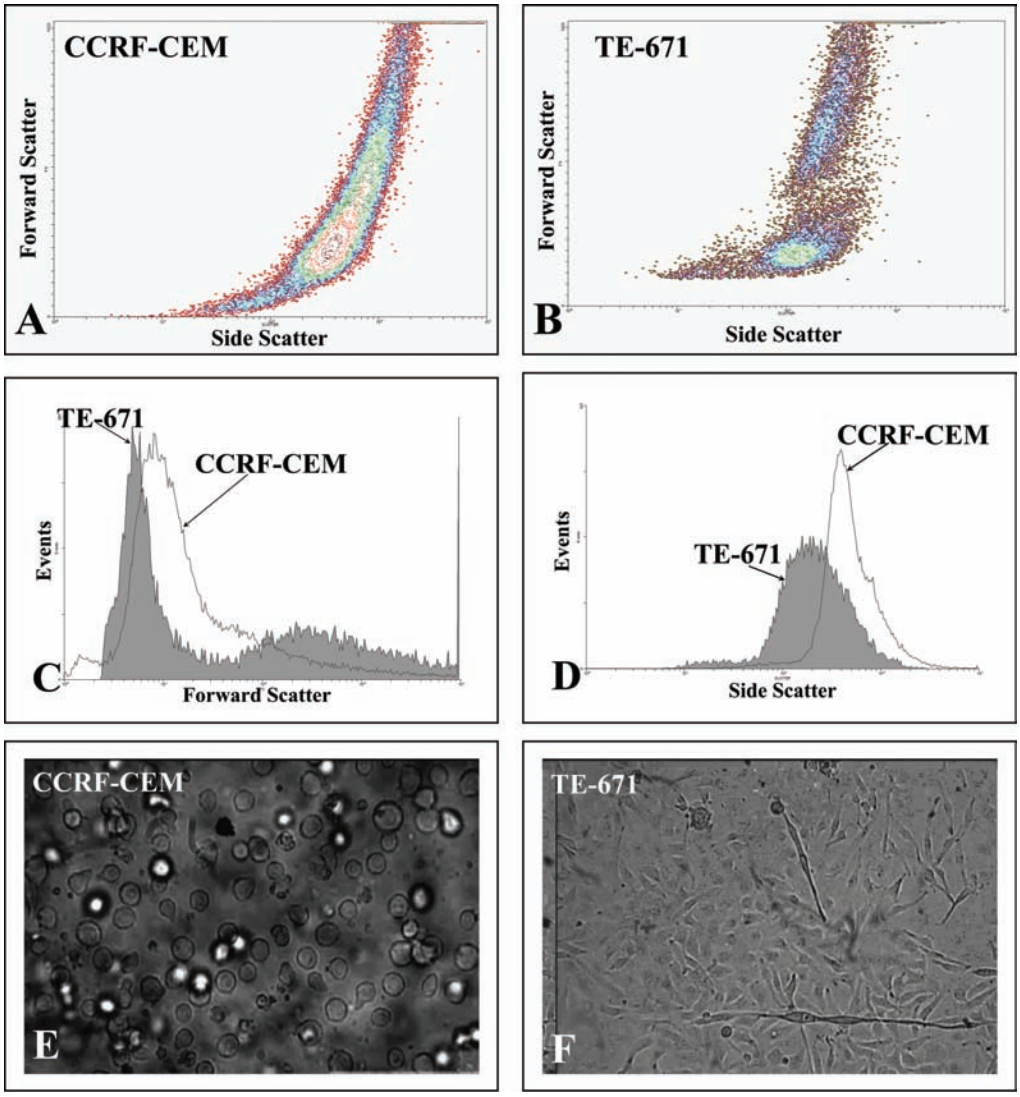
At 72h cells were harvested and further processed for microarray analysis. Several algorithms have been utilized for data normalization, as described in the previous section. Figure 3 presents the scatter plots for both microarray platforms (Figure 3A, 3E) and M-A plots for all genes on the chips (Figure 3B, 3F) as well as for genes filtered for SNR (Figure 3C, 3G) of the normalized data. In order to define over-, under- and equally-regulated genes, the 1-fold expression, on the $\log_2$ scale, was set as a threshold; for example, if a gene presented a $-1 \leq x \leq 1$ fold-value it was considered to be unchanged between the two cell types, and if a gene presented a $x<-1$ or $x>1$ it was considered to be under- and over-regulated respectively. Microarray analysis of the TAKARA and IMT platforms showed a total of 586 and 45 genes (including ESTs), i.e. good quality spots respectively. Overall, from the total of genes revealed, 228 are reportedly related to leukemia, 78 to rhabdomyosarcoma, 76 to the CCRF-CEM cell line and eight to the TE-671 cell line. At the same time, the normalization procedures followed were compared and the rank invariant normalization was the method of choice. Other normalization methods assume that the majority of genes remain unchanged. Yet, the rank invariant normalization makes no previous assumptions for gene expression and it calculates an invariant gene set that is used for further normalization. In Figure 4 the results of the rank invariant normalization are presented.

Since there was one microarray experiment, three main clusters were expected i.e. under-expressed, over-expressed and unchanged. However, in order to calculate the correlation between those genes an average hierarchical clustering analysis was performed (not shown). The only reason for performing cluster analysis in this dataset was to visualize neighboring genes and create the lists of genes that showed co-expression.

Chromosome Mapping

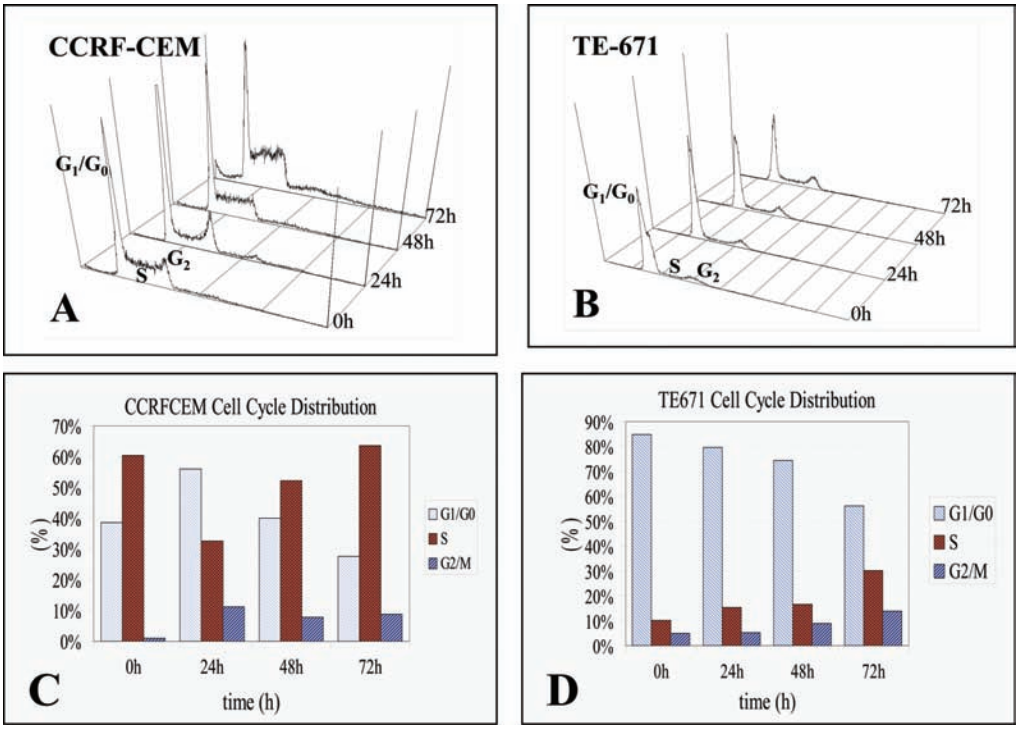
Genes were mapped on the 24 human chromosomes (Figure 5). There is a relatively equal distribution of genes on each chromosome, with the exception of chromosome 1 on which 57 (~9%) genes were mapped. Following this are chromosome 2 with 39 genes (6.6%), chromosome 6 with 31 genes (5.3%), chromosomes 4 and 10 with 28 and 27 genes respectively (~4.7%) and chromosomes 3, 5, 11, 12, 15, and 19 with ~25 genes (~4%). On the X chromosome 22 genes were mapped (3.7%). In Figure 5 B, we have calculated the median gene expression of all the genes belonging to a chromosome and have presented it in the form of a diagram. Both positive and negative values indicate expression measures; positive values mean over-expression of the genes in TE671 cells over those genes in CEM cells whereas negative values mean over-expression of CEM genes over TE671 genes. In this figure we observed that the diagram was in actual fact representing a difference between the two cell types. The CEM cells manifested a maximum of median activity on chromosome 8 while the TE671 cells manifested a peak median expression value on chromosome 15. In both cases median gene expression manifested an oscillatory

*Figure 1. Analysis of the CCRF-CEM and TE-671 cell lines characteristics as manifested by flow cytometry contour plot (***A, B***) respectively, not gated data of the forward (***C***) and side scatters (***D***) and morphology per microscopy (***E, F***) respectively.*



pattern. The pattern of gene expression across chromosomal distributions is a very interesting concept that needs further investigation. When we mapped the predicted TFBMs on chromosomes, we made an interesting observation. Despite the fact that maximum activity was observed for the TE671 cells at chromosome 15, no predicted TFBMs were mapped on this chromosome for unchanged, down-regulated, or up-regulated genes. This indicates that none of the known transcription factors regulating the present data set participates in gene activity on chromosome 15. On the other hand, on chromosome 8, where the higher CEM activity was observed, two transcription factors were mapped, the CEBPD (CCAAT/enhancer binding protein (C/EBP), delta) and the E2F5 (E2F transcription factor 5, p130-binding). These transcription factors appeared to be in the

*Figure 2. Cell cycle distribution for CCRF-CEM cells (***A, C***) and TE-671 (***B, D***). Experiments have been performed in duplicates. Data from a representative experiment are presented. Both cell types have been seeded at time -24h (see materials and methods section) and measured at 0h and every 24h thereafter. Both cell types enter S-phase at 0h with a rapid decrease at 24h and a gradual increase thereafter. G1-phase also manifests a gradual decrease from 24h to 72h. Finally, there is a small variance in the G2-phase over time.*



genes that remained unchanged and the same was predicted for the down-regulated genes, i.e. those genes over-expressed in the CEM cells (Figure 5 C, D, E, and F).

Gene Ontology (GO)

Due to the large number of genes revealed by the microarray experiments, we used gene ontology analysis in order to approach their functionality. GO analysis showed a high degree of relationship between the microarray genes and cellular processes or functions. The questions posed in the introduction were: a) which genes, under a certain limited set of genes, are typical for each cell type and at the same time appearing to the other, b) what are the similarities between the two cell types in tumor progression, cell cycle and secretory extracellular signaling, c) which are the common regulatory mechanisms for the two cell types, based on their expression profile and d) which cellular pathways are common to both cell types and whether these can be modeled in order to conclude common functions, e) is there any evidence for the origin of tumorigenesis?

Gene ontology relations for the general categories of *biological process*, *cellular compartment* and *molecular function* are presented in Figure 6 A, B and C. The genes of interest were those participating in biological process such as proliferation, cell cycle, differentiation, cell com-

*Figure 3. Representation of the microarray normalized data, in the form of scatter plots (***A***), (***E***), M-A plots for all genes on the two platforms (***B***), (***F***), M-A plots for the flagged genes, i.e. those that passed the SNR filtering criterion (***C***), (***G***) and intensity-dependent Z-scores (***D***) and (***H***) of the IntelliGene 4.8k chip and IMT 9.6k chip respectively.*

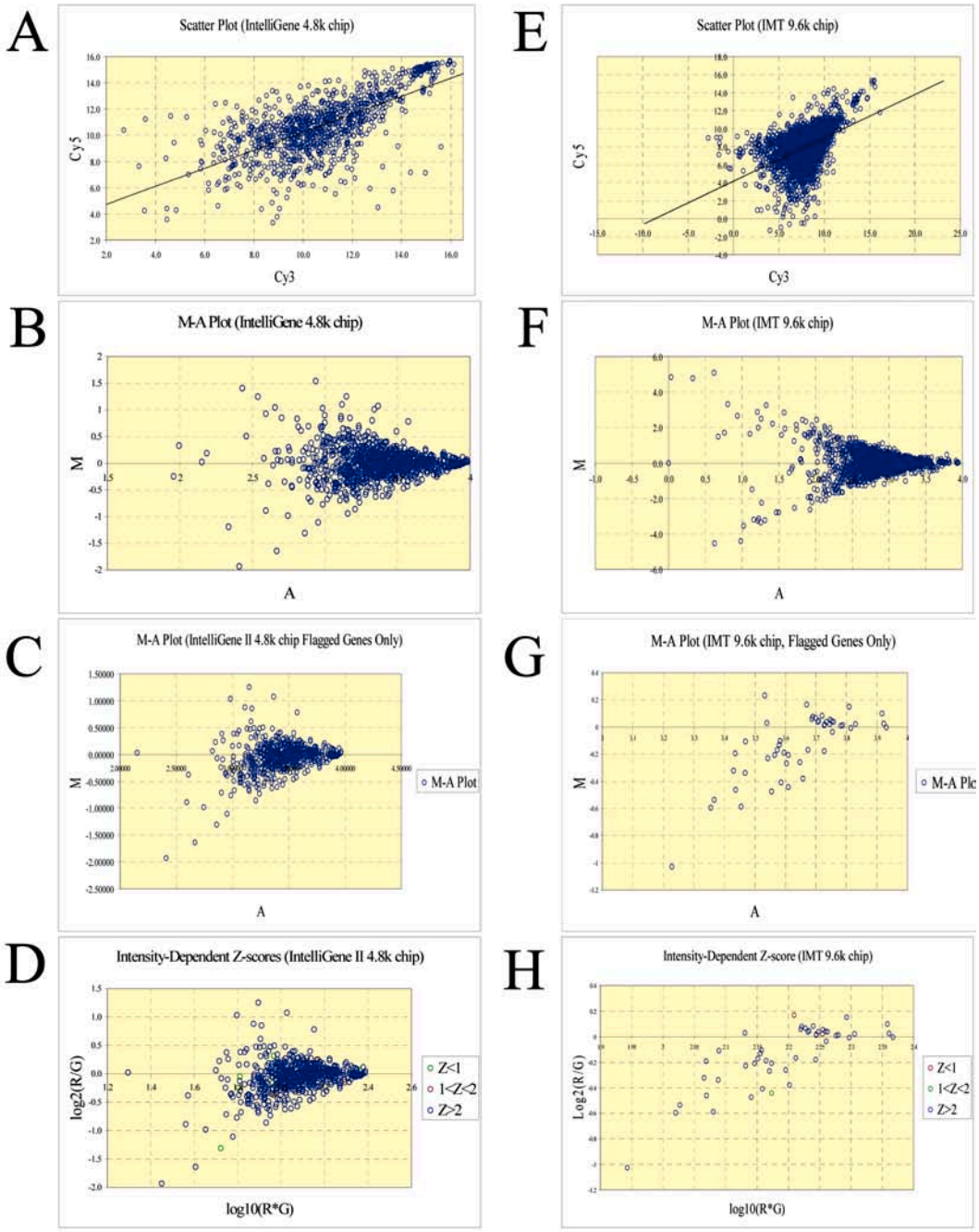
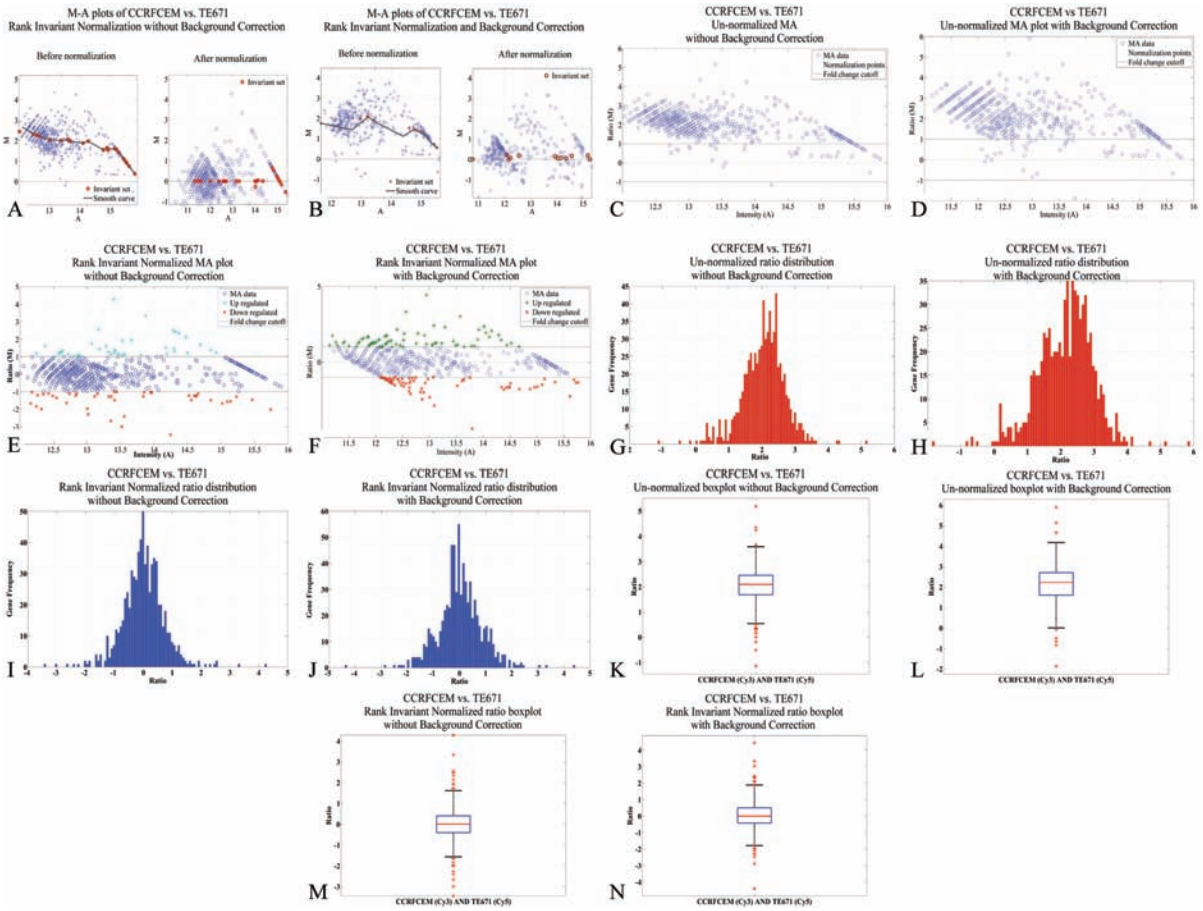


Figure 4. Rank invariant normalization of microarray dataset as compared with different variations, i.e. with and without background correction. The invariant genes used are presented in **A**, **B** *and in* **D**, **E**, **F** *the normalized dataset is presented with the cutoff lines for fold expression. In* **G**, **H**, **I**, **J** *the gene frequencies distributions are presented with and without background correction and with or without rank invariant normalization.*



munication (extracellular cell-cell signaling) and embryonal development.

Genes have been divided into seven major categories based on their function and unchanged expression profile: a) secretion molecule genes, which include CGRRF1, IGFBP7, PDGFB, PRC1, TGFB3, VEGFC, b) receptor/receptor binding/ cytokine activity genes (Figure 6. D, E, F), which include PDGFB, TGFB3, VEGFC, ADORA1, ALCAM, AVPR1A, CCRL1, CD40LG, CNR1, CSF3R, GABRA2, GLRA3, GPR44, HTR2B, IL2RA, IL9, LIFR, LRP1, MET, NPFF, PDYN, PTPN13, PTPN9, PTPRCAP, RXRB, SPRED2, TFRC, TLR3, TRIP13, c) cell communication/ cell-cell signaling genes (Figure 7. G, H, I), which include TGFB3, GABRA2, HTR2B, LIFR, NPFF, PDYN, SPRED2, ARHGAP6, CDC2, DDEF1, DDEF2, EFA6R, GNG12, KIAA0974, PLEKHG1, PSMA4, RAPGEF2, RUNDC3A, TIAM1, TME-PAI, d) cell cycle genes (including genes for mitosis regulation and cell cycle process) (Figure 7. J, K, L), which include CDC2, PRC1, AK021716, DLGAP5, H2AFV, PPAPDC1B, ZZEF1, e) cell proliferation genes, which include

*Figure 5. Chromosome distributions of known genes and predicted TFBMs. All differentially expressed genes were mapped on chromosomes (***A***) while median gene expression was mapped separately for each cell system based on their level of expression (designated as gene activity) (***B***). Predicted TFBMs were also mapped on chromosomes in order to find their distribution (***C***, **D**, **E**, **F***). Finally a heat-map of gene expression based on chromosomal distribution was also made in order to detect chromosomal areas that are mostly transcriptionaly active (***G***).*

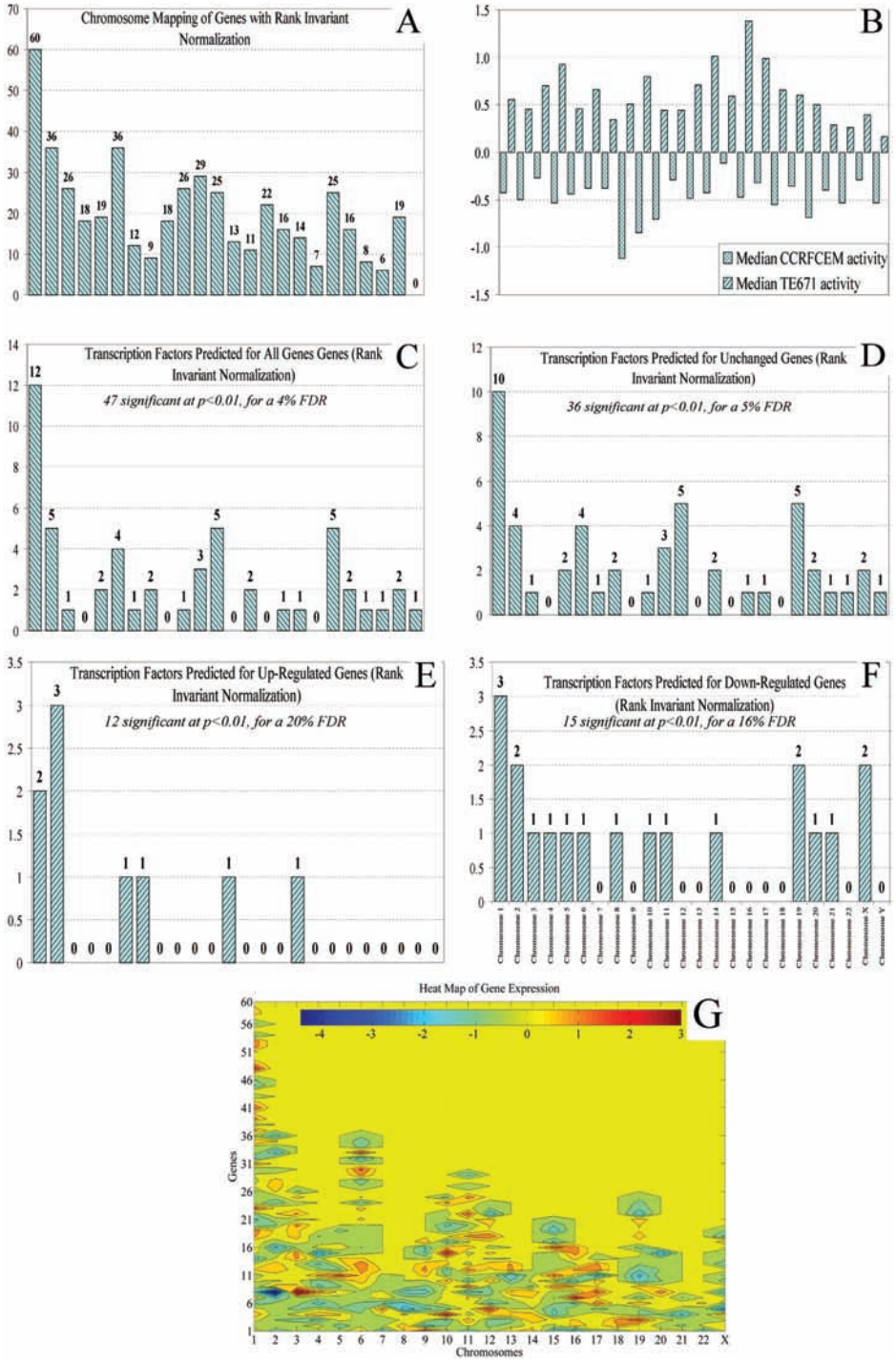
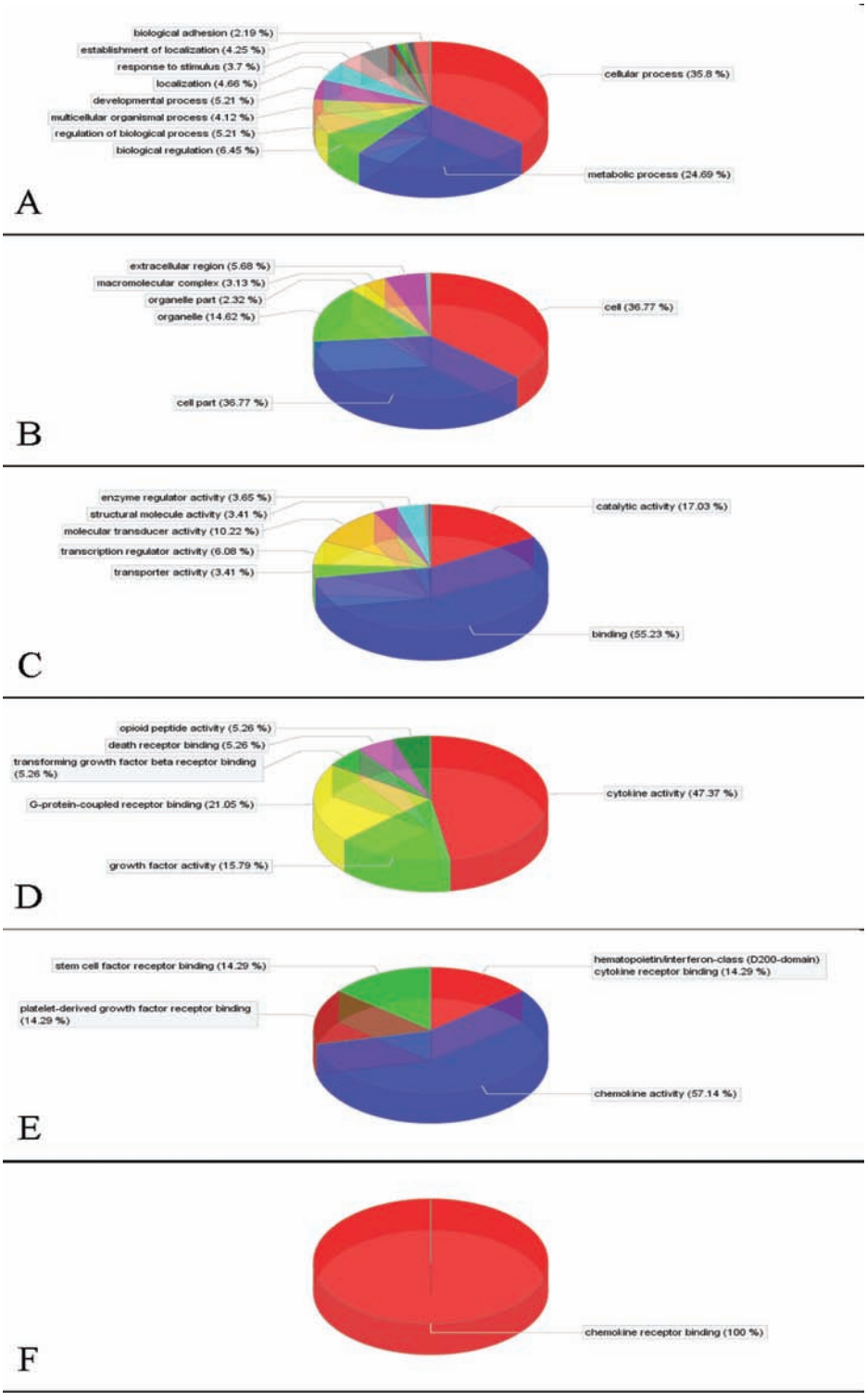


Figure 6. Gene Ontology (GO) results: Biological process (A), cellular component (B), molecular function (C), for the TAKARA and IMT platforms. Subsequent focusing onto the cellular process gave ontology results for receptor/receptor binding (D-F).



CDC2, GNL3, PDGFB, f) cell differentiation/ developmental/embryonic process genes (Figure 8. M, N, O), which include CDC2, VEGFC, CD40LG, IL2RA, ANXA4, BNIP3L, CD36, CFDP1, CUL7, FEM1B, FOXO1, MAB21L1, MBNL3, MMP2, NNAT, NP25, PDCD5, PDLIM7, PLAC1, RPS6KA3, RTTN, TCL1A, THBS4, ZNF313, and g) stem cell/stem cell differentiation related genes, which include CDC2, VEGFC, CD40LG, IL2RA, ANXA4, CFDP1, CUL7, FOXO1, MMP2, NNAT, NP25, PDCD5, PDLIM7, TCL1A, PDGFB, PRC1, DLGAP5, TGFB3, GABRA2, HTR2B, LIFR, NPFF, ARHGAP6, DDEF2, ADORA1, CCRL1, CNR1, CSF3R, GPR44, IL9, LRP1, MET, PTPN13, PTPN9, PTPRCAP, RXRB, TFRC, TLR3, CGRRF1, IGFBP7, FOSB, SIRT5, TCF4. Finally, in our search for negative or positive regulatory mechanisms, we found that two groups have studied which genes are negative and which are positive regulators of cellular processes. These two categories of genes include: ANXA4, CD36, FOSB, SIRT5, TCF4 for the genes with a negative regulatory effect, and FEM1B, as the gene with a positive regulatory effect. The detailed results of the *GO* analysis are presented in Table 1. Several genes have overlapping functions and this makes their analysis more complicated. In order to obtain a better view, genes have been sorted by function and by regulation, as presented in Table 2. where we have focused on genes specific for leukemia and rhabdomyosarcoma and their relations to secretion, receptors, cell cycle, cell proliferation and cell differentiation. Genes in the gray-shaded areas are considered to be unchanged or equally expressed in both cell types. In addition, in Figure 9 P-R, we present clusters of the differentially expressed genes with respect to gene annotations. Figure 9 P shows the genes that remain unchanged, Figure 6 Q shows the up-regulated genes, i.e. the ones activated in the TE671 cells, and Figure 6R shows the down-regulated genes, i.e. the ones activated in the CEM cells. Interestingly, Figure 9 R clearly shows that genes equally expresses in the two cell systems are genes that participate both in hematopoiesis and in myoblast maturation and skeletal muscle formation. This is in accordance with our previous notion of common developmental heritage while at the same time it shows that both cell types, despite their different phenotypes, retain common characteristics representative of their developmental history.

TFBMS

Transcription factor binding motif analysis (TFBM) has been performed with the complete population of the genes revealed by microarray analysis and also for each cluster separately. For this purpose, the on-line tool TELiS database was used; the TRANSFAC database was used for the identification of TFs. We analyzed the entire population of genes revealed by the microarray experiment for T*Fs* resulting in a total of 44 T*Fs* with a FDR 4% for $p<0.01$. Each cluster produced was analyzed separately for TFBMs in an attempt to detect T*FS* appearing only to one category of genes. In particular, three T*FS* were predicted to be common for all genes irrespectively of their expression cap (under-represented), Elk-1 (over-represented) and C/EBP (under-represented). Among the four types of analyses performed, one group of genes appeared to be regulating the unchanged genes. In addition, the analysis of the cluster containing the unchanged genes revealed that one of the TF CDP (over-represented) appeared only for this cluster. In Table 3 the TELiS results for all genes and for each cluster separately are presented in detail. The next step in our analysis was to detect which T*Fs* were predicted specifically for certain genes, as presented in Table 4. Combined analysis of the genes remaining unchanged and the transcription factors regulating them predicted eight T*FS*: C/EBPbeta (under-represented), c-Ets-1(p54) (over-represented), CREB (over-represented), c-Rel (over-represented), GATA-1 (under-represented),

Figure 7. Cell communication/cell-cell signaling (G-I), cell cycle (J-L).

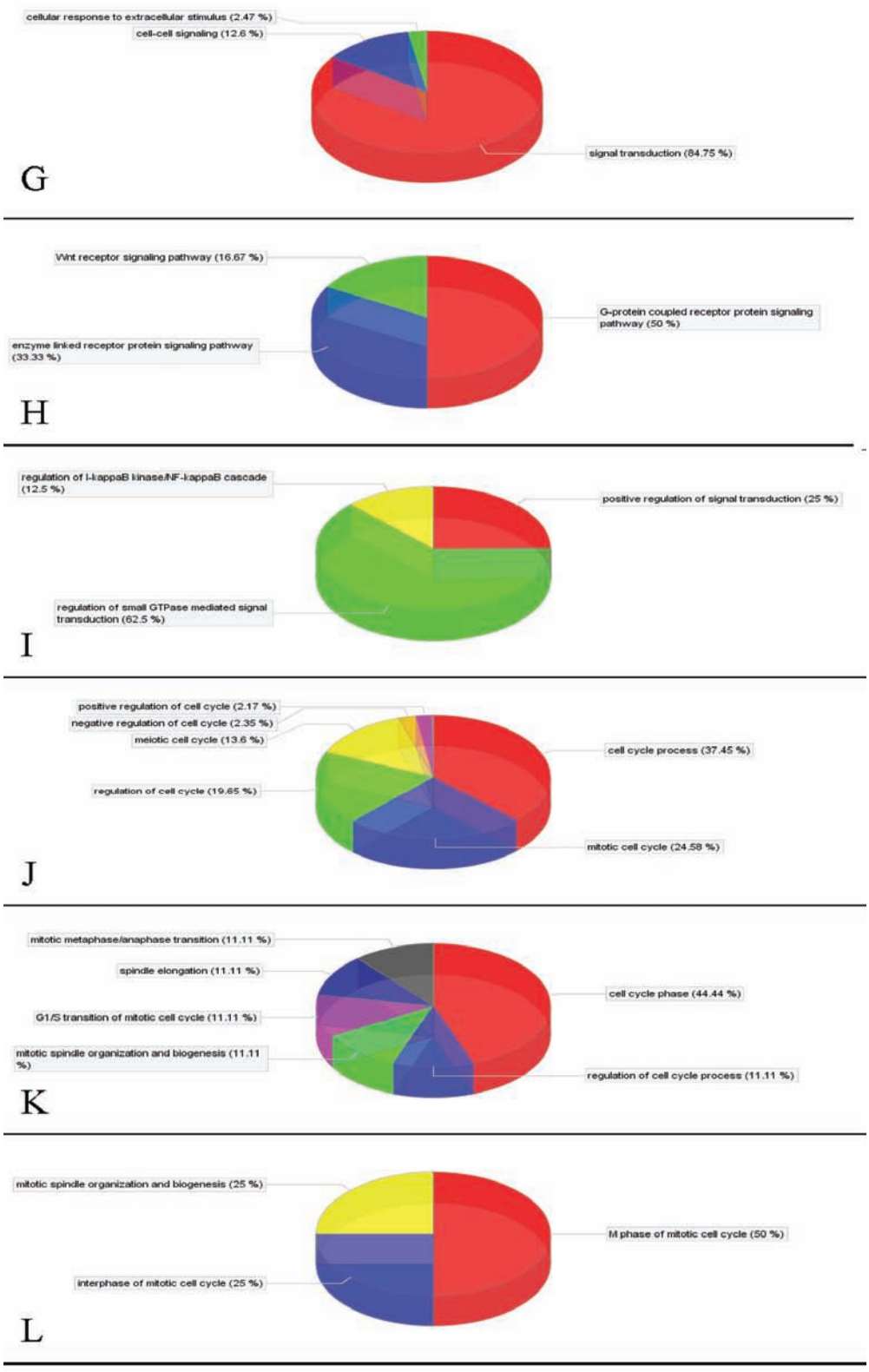
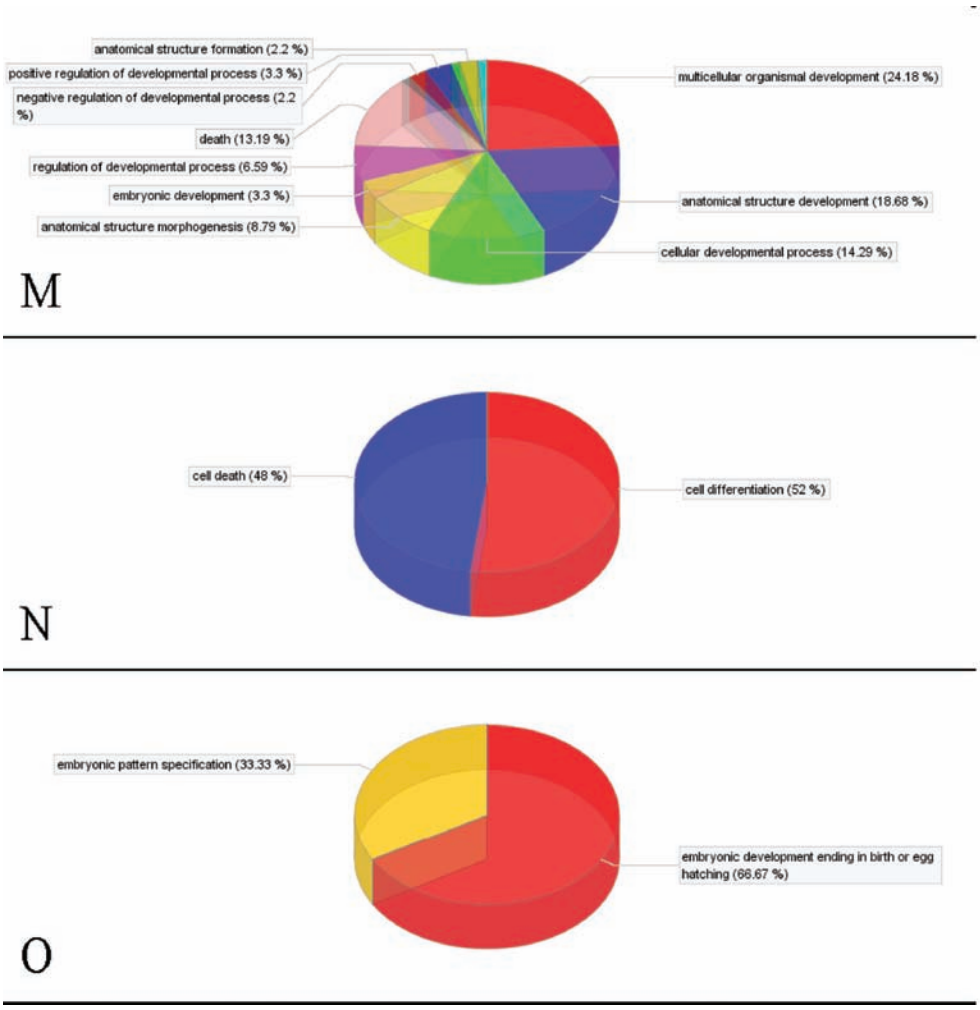


Figure 8. cell differentiation/developmental/embryonic process (M-O).



GATA-X (under-represented), MyoD (under-represented), Tax/CREB (over-represented).

BioNetwork Analysis

The relationships between genes have been investigated using two tools, PubGene (www.pubgene.org) and the MILANO microarray annotation tool.[1] Relations have also been searched for the specified genes with reference to cell cycle, cell proliferation, stem cell and developmental gene expression. Entering those terms in the website which the above link directs to produces the respective results.

Pathway Analysis

In order to understand the common mechanisms underlying the two different types of cancer we applied a pathway analysis. We aimed to understand which genes participate in which pathways and how they interact. Due to the complexity of the pathways and the transcripts revealed by the microarray analysis, we used the following approach: First of all, with the help of the Pathway Explorer software, we searched all available databases for the presence of the genes revealed by the microarray analysis in known pathways. Then we focused on the cell cycle pathway and

Table 1. List of genes selected for their function as revealed by GO analysis and partly hand curated

		secretion molecule	Receptor/receptor binding/cytokine activity	cell communication/cell-cell signaling	cell cycle	cell proliferation	cell differentiation/ developmental process/ embryonic development	negative regulation of cellular process	positive regulation of cellular process	Stem cell/ stem cell differentiation	Fold Expression
UniqueID	Gene Name										
NM_001105	ACVR1		X		X					X	-2.51450
NM_000674	ADORA1		X							X	0.35570
AK021716	AK021716				X						0.10320
Y10183	ALCAM		X								0.80360
NM_001153	ANXA4						X	X		X	-0.41550
NM_015957	APIP						X				1.02350
NM_001174	ARHGAP6			X						X	-0.00700
NM_001178	ARNTL		X							X	2.28990
NM_000706	AVPR1A		X								0.08890
NM_001196	BID		X	X			X				-1.01530
AL132665	BNIP3L						X				-0.38220
NM_025113	C13orf18			X							2.60510
NM_002986	CCL11	X	X							X	1.21250
NM_016557	CCRL1		X							X	-0.24970
BX091860.1	CD36						X	X			-0.11330
NM_000074	CD40LG		X				X			X	0.92110
NM_001786	CDC2			X	X	X	X			X	-0.28130
NM_017632	CDKN2AIP			X					X	X	-1.21870
NM_006324	CFDP1						X			X	-0.10830
NM_006568	CGRRF1	X								X	0.38330
NM_006614	CHL1						X			X	1.28840
NM_016083	CNR1		X							X	0.96720
NM_001842	CNTFR		X	X						X	-1.53090
NM_031476	CRISPLD2						X				1.45690
NM_000760	CSF3R		X							X	0.18160
NM_014780	CUL7						X			X	-0.65120
NM_005409	CXCL11	X	X							X	-1.74910
NM_006419	CXCL13	X	X							X	-2.84680
NM_001504	CXCR3		X							X	-1.93560
AB033075	DDEF1			X							0.15180
NM_003887	DDEF2			X						X	0.46100
NM_006182	DDR2		X							X	1.23050
NM_014750	DLGAP5				X					X	-0.16760

continued on following page

Table 1. Continued

		secretion molecule	Receptor/receptor binding/cytokine activity	cell communication/cell-cell signaling	cell cycle	cell proliferation	cell differentiation/ developmental process/ embryonic development	negative regulation of cellular process	positive regulation of cellular process	Stem cell/ stem cell differentiation	Fold Expression
AB051558	DOCK7						X				1.04090
NM_001938	DR1							X		X	1.83600
AK022156	DRIP80		X								1.13060
NM_006579	EBP			X						X	1.35930
NM_015310	EFA6R			X							-0.85590
NM_005228	EGFR		X				X			X	1.54280
NM_000119	EPB42						X				-1.62140
NM_001999	FBN2						X			X	1.30600
AB007856	FEM1B						X		X		0.09350
NM_000142	FGFR3	X	X	X						X	1.86900
NM_002019	FLT1		X				X			X	1.54220
NM_002027	FNTA			X							-1.62210
NM_006732	FOSB							X		X	0.59260
NM_002015	FOXO1						X			X	-0.52260
NM_000807	GABRA2		X	X						X	-0.11640
NM_003899	GEF			X						X	-1.23920
NM_006529	GLRA3		X								-0.17370
AK055914	GNG12			X							0.88830
NM_014366	GNL3					X					0.19610
NM_005292	GPR18		X	X							2.44570
NM_004778	GPR44		X							X	-0.81440
NM_000826	GRIA2		X							X	1.76800
NM_012341	GTPBP4				X						1.50740
AK025785	H2AFV				X						-0.13740
NM_004494	HDGF	X			X					X	2.25220
NM_000867	HTR2B		X	X						X	-0.69180
NM_002111	HTT						X			X	2.61230
NM_001553	IGFBP7	X								X	0.65840
NM_003855	IL18R1		X	X						X	1.53240
NM_000417	IL2RA		X				X			X	-0.05850
NM_000590	IL9		X							X	-0.08720
NM_002203	ITGA2		X							X	1.42800
NM_000885	ITGA4		X							X	1.93300
BC015394	KIAA0974			X							0.25090

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Table 1. Continued

		secretion molecule	Receptor/receptor binding/cytokine activity	cell communication/cell-cell signaling	cell cycle	cell proliferation	cell differentiation/ developmental process/ embryonic development	negative regulation of cellular process	positive regulation of cellular process	Stem cell/ stem cell differentiation	Fold Expression
NM_002290	LAMA4		X							X	-1.58140
NM_002310	LIFR		X	X						X	0.05560
NM_002332	LRP1		X							X	0.96870
NM_005584	MAB21L1						X				-0.21650
NM_003682	MADD			X						X	1.09220
NM_002446	MAP3K10						X		X	X	-1.95770
AL133625	MBNL3						X				0.59340
NM_000245	MET		X							X	-0.88820
NM_032711	MGC13090						X			X	2.95170
NM_004530	MMP2						X			X	-0.28880
NM_004536	NAIP						X			X	1.32050
NM_006534	NCOA3		X	X						X	-3.08980
NM_016418	NF2							X		X	1.45030
NM_021079	NMT1						X			X	-2.24310
NM_005386	NNAT						X			X	-0.69920
NM_013259	NP25						X			X	0.23170
NM_003717	NPFF		X	X						X	0.18860
NM_002530	NTRK3		X							X	2.53430
NM_016553	NUP62			X						X	1.48730
NM_004708	PDCD5						X			X	0.09580
NM_002608	PDGFB	X	X			X				X	-0.78240
NM_016205	PDGFC	X	X				X			X	1.56170
NM_005451	PDLIM7						X			X	-0.27470
NM_024411	PDYN		X	X							0.49460
NM_021796	PLAC1						X				-0.14430
AB033035	PLEKHG1			X							0.30110
NM_032483	PPAPDC1B				X						0.30310
NM_006243	PPP2R5A			X							1.66140
NM_003981	PRC1	X			X					X	0.39000
AK055714	PSMA4			X							-0.89000
AK024986	PTEN				X						2.94740
NM_006264	PTPN13		X							X	-0.17040
NM_002833	PTPN9		X							X	-0.51980
NM_005608	PTPRCAP		X							X	-0.29690

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Table 1. Continued

		secretion molecule	Receptor/receptor binding/cytokine activity	cell communication/cell-cell signaling	cell cycle	cell proliferation	cell differentiation/ developmental process/ embryonic development	negative regulation of cellular process	positive regulation of cellular process	Stem cell/ stem cell differentiation	Fold Expression
NM_014247	RAPGEF2			X							-0.37350
NM_002924	RGS7			X							3.08800
AB018283	RHOBTB1			X							2.83340
AK022869	RPS6KA3						X				0.48650
BC007359	RTTN						X				0.23600
NM_006695	RUNDC3A			X							0.97270
NM_021976	RXRB		X							X	-0.91460
NM_031244	SIRT5							X		X	-0.21510
NM_003825.2	SNAP23			X							1.50180
AK056479	SPRED2		X	X							-0.24150
NM_012448	STAT5B						X			X	1.29530
NM_014574	STRN3				X					X	1.48520
NM_022771	TBC1D15			X							2.81000
NM_003199	TCF4							X		X	0.78030
NM_021966	TCL1A						X			X	0.21240
NM_003234	TFRC		X							X	-0.18980
NM_003238	TGFB2	X					X			X	-1.02150
NM_003239	TGFB3	X	X	X						X	0.87070
NM_004257	TGFBRAP1		X	X						X	1.16430
NM_003248	THBS4						X				-0.49600
U90902	TIAM1			X							-0.72830
NM_003265	TLR3		X							X	0.49000
AF305616	TMEPAI			X							0.01560
NM_002160	TNC		X							X	1.66420
NM_001244	TNFSF8		X				X		X	X	-4.13020
NM_006073	TRDN		X							X	-2.93200
NM_004237	TRIP13		X								0.18310
NM_005429	VEGFC	X	X				X			X	0.20610
NM_002995	XCL1	X	X							X	1.78420
NM_019028	ZDHHC13			X					X		-1.16820
BC013695	ZNF313						X				-0.44100
AB007859	ZZEF1				X						0.01690

Table 2. List of unchanged genes from Table 1. Genes are included whose fold expression remains within the threshold of -1<x<1

		secretion molecule	Receptor/receptor binding/cytokine activity	cell communication/cell-cell signaling	cell cycle	cell proliferation	cell differentiation/ developmental process/ embryonic development	negative regulation of cellular process	positive regulation of cellular process	Stem cell/ stem cell differentiation	Fold Expression
UniqueID	**Gene Name**										
NM_000674	ADORA1		X							X	0.35570
AK021716	AK021716				X						0.10320
Y10183	ALCAM		X								0.80360
NM_001153	ANXA4						X	X		X	-0.41550
NM_001174	ARHGAP6			X						X	-0.00700
NM_000706	AVPR1A		X								0.08890
AL132665	BNIP3L						X				-0.38220
NM_016557	CCRL1		X							X	-0.24970
BX091860.1	CD36						X	X			-0.11330
NM_000074	CD40LG		X				X			X	0.92110
NM_001786	CDC2			X	X	X	X			X	-0.28130
NM_006324	CFDP1						X			X	-0.10830
NM_006568	CGRRF1	X								X	0.38330
NM_016083	CNR1		X							X	0.96720
NM_000760	CSF3R		X							X	0.18160
NM_014780	CUL7						X			X	-0.65120
AB033075	DDEF1			X							0.15180
NM_003887	DDEF2			X						X	0.46100
NM_014750	DLGAP5				X					X	-0.16760
NM_015310	EFA6R			X							-0.85590
AB007856	FEM1B						X		X		0.09350
NM_006732	FOSB							X		X	0.59260
NM_002015	FOXO1						X			X	-0.52260
NM_000807	GABRA2		X	X						X	-0.11640
NM_006529	GLRA3		X								-0.17370
AK055914	GNG12			X							0.88830
NM_014366	GNL3					X					0.19610
NM_004778	GPR44		X							X	-0.81440
AK025785	H2AFV				X						-0.13740
NM_000867	HTR2B		X	X						X	-0.69180
NM_001553	IGFBP7	X								X	0.65840
NM_000417	IL2RA		X				X			X	-0.05850
NM_000590	IL9		X							X	-0.08720
BC015394	KIAA0974			X							0.25090
NM_002310	LIFR		X	X						X	0.05560
NM_002332	LRP1		X							X	0.96870

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Table 2. Continued

		secretion molecule	Receptor/receptor binding/cytokine activity	cell communication/cell-cell signaling	cell cycle	cell proliferation	cell differentiation/ developmental process/ embryonic development	negative regulation of cellular process	positive regulation of cellular process	Stem cell/ stem cell differentiation	Fold Expression
NM_005584	MAB21L1						X				-0.21650
AL133625	MBNL3						X				0.59340
NM_000245	MET		X							X	-0.88820
NM_004530	MMP2						X			X	-0.28880
NM_005386	NNAT						X			X	-0.69920
NM_013259	NP25						X			X	0.23170
NM_003717	NPFF		X	X						X	0.18860
NM_004708	PDCD5						X			X	0.09580
NM_002608	PDGFB	X	X			X				X	-0.78240
NM_005451	PDLIM7						X			X	-0.27470
NM_024411	PDYN		X	X							0.49460
NM_021796	PLAC1						X				-0.14430
AB033035	PLEKHG1			X							0.30110
NM_032483	PPAPDC1B				X						0.30310
NM_003981	PRC1	X			X					X	0.39000
AK055714	PSMA4			X							-0.89000
NM_006264	PTPN13		X							X	-0.17040
NM_002833	PTPN9		X							X	-0.51980
NM_005608	PTPRCAP		X							X	-0.29690
NM_014247	RAPGEF2			X							-0.37350
AK022869	RPS6KA3						X				0.48650
BC007359	RTTN						X				0.23600
NM_006695	RUNDC3A			X							0.97270
NM_021976	RXRB		X							X	-0.91460
NM_031244	SIRT5							X		X	-0.21510
AK056479	SPRED2		X	X							-0.24150
NM_003199	TCF4							X		X	0.78030
NM_021966	TCL1A						X			X	0.21240
NM_003234	TFRC		X							X	-0.18980
NM_003239	TGFB3	X	X	X						X	0.87070
NM_003248	THBS4						X				-0.49600
U90902	TIAM1			X							-0.72830
NM_003265	TLR3		X							X	0.49000
AF305616	TMEPAI			X							0.01560
NM_004237	TRIP13		X								0.18310
NM_005429	VEGFC	X	X				X			X	0.20610
BC013695	ZNF313						X				-0.44100
AB007859	ZZEF1				X						0.01690

Table 3. List of all transcription factors as revealed by TELiS analysis. Italicized TFs are under-represented while underlined TFs are over-represented. The whole set of genes was first analyzed with TELiS database, which gave 44 significant results for a 4% FDR at p<0.01. Then genes were divided into the three main groups, as revealed by gene clustering, and an additional analysis was performed with each cluster separately. Down-regulated genes revealed 13 significant results for an 18% FDR at p<0.01, up-regulated genes revealed 28 significant results for an 8% FDR at p<0.01 and unchanged genes revealed 28 significant results for an 8% FDR at p<0.01. These TFs were sorted in order to find patterns of similar regulation among the different clusters. At the end of this table are presented the TFS found in the clusters when analyzed separately, which did not appear in the complete gene analysis.

		TRANSFAC (1 Group Analysis)				
		Transcription Factors (600/90)				
		44 significant at p <.01, for a 4% false discovery rate. Details	**13 significant at p <.01, for a 18% false discovery rate. Details**	**28 significant at p <.01, for a 8% false discovery rate. Details**	**28 significant at p <.01, for a 8% false discovery rate. Details**	
Symbol	**Official Symbol**	**TELiS Symbol**	**TELiS Symbol**	**TELiS Symbol**	**TELiS Symbol**	**Description**
Transcription Factor Common for both Down-regulated, Unchanged andUp-regulated Genes						
		All Genes	**Down-regulated**	**Unchanged**	**Up-regulated**	
cap	*CAP1*	*3.V$CAP_01*	*7.V$CAP_01*	*7.V$CAP_01*	*3.V$CAP_01*	cap signal for transcription initiation
Elk-1	ELK1	5.V$ELK1_02	3.V$ELK1_02	13.V$ELK1_02	10.V$ELK1_02	Elk-1
C/EBP	*CEBPA*	*18.V$CEBP_01*	*10.V$CEBP_01*	*26.V$CEBP_01*	*19.V$CEBP_01*	CCAAT/enhancer binding protein
GATA-1	*GATA1*	*6.V$GATA1_01*	*5.V$GATA1_01*	*3.V$GATA1_01*		GATA-binding factor 1
GATA-2	*GATA2*	*8.V$GATA2_01*	*6.V$GATA2_01*	*5.V$GATA2_01*		GATA-binding factor 2
GATA-3	*GATA3*	*10.V$GATA3_01*	*11.V$GATA3_01*	*6.V$GATA3_01*		GATA-binding factor 3
YY1	*YY1*	*16.V$YY1_01*	*12.V$YY1_01*	*19.V$YY1_01*		Yin and Yang 1
STAT1	STAT1	24.V$STAT1_01	13.V$STAT1_01	17.V$STAT1_01		signal transducer and activator of transcription 1
SRF	SRF	39.V$SRF_01	1.V$SRF_01			serum response factor
AP-2	TFAP2A	4.V$AP2_Q6		2.V$AP2_Q6	11.V$AP2_Q6	activator protein 2
Sp1	SP1	7.V$SP1_Q6		11.V$SP1_Q6	2.V$SP1_Q6	stimulatig protein 1
Sp1	SP1	9.V$SP1_01		9.V$SP1_01	4.V$SP1_01	stimulatig protein 1
CdxA		*11.V$CDXA_02*		*10.V$CDXA_02*	*16.V$CDXA_02*	CdxA
GC		12.V$GC_01		14.V$GC_01	6.V$GC_01	GC box elements
14.V$CDXA_01	*CdxA*	*13.V$CDXA_01*		*8.V$CDXA_01*		CdxA
22.V$OCT1_03	*1-Oct*	*15.V$OCT1_03*		*12.V$OCT1_03*		octamer factor 1
26.V$GATA1_04	*GATA-1*	*19.V$GATA1_04*		*23.V$GATA1_04*		GATA-binding factor 1
25.V$CREB_02	CREB	22.V$CREB_02		24.V$CREB_02		cAMP-responsive element binding protein
21.V$E2F_02	*E2F*	*26.V$E2F_02*		27.V$E2F_02		E2F

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Table 3. Continued

		TRANSFAC (1 Group Analysis)				
		Transcription Factors (600/90)				
		44 significant at p <.01, for a 4% false discovery rate. Details	13 significant at p <.01, for a 18% false discovery rate. Details	28 significant at p <.01, for a 8% false discovery rate. Details	28 significant at p <.01, for a 8% false discovery rate. Details	
Symbol	**Official Symbol**	**TELiS Symbol**	**TELiS Symbol**	**TELiS Symbol**	**TELiS Symbol**	**Description**
	Tax/CREB	**1.V$TAXCREB_02**		**4.V$TAXCREB_02**		Tax/CREB complex
	Brachyury	**2.V$BRACH_01**		**1.V$BRACH_01**		Brachyury
	c-Ets-1(p54)	**17. V$CETS1P54_01**		**18. V$CETS1P54_01**		c-Ets-1(p54)
	GATA-X	***21.V$GATA_C***		***15.V$GATA_C***		GATA binding site
	GATA-1	***23.V$GATA1_02***		***20.V$GATA1_02***		GATA-binding factor 1
	MyoD	***25.V$MYOD_Q6***		***22.V$MYOD_Q6***		myoblast determining factor
	CREB	**27.V$CREB_Q4**		**28.V$CREB_Q4**		cAMP-response element binding protein
	C/EBPbeta	***31.V$CEBPB_01***		***25.V$CEBPB_01***		CCAAT/enhancer binding protein beta
	c-Rel	**44.V$CREL_01**		**16.V$CREL_01**		c-Rel
5.V$NRF2_01	**NRF-2**	**14.V$NRF2_01**				nuclear respiratory factor 2
24.V$SRY_02	***SRY***	***20.V$SRY_02***				sex-determining region Y gene product
13.V$S8_01	***S8***	***28.V$S8_01***				S8
23.V$CREB_Q2	**CREB**	**29.V$CREB_Q2**				cAMP-response element-binding protein
15.V$SRY_01	***SRY***	***30.V$SRY_01***				sex-determining region Y gene product
20.V$ATF_01	**ATF**	**35.V$ATF_01**				activating transcription factor
NGFI-C	**EGR4**	**36.V$NGFIC_01**			**7.V$NGFIC_01**	nerve growth factor-induced protein C
CRE-BP1	**ATF2**	**33.V$CREBP1_Q2**			**18.V$CREBP1_Q2**	CRE-binding protein 1
MZF1	**MZF1**	**32.V$MZF1_01**				MZF1
Sox-5	***SOX5***	***34.V$SOX5_01***				Sox-5
N-Myc	**MYCN**	**37.V$NMYC_01**				N-Myc
Egr-1	**EGR1**	**38.V$EGR1_01**				Egr-1/Krox-24/NGFI-A immediate-early gene product
USF	***USF1***	***40.V$USF_02***				upstream stimulating factor
USF	***USF1***	***41.V$USF_C***				USF binding site
GATA-1	***GATA1***	***42.V$GATA1_03***				GATA-binding factor 1
Pbx-1	**PBX1**	***43.V$PBX1_01***				Pbx-1

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Table 3. Continued

		TRANSFAC (1 Group Analysis)				
		Transcription Factors (600/90)				
		44 significant at p <.01, for a 4% false discovery rate. Details	13 significant at p <.01, for a 18% false discovery rate. Details	28 significant at p <.01, for a 8% false discovery rate. Details	28 significant at p <.01, for a 8% false discovery rate. Details	
Symbol	**Official Symbol**	**TELiS Symbol**	**TELiS Symbol**	**TELiS Symbol**	**TELiS Symbol**	**Description**
		All Genes	**Unique for Down-regulated**	**Unique for Unchanged**	**Unique for Up-regulated**	
CCAAT	**NFYA**		**2.V$CAAT_C**			Retroviral CCAAT box
SRF	**SRF**		**4.V$SRF_Q6**			serum response factor
IRF-2	**IRF2**		**8.V$IRF2_01**			interferon regulatory factor 2
IRF-1	**IRF1**		**9.V$IRF1_01**			interferon regulatory factor 1
CDP	**CUX1**			**21.V$CDP_01**		cut-like homeodomain protein
Egr-1	**EGR1**				**1.V$EGR1_01**	Egr-1/Krox-24/NGFI-A immediate-early gene product
ARP-1	**APOA1**				**8.V$ARP1_01**	apolipoprotein AI regulatory protein 1
					9.V$EGR3_01	early growth response gene 3 product
					12.V$ELK1_01	**Elk-1**
					17.V$EGR2_01	Egr-2/Krox-20 early growth response gene product
					27.V$IK2_01	Ikaros 2
					28.V$TATA_01	cellular and viral TATA box elements

on signal transduction pathways. We prepared an overview of the pathway-to-pathway interactions based on the KEGG pathway database (Figure 10). We discovered a common presence of extracellular signaling molecules. The two cell types share three main axons of signaling, among others, for cell cycle regulation and cell proliferation: a) an ECM-receptor/focal adhesion/MAPK signal transduction, b) a Cytokine-cytokine-receptor/MAPK/ cell cycle signal transduction pathway and c) a Jak-STAT/MAPK pathway signal transduction. All three combinations participate in cell proliferation, differentiation, cell fate determination and anti-apoptosis. In Figures 12, 13, 14, and 15, we have prepared a summary of these pathways with the genes that remain unchanged for the two cell types.

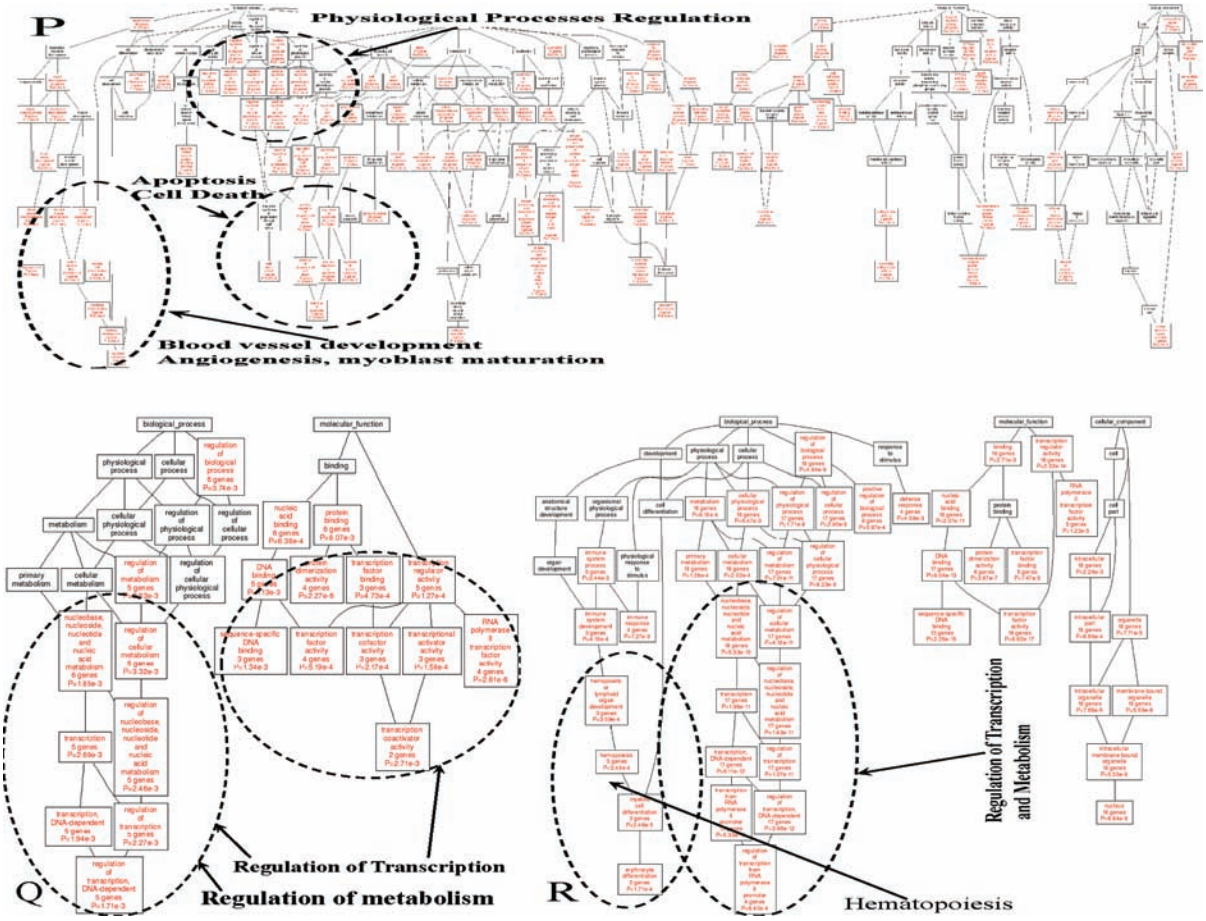
Going into more detail in the predicted pathways, we have classified them in categories and the observed findings are listed below.

Cytokine/Cytokine-Receptor Interactions

The Interleukins

Our analysis has predicted 25 unique targets mapped on the cytokine/cytokine-receptor pathway. Cytokines are important molecules participating in hemopoietic stem cell differentia-

Figure 9. Clusters of gene functions are presented in P-R, as revealed by webgestalt analysis tool.



tion, gamma interleukins in particular, reported to have proliferative and differentiation effects on T-cell acute lymphoblastic leukemia primary cells (Barata et al., 2004). Despite the differences between normal and malignant blood stem cells, both cell types use the cytokine signaling for progression and differentiation (Kiss, Benko, & Kovacs, 2004). In our analysis IL8 and IL9, along with the IL2 receptor, where predicted.

The Chemokines

None of the CXCL or the CXCR family of molecules appeared to have similar expression patterns, but are predicted to be over-expressed in the CCRF-CEM cell line. This confirms the predictions made by the microarray experiment. Interestingly, it has been reported that for certain cell lines, in order to produce these chemokines, the constitutive presence of NF-kB in the nucleus is required (Hiroi & Ohmori, 2003a). The CCRF-CEM cells have shown that the NF-kB is constitutively present in the nucleus (Lambrou, et al., 2009), thus supporting the evidence of chemokine expression.

The Hematopoietins

The two cell types seem to also share a common expression for the LIFR gene. This protein participates in embryo implantation, in the differentiation of neural stem cells to astrocytes (de

Table 4. List of the GO genes with unchanged fold expression and their respective predicted TFs. Under-represented TFs are italicized and the over-represented TFs are underlined.

		Fold Expression	*C/EBPbeta*	c-Ets-1(p54)	CREB	c-Rel	*GATA-1*	*GATA-X*	*MyoD*	Tax/CREB
NM_000674	ADORA1	0.35570	**X**						**X**	
AK021716	AK021716	0.10320	**X**							
Y10183	ALCAM	0.80360	**X**				**X**		**X**	
NM_001153	ANXA4	-0.41550	**X**				**X**	**X**		
NM_001174	ARHGAP6	-0.00700							**X**	
NM_000706	AVPR1A	0.08890	**X**				**X**	**X**	**X**	
AL132665	BNIP3L	-0.38220	**X**		**X**	**X**			**X**	
NM_016557	CCRL1	-0.24970	**X**	**X**			**X**	**X**		
NM_000074	CD40LG	0.92110		**X**			**X**	**X**		
NM_001786	CDC2	-0.28130	**X**						**X**	
NM_006324	CFDP1	-0.10830	**X**	**X**			**X**	**X**	**X**	
NM_006568	CGRRF1	0.38330	**X**							
NM_016083	CNR1	0.96720	**X**			**X**				
NM_000760	CSF3R	0.18160	**X**	**X**					**X**	
NM_014780	CUL7	-0.65120	**X**			**X**				
AB033075	DDEF1	0.15180	**X**	**X**					**X**	
NM_003887	DDEF2	0.46100							**X**	
NM_014750	DLGAP5	-0.16760	**X**	**X**		**X**			**X**	
NM_015310	EFA6R	-0.85590	**X**				**X**			
AB007856	FEM1B	0.09350				**X**				
NM_006732	FOSB	0.59260	**X**	**X**	**X**	**X**				
NM_002015	FOXO1	-0.52260	**X**							
NM_000807	GABRA2	-0.11640								
NM_006529	GLRA3	-0.17370		**X**		**X**			**X**	
AK055914	GNG12	0.88830	**X**	**X**						
NM_014366	GNL3	0.19610		**X**		**X**			**X**	
NM_004778	GPR44	-0.81440	**X**	**X**					**X**	
AK025785	H2AFV	-0.13740	**X**							
NM_000867	HTR2B	-0.69180	**X**	**X**						
NM_001553	IGFBP7	0.65840		**X**						
NM_000417	IL2RA	-0.05850	**X**	**X**						
NM_000590	IL9	-0.08720	**X**			**X**	**X**	**X**		
NM_002310	LIFR	0.05560								
NM_002332	LRP1	0.96870		**X**						
NM_005584	MAB21L1	-0.21650	**X**						**X**	

continued on following page

Table 4. Continued

		Fold Expression	*C/EBPbeta*	c-Ets-1(p54)	CREB	c-Rel	*GATA-1*	*GATA-X*	*MyoD*	Tax/CREB
AL133625	MBNL3	0.59340	**X**				**X**		**X**	
NM_000245	MET	-0.88820		**X**					**X**	
NM_004530	MMP2	-0.28880	**X**	**X**		**X**			**X**	
NM_005386	NNAT	-0.69920	**X**	**X**						
NM_013259	NP25	0.23170		**X**		**X**	**X**	**X**	**X**	
NM_003717	NPFF	0.18860	**X**	**X**		**X**	**X**	**X**		
NM_004708	PDCD5	0.09580		**X**			**X**	**X**		
NM_002608	PDGFB	-0.78240					**X**		**X**	
NM_005451	PDLIM7	-0.27470	**X**			**X**				
NM_024411	PDYN	0.49460		**X**				**X**		
NM_021796	PLAC1	-0.14430	**X**				**X**	**X**		
AB033035	PLEKHG1	0.30110	**X**	**X**			**X**	**X**		
NM_032483	PPAPDC1B	0.30310		**X**						
NM_003981	PRC1	0.39000			**X**	**X**		**X**		
AK055714	PSMA4	-0.89000	**X**				**X**		**X**	
NM_006264	PTPN13	-0.17040		**X**						
NM_002833	PTPN9	-0.51980					**X**	**X**		
NM_005608	PTPRCAP	-0.29690		**X**				**X**	**X**	
NM_014247	RAPGEF2	-0.37350								
AK022869	RPS6KA3	0.48650								
BC007359	RTTN	0.23600		**X**						
NM_006695	RUNDC3A	0.97270		**X**	**X**		**X**	**X**	**X**	
NM_021976	RXRB	-0.91460	**X**	**X**		**X**				
NM_031244	SIRT5	-0.21510		**X**						
NM_003199	TCF4	0.78030	**X**							
NM_021966	TCL1A	0.21240		**X**		**X**				
NM_003234	TFRC	-0.18980	**X**	**X**			**X**		**X**	**X**
NM_003239	TGFB3	0.87070	**X**						**X**	
NM_003248	THBS4	-0.49600	**X**	**X**					**X**	
U90902	TIAM1	-0.72830		**X**						
NM_003265	TLR3	0.49000	**X**				**X**	**X**		
AF305616	TMEPAI	0.01560	**X**		**X**				**X**	
NM_004237	TRIP13	0.18310		**X**					**X**	
NM_005429	VEGFC	0.20610					**X**	**X**		
BC013695	ZNF313	-0.44100		**X**					**X**	
AB007859	ZZEF1	0.01690		**X**		**X**	**X**	**X**		

Figure 10. Overview of pathway-pathway interactions using the KEGG pathways database. Numbers in parenthesis indicate the amount of microarray genes mapped in each respective pathway. Microarray genes have been mapped on these pathways using the Pathway Explorer software. In particular, pathways include: Cell Cycle (A), MAPK Signaling Pathway (B i-iv), Integrin-mediated Cell Adhesion (C), Wnt Signaling Pathway (D i-iii), TGF-beta Signaling Pathway (E), mTOR Signaling Pathway (F), VEGF Signaling Pathway (G), Calcium Signaling Pathway (H), Focal Adhesion Pathway (I), Jak-STAT Signaling Pathway (J), Notch Signaling Pathway (K)

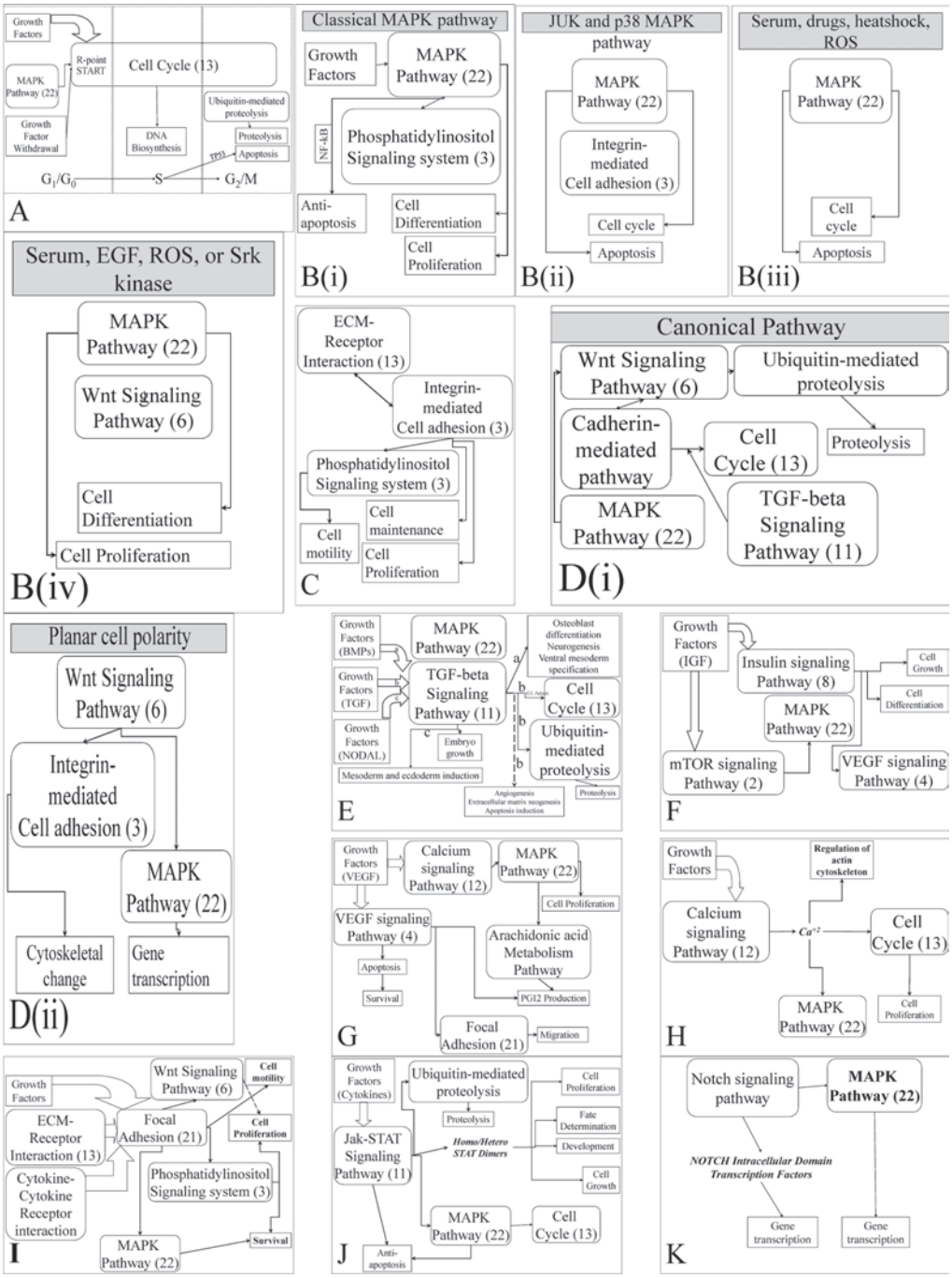
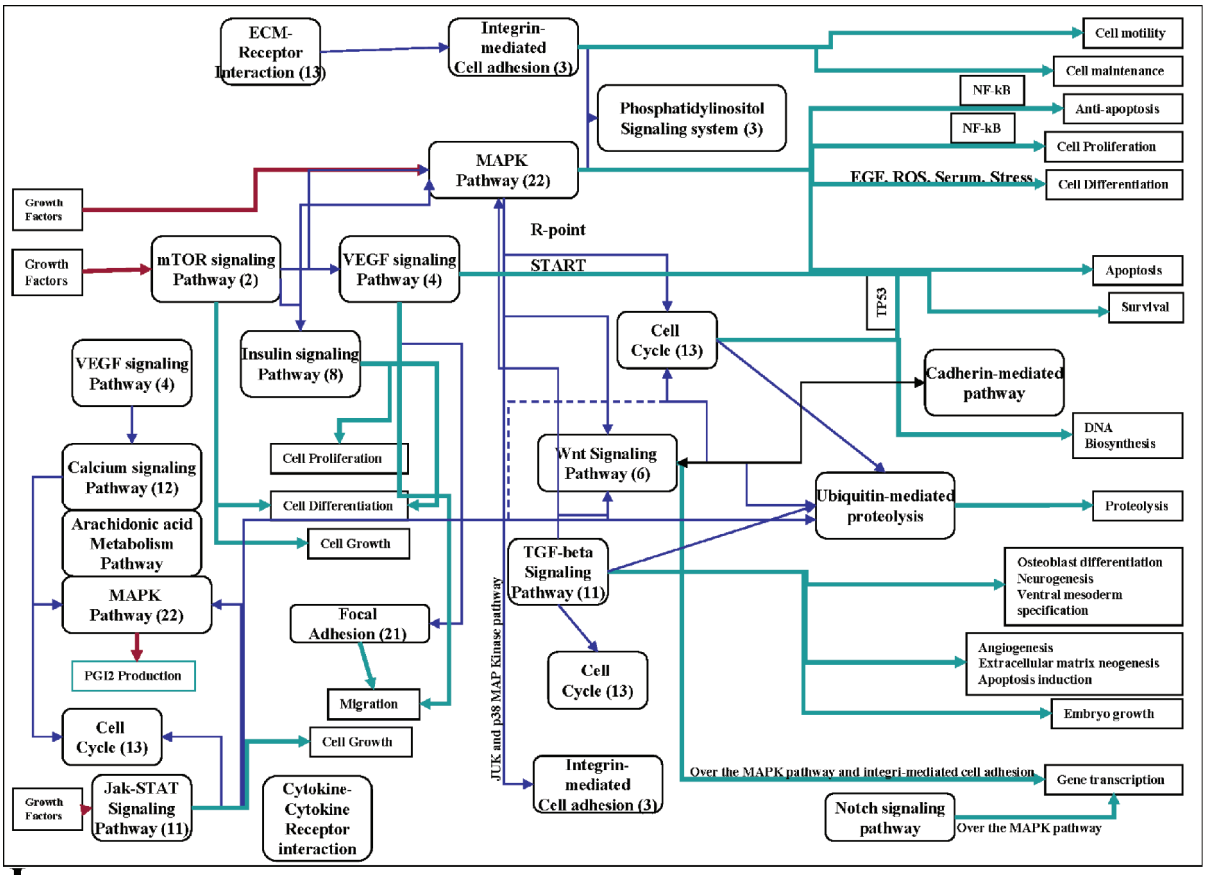


Figure 11. Complete overview of all mentioned pathways (L).



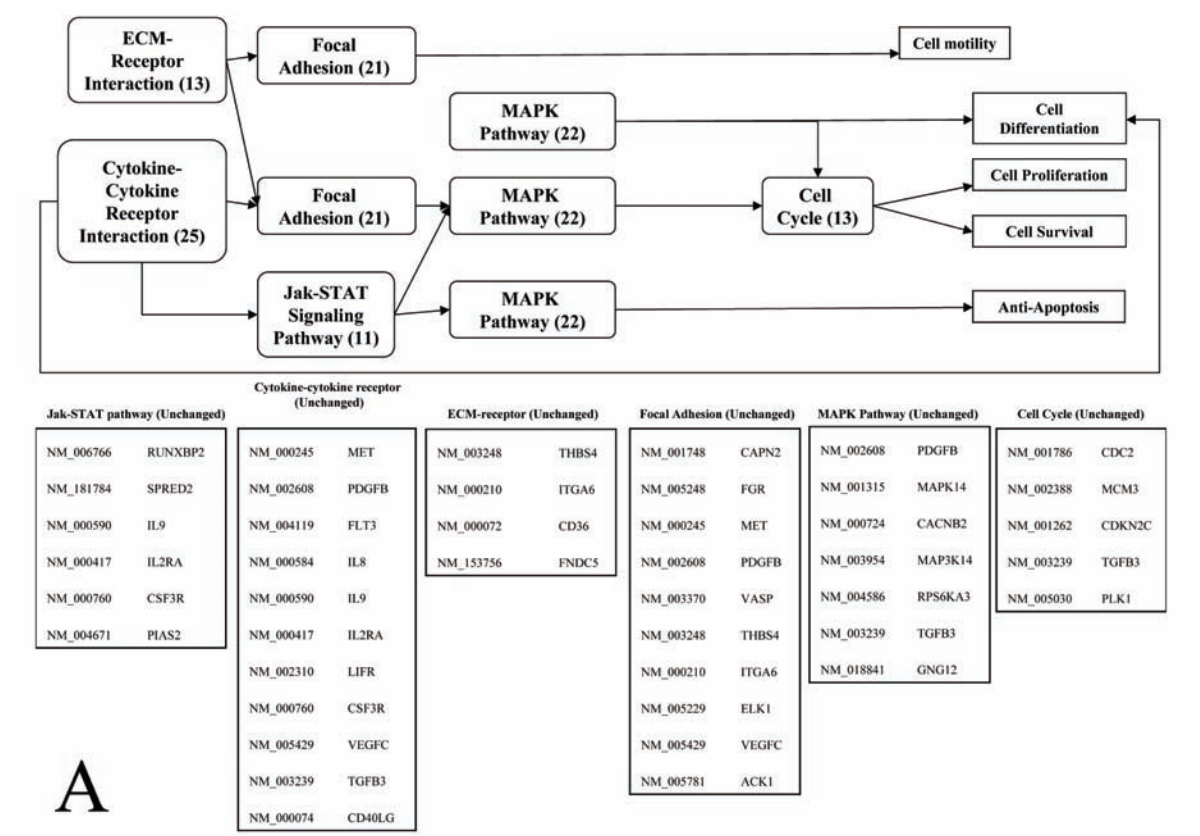
L

la Iglesia et al., 2008) as well as in the conversion of mesenchyme to epithelial cells(Yoshino, Monkawa, Tsuji, Hayashi, & Saruta, 2003). The role of LIFR has been reported to be connected to PTEN-Akt-FOXO axis and STAT3 (de la Iglesia, et al., 2008). There are no reports linking this gene with acute lymphoblastic leukemia or rhabdomyosarcoma. The role of LIFR is unclear in the present system. Another important gene predicted is CSF3R, previously reported for its role in hemopoiesis, since it is expressed only in the myeloid lineages of the hematopoietic cells (Niini et al., 2002). There are no reports linking this gene to rhabdomyosarcoma. A common developmental mechanism could however be utilized by both cell types for their growth and tumor progression.

The PDGF Family

Our study predicted that within this family of genes the two cell types share a common expression for the secretory molecules PDGFB, VEGFC and the receptors MET and FLT3. PDGF regulates clonal proliferation in pre-B cell lines and has been found to be over-expressed in B-chronic leukemia (Ho, Hsu, Phyliky, & Li, 2005). Both the VEGF and PDGF families are involved in neo-angiogenesis in the embryo and in tumors (Karamysheva, 2008). Rhabdomyosarcomas have been reported to express IGF and PDGF receptors (Blandford, et al., 2006). However, receptor expression is independent of signal molecule expression and expression of PDGF is associated with poor prognosis (Blandford, et al., 2006). MET is the

Figure 12. Summary review of the coupled pathways participating in our system of study (A)



receptor of the hepatocyte growth factor, produced by bone marrow stromal cells (Weimar et al., 1998) specific for the acute phase of T-cell ALL (Choi et al., 2007). MET is also known to play a role in chronic myeloid leukemia (Zhelyazkova, Tonchev, Kolova, Ivanova, & Gercheva, 2008) and to be expressed in B-ALL cells possessing the TEL-AML1 translocation (Accordi et al., 2007). Similarly, a mutated form of the FLT3 receptor is implicated in AML (Scholl, Gilliland, & Frohling, 2008). Members of the PDGF family of signaling molecules appear to be active in both of the cell lines studied. This suggests that both cell types utilize the same molecules for their signaling purposes. Since this family of molecules is active in embryogenesis and hemopoietic cell differentiation, it can be assumed that in the present system they pose a developmental mechanism still active after malignancy has taken place.

The ECM-Receptor Interactions

In this signaling pathway the two cell types share two genes with equal expression: Thrombospondin-4 (THBS4) and ITGA6. The integrin gene is known to be expressed in childhood hematologic malignancies (Hara et al., 2000) while there are no reports characterizing the THBS4 gene with respect to leukemia or rhabdomyosarcoma. Both are signaling molecules implicated in ECM-receptor interaction.

Figure 13. Genes below each pathway represent similarly regulated i.e. equally expressed, genes. Mapped genes with the respective expression values per channel are presented for the MAPK pathway. This facilitates the comparison of expression between equally expressed, unchanged, genes (B)

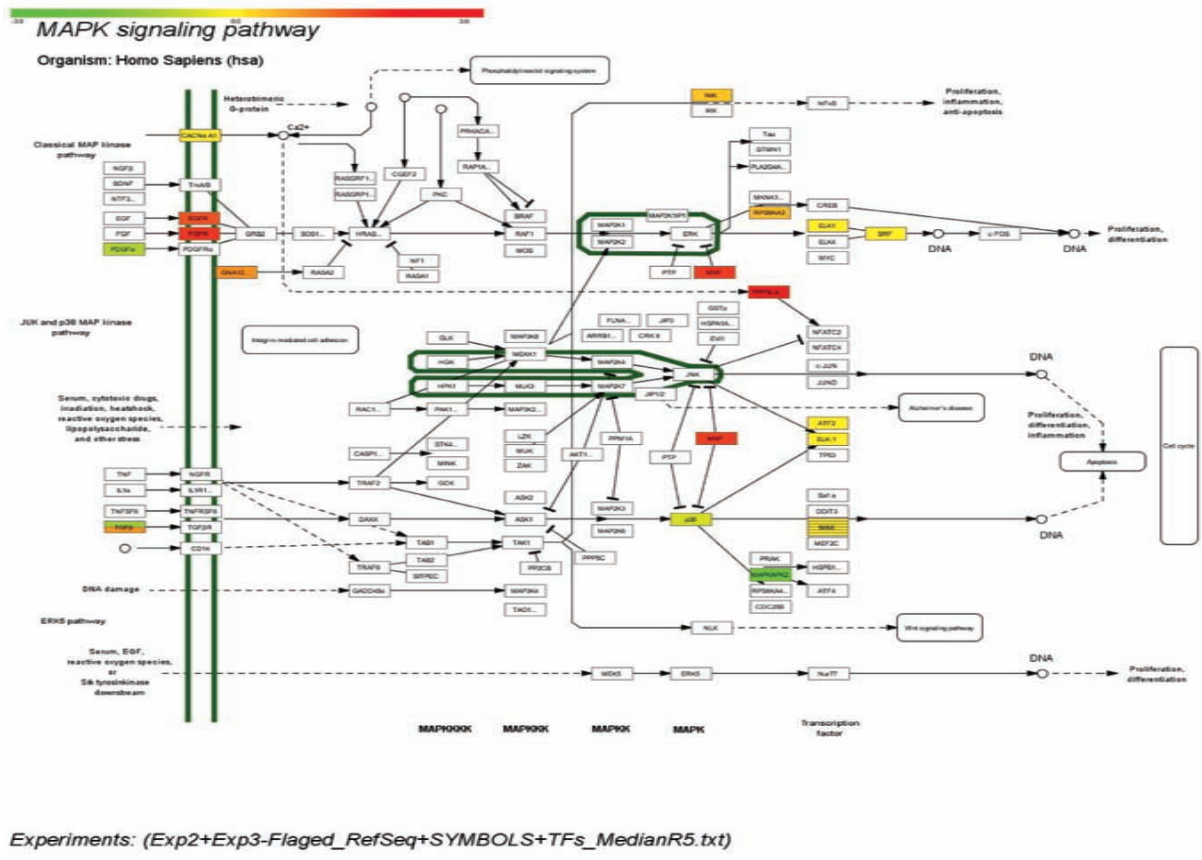


Figure 14. Genes have been plotted in increasing order with respect to normalized channel intensities (B).

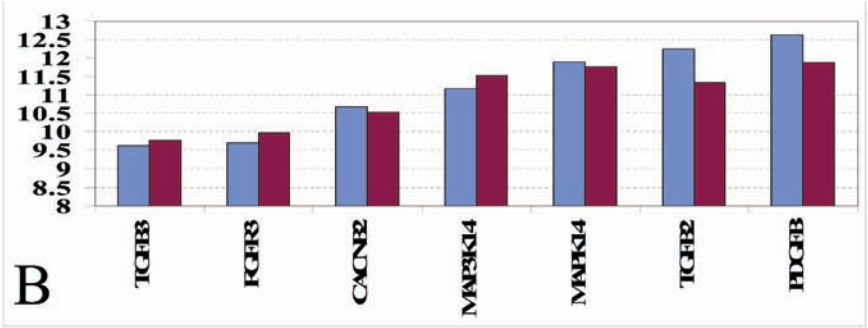
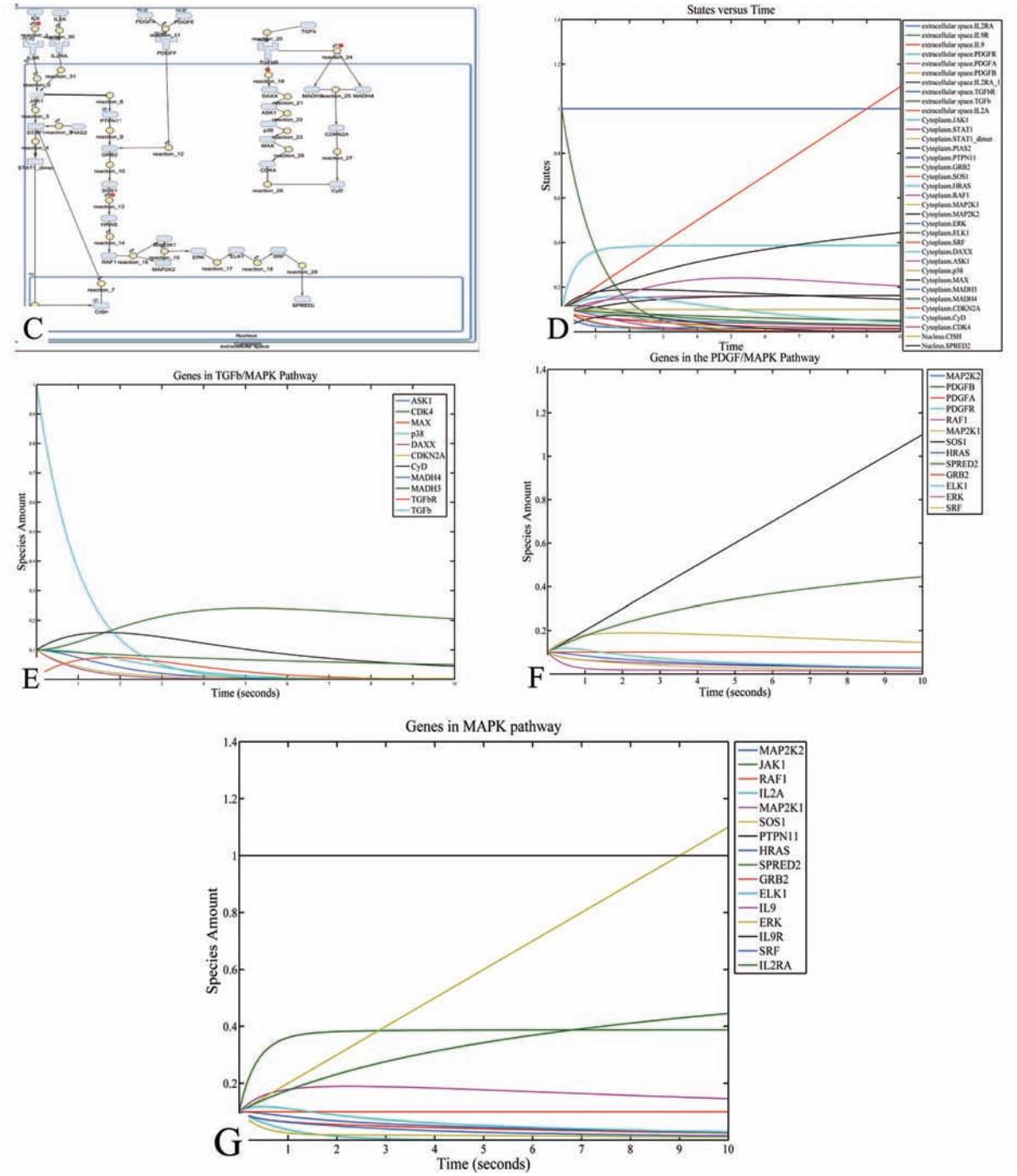


Figure 15. In C-G simulations of common genes for coupled pathways are presented.



Focal Adhesion

Both the ECM-receptor and the cytokine-receptor interaction signaling are interrelated to focal adhesion. Our system predicted that there are two courses of signal transduction, as presented in Figure 8: One follows the ECM-receptor signaling over LAMB2-ITGA6-CAPN2-VASP-cell motility and the other follows cytokine induction over VEGF-MET/ACK1/-ELK1-cell proliferation. Elk1 was not found to be expressed in the microarray screening but it was rather correctly predicted in the TFBM analysis.

CAPN2 is a known regulator of cell migration of various cell types (Raynaud, Marcilhac, Chebli, Benyamin, & Rossel, 2008) but no connection of this gene in either leukemia or rhabdomyosarcoma has been reported. Similarly, no reports link LAMB2 to leukemia or rhabdomyosarcomas. In our system it is predicted that these two molecules play a role in migration for both of the cell types studied. ACK1 is a known cell cycle regulator whose over-expression is related to cancer and to EGFR down-regulation (Grovdal, Johannessen, Rodland, Madshus, & Stang, 2008; Shen, Lin, Gu, Childress, & Yang, 2007). Interestingly, in the present system ACK1 is equally expressed in both cell lines, while EGFR seems to be over-expressed in the TE-671 cells. Elk-1, the predicted transcription factor, appears to be involved in direct association with Focal Adhesion Kinase and MAP kinase inducing anti-apoptosis (Mamali, Kotsantis, Lampropoulou, & Marmaras, 2008). As shown in Figures 12, 13, 14, and 15, there is a direct connection of Focal adhesion pathway to cell cycle regulation over the MAPK pathway. Our system correctly predicted that the mediator for this transition, in both cell types, is the Elk-1 transcription factor.

The Jak-STAT/MAPK Signaling Pathways

Signaling in the Jak-STAT pathway is predicted to start with the interleukins IL2, IL8, IL9 and their receptors in the present system appear to be the same for both cell types. The interleukin receptor binds to the JAK kinase, which then activates (Rogers, Horvath, & Matunis, 2003) the STAT1 transcription factor by phosphorylation (Kisseleva, Bhattacharya, Braunstein, & Schindler, 2002; Levy & Darnell, 2002). The STAT1 transcription factor remains dormant in the cytoplasm until activation by JAK takes place and then it translocates to the nucleus signaling the expression of genes responsible for proliferation, growth and differentiation. The STAT1 and STAT5B transcription factors appeared to be expressed in the present system while the STAT1 factor was also predicted by TFBM analysis. Both factors appear to be over-expressed in the TE-671 cells, as compared to CCRF-CEM cells. At the same time, both cell types seem to express PIAS2 (PIASxalpha), a known STAT1 inhibitor acting as a E3 ligase.(Rogers, et al., 2003) When STAT1 translocates to the nucleus, it interacts with histone acetyltransferase (CREB-binding protein) co-factor (Korzus et al., 1998) and NF-kB to initiate transcription (Hiroi & Ohmori, 2003b). This part of the Jak-STAT pathway can initiate gene transcription leading to proliferation, differentiation and development. On the other hand, there is the alternative regulation of cell proliferation by cytokines through activation of the MAPK pathway via protein tyrosine phosphatases and the Ras/MAPK pathway (So, Oh, Jang, Kim, & Lee, 2007). Activation through the Ras/MAPK pathway involves signaling from cytokines such as PDGFA or PDGFB, both expressed in our system, activation of HRAS and subsequent activation of RAF/MAPK/ERK, also known to be involved in myeloid cell terminal differentiation (Dorsey, Cunnick, Mane, & Wu, 2002). Following these events, a new protein of the serine/threonine

kinases family (RPS6KA3) participates in the Ras/Raf/MAPK pathway (Delaunoy et al., 2001); RPS6KA3 interacts with CREB transcription factor, predicted by TFBM analysis, leading to gene regulation of cell proliferation. Alternative to this process is the activation of the Elk-1 transcription factor from the ERK kinase. The Elk-1 transcription factor is phosphorylated by the MAP kinases (Yang, Shore, Willingham, Lakey, & Sharrocks, 1999) and is recruited by the SRF transcription factor (Sharrocks, 2001; Shaw & Saxton, 2003). The SRF factor binds the gene's promoters that posses the Serum Response Elements (SREs) in order to induce the expression of immediate early (IE) genes such as c-FOS and EGR-1 (H. M. Zhang et al., 2008). Interestingly, the SRF transcription factor was predicted by the TFBM analysis while the genes EGR-1, -2, -3, -4 and FOSB appeared to be expressed in our system of study. In addition, it has been reported that TGF-beta activates the MAPK signaling pathway over the p38/MAPK pathway (Ohshima & Shimotohno, 2003). Interestingly, the two key molecules TGF-beta and p38 appeared not only to be expressed in the two cell types under study but also to be equally regulated, suggesting a common utilization of this pathway for cell proliferation. In particular, TGF-beta activates members of the MAPK pathway, such as c-JUN, which in turn activate the stress-activated kinase p38 (Engel, McDonnell, Law, & Moses, 1999; Hocevar, Brown, & Howe, 1999; Hu et al., 1999; Yue & Mulder, 2000). The p38 kinase is known to interact with Elk-1 and ATF-2 transcription factors (Price, Cruzalegui, & Treisman, 1996; Raingeaud et al., 1995; Raingeaud, Whitmarsh, Barrett, Derijard, & Davis, 1996; Whitmarsh, Yang, Su, Sharrocks, & Davis, 1997). The ATF-2 transcription factor was also predicted by the TFBM analysis. The mechanisms described appear to be common for both the leukemia and rhabdomyosarcoma cells described in our study. To our knowledge, several of these mechanisms are known to manifest in leukemic cells but there are no up to date reports linking them to sarcoma or rhabdomyosarcoma cells. Finally, another pathway also predicted by our analysis is the interaction of the p38 kinase with the MAX transcription factor (MYC associated factor X), known to form complexes with other proteins for gene regulation (Lee, Onesime, Reddy, Dhanasekaran, & Reddy, 2002). The Jak-STAT pathway, in particular, is essential for embryonic stem cell renewal and proliferation (Rho et al., 2006) implying an active presence in our system of study.

Cell Cycle

Even though we have described the signaling pathways affecting cell proliferation, we have not described how these are implemented in the cell cycle itself.

A key regulator of cell cycle progression is the MAPK pathway. Depending on the signaling of MAPK, the cell cycle pathway receives signals in order to proceed. It seems that in our system key-molecules in the MAPK pathway signal the activation of cell cycle progression. Especially for the two cell types under study, it was shown that cell cycle follows a time-dependent shift from the G1- to the S phase, while G2-phase remains practically constant (Figure 2 C, D). The CCRF-CEM cell line has a defective TP53 gene (Strasser-Wozak et al., 1998) which is unable to interact with PCNA. Also, PCNA expression is linked to S-phase transition (Tonkinson et al., 1997); this is in agreement with our results indicating that PCNA is over-expressed in the leukemic cells, which progress more rapidly into S-phase than the rhabdomyosarcoma cells. Interestingly, histone deacetylase 7 (HDAC7), expressed in our system, is a known tissue-specific class II histone deacetylase (Verdin, Dequiedt, & Kasler, 2003). This class of histone deacetylases are anti-apoptotic factors found in thymocytes (Dequiedt et al., 2003) and were found to be over-expressed in the rhabdomyosarcoma cells.

In conclusion, although the two cell types studied were different, they possessed similar mechanisms of progression, conserved from their embryonic history. The key pathway predicted appeared to be the MAPK along with Jak-STAT pathway. This could probably be a hint for the "stemness" of cancer. Also, the two cell types possessed common extracellular signaling molecules opening the way for common biomarker discovery. In Figure13 and 14 B we plotted the expression of genes participating in the MAPK pathway. In order to investigate the levels of gene expression, we plotted the normalized intensities of the individual channels for each cell, instead of plotting expression ratios, as the latter would only give us the relative expression of one cell type against the other. Yet, with this presentation we were able to predict that both cell types use two common extracellular signaling mechanisms which at the same time participate in the MAPK pathway, i.e. TGFb and PDGFb growth factors. Further on, in order to obtain a view of the dynamics of the studied system, one extra step needed to be taken. That was the simulation of genes as they interact within a pathway, a very elaborate task requiring knowledge, not only of the literature but also of the obtained data, of the mapped genes, of their pathways and their interactions. We have, therefore, simulated some of the commonly regulated genes as they are presented in Figure 12 A. Although, there are numerous available pathways for several signal transduction cascades, we chose to design a pathway including the sum of genes participating in different known pathways (Figure 15 C). Simulations were performed thereafter and the results are shown in Figures 15 D-G. Figure 15 D shows the simulation for the total of the genes mapped. We then separated genes (i.e. proteins in the pathway) into smaller functional groups in order to see the dynamics of the system. We should mention that these genes were the ones equally regulated in both cell systems. This means that the dynamics observed are similar for both cell systems. For example, both cell types use TGFb and PDGFA, as extracellular signals, in a very similar way. The two extracellular molecules have as common denominator the activation of SPRED2 which, together with BCL2, is a regulator of apoptosis and anti-apoptosis respectively.

CONCLUSION

As we mentioned at the beginning of this chapter, there is not direct answer to the question of how computational and systems biology can contribute to the benefit of healthcare. It is only through the understanding of cell physiology and mechanics that these disciplines can contribute to the benefit of healthcare. This of course is also the purpose of the classical approaches, with the only difference that classical approaches deal with a limited set of factors simultaneously. If we recall the words of *Henri Poincaré*, also mentioned at the beginning of the chapter, the answer to the mysteries of life is through comprehension of the holistic system called the cell. In that sense, systems biology can make the major contribution possible. One of the main reasons that many things are still unknown is because we do not have the "big picture" of what we study. We see stacks of "trees" individually and not how the "forest" is organized. We mentioned that an immediate application of systems biology would be in the area of drug design. Yet the understanding of cell mechanics and designing effective drugs are so interconnected that it might be impossible to separate them as disciplines. Hence, the application of systems biology methodologies to the discovery of mechanisms that underlie biological systems would have as a result the production of more efficient and more feasible financially drugs.

Naturally, systems biology does not escape the net of ethics. The more we understand biological systems the more we will be able to manipulate them to our benefit. This, however, could function as a double-edged knife. This dual character is due to the fact that manipulation of biological systems

means intervention, which however can range from the making of hyper-effective biological weapons to manipulation of human cells for eugenics or military scopes. On the other hand, one of the main beneficial uses would be the discovery of efficient drugs to cure human diseases or invent ways for more efficient crops. Referring a little more to drug discovery, cheap and efficient drugs would be available for the benefit of all humanity, especially for cancer treatments. Despite the fact that many diseases can be cured today with only a few cents, mainly due to expired patents, hundreds of millions of humans still die from simple diseases (e.g. Inflammations, tetanus) every year, because they do not have access to even this type of cheap medication. This brings on top again the aspect of double-edged character of systems biology.

Therefore, systems biology, and if we put in a more general frame systems approaches, is a one-way road as far as the understanding of biological phenomena is concerned.

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KEY TERMS AND DEFINITIONS

Antisense Amplified RNA (aaRNA or complementary RNA (cRNA): The RNA molecule that is produced from an *in vitro* transcription reaction. It was first described by *Van Gelder et al.* in 1990 (Van Gelder, et al., 1990). This methodology is based on the linear amplification of dsDNA transcribed to cRNA with the use of *T4 DNA Polymerase, T3 RNA Polymerase* and *T7 RNA Polymerase*. It is a very powerful technique since it can amplify 20ng to 2ug of total RNA yielding an approximately 5000-fold amplification. This means that samples of 200ng could yield 40ug of cRNA or samples of 2ug of total RNA could yield up to 100ug of *cRNA* (complementary RNA), also called *aaRNA* (*anti-sense amplified RNA*).

cDNA: Also known as complementary DNA. This is the product of *in vivo* or *in vitro* reactions where RNA is reverse transcribed to DNA (cDNA). Usually, the parts of the RNA that are reversely transcribed are the exons or the protein-coding gene segments.

Comparative Genomic Hybridization (CGH): Comparative genomic hybridization (CGH or Chromosomal Microarray Analysis (CMA) is a high throughput method for the analysis of copy number changes (gains/losses) in the DNA content of a given subject's DNA and often in tumor cells. CGH will detect only unbalanced chromosomal changes. Structural chromosome aberrations such as balanced reciprocal translocations or inversions cannot be detected, as they do not change the copy number.

CHIP-chip (CHIP-on-chip) Microarrays: ChIP-chip (also known as ChIP-on-chip) is a high throughput technique that combines chromatin immunoprecipitation (*ChIP*) with microarray technology (*chip*). ChIP-on-chip is used to investigate interactions between proteins and DNA *in vivo*. Whole-genome analysis can be performed to determine the locations of binding sites for almost any protein of interest.

Clustering: Clustering or Cluster analysis is the assignment of a set of observations into subsets (called *clusters*) so that observations in the same cluster are in a sense similar. Clustering is a method of unsupervised learning and a common technique for statistical data. It is usually applied through the similarity measurement of data. Methods for clustering, among others, include: *uncentered Pearson correlation coefficient, squared correlation coefficient, averaged dot product, cosine correlation coefficient, covariance, Euclidian distance, Manhattan distance, mutual formation, Spearman Rank-Order correlation* and *Kendall's Tau*.

Cy3 and Cy5 (Cyanines): Cyanine is a non-systematic name of a synthetic dye family belonging to the polymethine group. Cyanines have many uses as fluorescent dyes, particularly in biomedical imaging. Depending on the structure, they cover the spectrum from IR to UV. Cyanines were first synthesized over a century ago and a large number of this family is reported in the literature.

Dendrogram: This is the tree-like diagram or graph used to represent the arrangement of clusters, which are usually produced by hierarchical clustering.

Euclidian Distance: In general the Euclidian distance or Euclidian metric is the distance between two points and it is derived from the Pythagorian Theorem ($\alpha^2 = \beta^2 + \gamma^2$). Hence, the distance *d* between two points *a* and *b* with coordinates $a(x_1, x_2,\ldots, x_n)$ and $b(y_1, y_2,\ldots,y_n)$ is given by Equation 1:

$$d(a,b) = \sqrt{(x_1 - y_1) + (x_2 - y_2) + \ldots + (x_n - y_n)} = \sqrt{\sum_{i=1}^{n} (x_i - y_i)^2}$$

Exons: Exons are nucleic acid sequences appearing in the RNA molecule after selective splicing. In many genes each exon contains a part from Open Reading Frames (ORFs), which code for the specific region of a protein.

Fluorochromes: Fluorochrome or also known as fluorophore, in analogy to the chromophore, is the part of a molecule that attributes fluorescence to it. That is the emission of light after it has been stimulated by an energy source, usually an electromagnetic wave.

Hybridization: This is the process through which two complementary sequences, usually nucleic acids, attach to form a dimmer. It finds applications in many methodologies such as: Southern and Northern Blots, all sorts of microarrays, in-situ hybridization and others.

Kendall's Tau: Kendall rank correlation coefficient, more commonly referred to as Kendall's tau (τ) coefficient or a tau test, is a non-parametric statistic used to measure the association or statistical dependence between two measured quantities. It is defined as Equation 2:

$$\tau_A = \frac{n_c - n_d}{\frac{1}{2} n(n-1)}$$

k-means Clustering: *k*-means is a method of cluster analysis, which partitions *n* observations into *k* clusters, in which each observation belongs to the cluster with the nearest mean. Given a set of

observations ($x_1, x_2, ..., x_n$), where each observation is a *d*-dimensional real vector, then *k*-means clustering aims to partition the *n* observations into *k* sets ($k < n$) S=$\{S_1, S_2, \ldots, S_k\}$ so as to minimize the within-cluster sum of squares.

M-A Plots: M-A plots are used for the comparison of data from one sample against another. This is usually the case for two-channel DNA microarrays where both samples are hybridized on the same chip or with single channel arrays where each sample is hybridized on pairs of chips. *M* is the intensity ratio and *A* is the average intensity for a dot in the plot. M-A plots are then used to visualize the intensity-dependent ratio of raw microarray data. The M-A plot uses *M* as the *y*-axis and *A* as the *x*-axis. The M-A plot gives a quick overview of the data. The majority of the points on the *y* axis (*M*) would be located at 0, since $log_2(1)$ is 0. *M* and *A* are given by Equation 3.

$$M = \log_2 R - \log_2 G$$
$$A = \frac{1}{2} \cdot (\log_2 R + \log_2 G)^{'}$$

where, *R* and *G* are the intensities of the fluorescent dyes measured during an experiment.

Mismatch and Perfect Match: These terms have been utilized by Affymetrix microarray chips and certain methodologies for data analysis. In particular, a probe pair consists of a 25-mer oligonucleotide perfectly complementary to the sequence of a gene (*Perfect Match PM*) and a 25-mer oligonucleotide that differs from the perfect match probe by a single mismatched nucleotide at the central position (13th position; *Mismatch: MM)*. This combination of a 25-mer pair, *PM* and *MM*, offers the highest sensitivity and specificity, while it solves, in part, co-hybridization problems. The difference between *PM* intensities minus *MM* intensities averaged across the probe pairs gives an estimation of the hybridization intensity. The intensities measured using *Affymetrix* arrays are considered to be the absolute expression levels: the less efficient hybridization of the *MM* probe to the target allows the PM intensity minus the MM intensity of the probe to offer a better estimation of intensity. Background correction (*BC*) on the intensities is done automatically (GeneChipTM software) using a regionalized method, i.e. dividing the array in several rectangular zones.

Normalization: This is the process of removing statistical bias from measured data. It applies to many forms of data and in many disciplines. In this context it mainly refers to microarray data normalization. Since it is known that microarray data entail a lot of bias, methods have been developed in order to remove systematic errors. Such methods include Global Median division, Lowess, Loess, Robust Lowess, Rank Invariant and others.

Spearman Rank-Order Correlation: Spearman's rank correlation coefficient or Spearman's rho, denoted by the Greek letter ρ (rho) or as r_s, is a non-parametric measure of statistical dependence between two variables. It assesses how the relationship between two variables can be described using a monotonic function. If there are no repeated data values, a perfect Spearman correlation of +1 or −1 occurs when each of the variables is a perfect monotone function of the other. The *n* raw scores X_i, Y_i are converted to ranks x_i, y_i, and the differences $d_i = x_i - y_i$ between the ranks of each observation on the two variables are calculated. If there are no tied ranks, then ρ is given by Equation 4:

$$\rho = 1 - \frac{6\sum d_i^2}{n(n^2 - 1)}.$$

If tied ranks exist, Pearson's correlation coefficient between ranks should be used for the calculation (Equation 5):

$$r = \frac{\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \overline{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \overline{y})}},$$

Acute Lymphoblastic Leukemia (ALL): Neo-plasmatic disease of the blood. It appears mainly during childhood whereas it's occurrence is less frequent in adolescence and the elderly.

Rhabdomyosarcoma (RMS): A rare childhood cancer comprising 5-8% of all tumors that emerge during childhood. In general the sarcomas of head and neck comprise 12% of all childhood neoplasias with an incidence rate of 250 cases every year in the United States. Rhabdomyosarcoma is a malignant tumor of mesenchymal origin and is within the category of small blue cell tumors, including neuroblastoma and primitive neuroectodermal tumors. The embryonal form is most common at birth whereas the alveolar form peaks in childhood and adolescence. Embryonal rhabdomyosarcoma manifests a spindle cell and botryoid form and has a better prognosis.

Cancer Germ-line Gene (CGG): Genes which are active during developmental stages and which are silenced thereafter in somatic cells. When referring to cancer these genes are also active in somatic cells due to the undifferentiated nature of tumor cells.

Primitive Neuroectodermal Tumor (PNET): This is a neural crest tumor. It is a rare tumor usually occurring in children under 10 years old. It has a survival rate of less than 40%. Its name derives from the fact that the majority of cells in the tumor come from the neuroectoderm but have not developed and differentiated in the way a normal neuron would, hence the "primitive" appearance of cells. For example, medulloblastoma is known to derive from neural stem cell precursors, but because of its unusual appearance for years it was thought to be a glioma. PNET belongs to the Ewing family of tumors.

Nuclear Factor of Kappa in B-cells (NF-κB): A transcription factor. It translocates to the nucleus and plays a major role, among others, in response to inflammation and glucocorticoid resistance.

Transcription Element Listening System (TELiS) Database: Transcription Element Listening System Database. On-line tool for transcription factor binding motifs prediction.

False Discovery Rate (FDR): A statistical method used in multiple hypothesis testing to correct for multiple comparisons. In a list of rejected hypotheses, FDR controls the expected proportion of incorrectly rejected null hypotheses (type I errors). In practical terms, the FDR is the expected false positive rate; for example, if 1000 observations were experimentally predicted to be different, and a maximum FDR for these observations was 0.10, then 100 of these observations would be expected to be false positives.

Transcription Factors (TFs): Proteins that bind to specific DNA sequences, thereby controlling the transfer (or transcription) of genetic information from DNA to mRNA.

Forward Scatter (FS): A measure for cell size in flow cytometry. It is actually the scattering of light as cells pass across a laser beam.

Side Scatter (SS): Similar to forward scatter but with the difference that side scatter is a measure of cell's granulation.

Propidium Iodide (PI): A fluorescent dye with high binding affinity for nucleotides. It is used for flow cytometric studies. It facilitates the measurement of necrosis and DNA content.

Gene Ontology (GO): A major attempt to classify and organize all known functions for known genes. It contains annotations for known genes and their products.

Stemness of Cancer: A term referred to the existence of cancer stem cells. The ability of cancer cells to divide in an uncontrollable manner has fueled the idea that stem cells and cancer may share some common characteristics. This includes the existence of stem cells with a cancer cell population.

ENDNOTE

[1] http://tinyurl.com/3ssxe2g

Section 4

Healthcare Quality Assessment

Chapter 8
Planning of a Specialized Pedagogic Environment and Defining Ethical Requirements in Educational Practice for Healthcare Quality

Vassiliki Ioannidi
University of Peloponnese, Greece

Antonios K. Travlos
University of Peloponnese, Greece

Sofia Vasileiadou
University of Athens "Attikon" Hospital, Greece

Evangelia Loukidou
Loughborough University, UK

ABSTRACT

Teaching is a continuous procedure of dissolving puzzling situations (Matsaggouras, 2002, p. 418) People's health is based on a complicated combination of conditions; for example, public health is connected to society, culture, economy, politics. Consequently, in order to achieve, preserve, protect, and promote health, people need to act under the idea of solidarity and not of people's exploitation. Incomplete accommodation, unemployment, poverty, racial discrimination, social and cultural stereotypes have a negative effect on individual and public health. Well coordinated efforts, individual and public acts, as well as the development of public interest on health ethics, are all demanded in order to protect and promote citizens' health. In this social and cultural framework, the role of Nurse Science becomes very significant, as health is considered to be the means of well being. Didactical interventions are important to that aim. On the other hand, research is significant for the improvement of health and quality of life. Science and technology are rapidly improving, and legislation is following on an attempt to cover major issues on ethics and deontology. Nurses' and other health scientists' and officers' views, perceptions

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and attitude on the concept of health and health promotion, as well as on other basic parameters, are critical. The philosophy of the present chapter consists of the following ideas: Presentation and discussion of theoretical pedagogic and ethical dimensions on health care and safety; Research and analysis of special didactical methods in a sustainable administration of health, aiming at ethics and education for health, quality and safety in health care.

INTRODUCTION

Human health depends on a multifaceted combination of conditions; for instance, public health is linked to society, culture, economy and politics. Consequently, all acts related to health acquisition, maintenance and promotion should be based on the principle of sympathy and not exploitation. Homelessness, unemployment, poverty, racism, discrimination, social and cultural stereotypes have a negative effect on individual and public health. In order to minimise such phenomena and to protect and promote citizens' health, there is a need for coordinated social efforts, individual as well as group action and active interest for the ethical standards that exist in the health-care domain (see also Johnstone, 1999).

In the existing social and cultural framework, the nursing science role becomes vital in respect to the new conceptualization of health as a means for "well-being". Educational interventions can play a major role in the conquest of "well-being". On the other hand, research is very important for the improvement of health and quality of life. Science and technology are rapidly progressing, whilst legislation follows in an attempt to cover major arising issues of ethics and deontology. To this direction, the beliefs, perceptions and attitudes of nurses, and health professionals in general, regarding health, health promotion and other basic parameters is of major significance[1].

Based on the above, the rationale that underlies the present chapter was developed through the association of the following concepts:

- Discussion and presentation of pedagogical and ethical dimensions of health care and health safety,
- Research and analysis of specialized didactical techniques in the context of sustainable health management aiming at ethics and education for health, as well as quality and safety in health care.

SEARCHING FOR A THEORETICAL MODEL OF NURSE EDUCATIONAL INTERVENTION FOR HEALTHCARE QUALITY

Guiding Principles

Teaching and learning are the two cornerstones of nurse education, both at clinical and theoretical level. They promote the evolution of the existing knowledge, produce new knowledge and cultivate new abilities and skills on nursing practice and management. Nursing education, as well as of any discipline, is diversified according to the aims that it sets and the approaches that it employs in order to achieve those aims. According to Karseth (2004) education may be oriented towards knowledge mastery or the development of professionals who would fit the needs of a particular job-market or, lastly, towards the moral and ethical aspects of a specific profession.

We are aiming at:

- Guiding, sensitizing and informing students about their personal aptitudes and interests, professional options and oppor-

tunities, social data and job market requirements, etc,
- Connecting students' scientific activity with the wider scientific, professional and social orientations of nurse education, research and practice,
- Developing students' confidence, in terms of helping them to assume responsibility for their education.

In order to achieve such goals, there is a need not only to teach students about the ethical values upon which nursing research and practice are (or should be) based, but to do so through active listening to their needs, interests and inclinations.

Educational Goals and Ethical Expectations

The demand from one to be moral, either as a health professional or as an educator and to serve the highest ethical standards while working, constitutes at the same time a request for his/her personal, professional and social self-fulfillment. International literature from various disciplines has dealt with this issue however it would be beyond the scope of the present chapter to review the subject. Nevertheless, it is our belief that pointing out the educational targets and the ethical demands that contribute to the development of proper professional choices and models, completes the issues and perspectives that this chapter addresses.

In our view, nursing educational interventions should be based on specific goals that would also function as criteria for any educational proposition in nursing education. Such goals/criteria might be[2]:

- Interdisciplinarity
- Critical extension
- Reinforcement
- Pedagogical innovation
- Aligned theory and practice
- Efficiency
- Continuing life education
- Change management
- Promotion of humanistic values
- Accountability towards society and culture
- Vision, in the local and national context and in the frame of globalization.

However, no educational or/and nursing intervention can be ethical, scientific and professional without having ethical criteria and dimensions. This idea can also be reinforced by the following:

1. Nurses are committed to patterns of professional behavior that are presented in public through legal papers, for example professional deontology, guidelines regarding the profession and practice, ethical codes, etc. The contemporary ethical basis of nurse science is connected to the ideas of respecting human rights, protecting and rehabilitating health, preventing illness and alleviating human suffering, especially of vulnerable social groups. Even the simplest practices of nursing care are ethically important, as they could cause injuries, ethical harm, lost of trust, etc (Johnstone, 1999).
2. Given the above, the two concepts that affect nurses' professional training and professional efficacy are "ETHICS" and "LAW". More specifically, ethics provides the structure to put behavior into action. Values are personal beliefs concerning reality and evaluations of thoughts, objects or behavioral patterns. Over the course of time values might change or form diverse subcategories, such as personal, professional and social. Finally, values provide guidance and determine the everyday life choices. The nature of ethics and values is individualistic, since they are formed to a great extent by past experiences gained through education and the environment. The legal system is founded on rules and regulations that guide society to a formal and binding pattern of conformity. Created by human and being able

to change, the legal system gives continuing direction to those who provide health care. It is clear that nurses apply a variety of ethical values in every day clinical practice; values that define their professional choices and form their scientific and professional profile. Indicative of these values are (Guido, 2006):

- *Autonomy:* meaning personal freedom and self-determination. There is the right to choose, respect for the patients and their rights, who are free to make their own decisions over their treatment.
- *Beneficence:* meaning acts aiming at patients' wellness and encouragement as they go through painful processes.
- *Truthfulness/Honesty/Veracity:* meaning answering patients' questions and providing information.
- *Loyalty*: meaning keeping promises and not promising when not being able to meet expectations.
- *Justice*: meaning the provision of equal health-care services for all people.
- *Respect for others*: main principle, as it helps overcoming other problems and difficulties.

It is therefore a basic goal and simultaneously an inherent role of education to inflict to nursing students the ethical values upon which nursing practice is (or should be) based. Students' educational experiences as well as instances from their clinical practice can convert through appropriate teaching strategies to *learning cases* of ethical behaviour.

Teaching and Communicative Collaboration Strategies Through Experiential Learning

The question that follows the above presentation is: what are the educational strategies and how can they contribute to the development of students' ethical professional identity? It has been asserted that in order to augment the quality of nurse education there is a need to shift power from teachers towards students, to apply the concept of experiential learning and to get students "involved" in the learning process and in the actual practice of nursing (Morrall, 2005).

1. According to Wallace and Hellmundt (2003), the learning process is strengthened when the emphasis is given to students' educational needs and personal style and facilitates their interaction. The consequences of such emphasis would be students' active participation in the learning process and better understanding of the learning product, that is, the knowledge. The ultimate result of this process would be having educators' and students' expectations met. More specifically, such process of familiarizing with knowledge, acquiring information and developing skills requires:
   A. *Placing the focus of teaching on students*, for instance creating student-centered cooperative activities·
   B. *Students' encouragement by educators*, after the latter have clarified the learning goals, the teaching methods and style and after they have evaluated the cultural diversity of the students' group·
   C. *Facilitating the development of students' relationships*, through the discussion of ideas, opinions and issues in a holistic manner. Creating a climate of partnership has a dual effect: first, it may discard from teachers the role of passively transmitting information. The second effect regards the role of students as responsible agents who actively develop academic skills, such as critical thinking and analysis, assessment and reinforcement of a system of

beliefs that is personal yet spherical, specialized knowledge on legal and professional matters regarding clinical decision-making, etc·

D. *Developing small work-teams*, in order to provide students the opportunity to familiarize with each other through discussions and case- analyses, to enhance their self-esteem since it is easier for one to expose personal opinions in small groups and ultimately to abolish stereotypes in collaborative framework.

2. Moreover, according to Lambert and Glacken (2004), the aim is to highlight the supportive roles as a didactical method in nurse education, as well as their efficiency and functionality. Introducing supportive roles in education signifies the need for close interaction between theory and practice in order for a successful and efficient teaching and learning procedure to occur. Under no circumstances can theory be isolated from practice, as it cannot captivate the complexity of practice as this takes place in a clinical environment. The clinical environment is identified as basic element for nurse students' learning, in which theory and practice are connected through clinical parameters and supportive structures. The identification of ways to build strong bridges between healthcare services and academic institutions has been a central theme of research[3]. A basic proposition for enhancing the effectiveness of training regards the introduction of people who will act supportively to teaching in clinical settings. The vision for the development of this supportive role aims at meeting leaning needs and reinforcing clinical practice; for instance, clinical supportive roles are necessary as didactical methods for enhancing students' education through a holistic educational model between theory and practice.

3. This interweaving of theory and practice consists of pedagogical interactions and communication and aims at the duality *Knowledge* (referring to cognition and to teaching methodology and planning) and *Relation* (referring to the communication and to the climate created in the class that transform the educational experience into a free and productive relationship and that constitute a learning procedure). We aim at gaining knowledge relative to the cognitive subject and at developing skills and abilities, motives and interests in order to engage in constructive interaction within the context of a pedagogical relationship (Mpakirtzis, 1998, p. 101).

The rapid changes in healthcare systems and the increasing demand for interdisciplinarity highlight the need for effective collaboration between health professionals (Posey & Pintz, 2006). It is therefore an imperative for nursing education to incorporate activities that prepare students for future collaborative processes. Furthermore these collaborative approaches that incorporate experiential learning, team-work, clinical practice may improve not only the quality of learning but also the nature of relationship in-between students and between students and teachers (Morrall, 2005).

Didactical and Methodological Procedure During the Intervention

The contemporary role of educators is characterized as dynamic and it progressively becoming more complex and demanding within the context of globalization. It necessitates cognitive as well as emotional analytical and communication skills, such as academic excellence, understanding, empathy and imagination in order to inspire and guide young students.

Teacher's contribution to the quality of learning, in terms of constructively associating theory and practice, is aligned with students'

role through the concept of *guided supervision* (a term introduced by the great philosopher and teacher J. Dewey, who had a great influence on the evolution pedagogy of the 20th century[4]. Dewey emphasized students' engagement in the learning process, which is activated according to their interests and motives and which results in learning through experience).

Consequently, this learning, didactical and educational process may consist of:

- *Discussion*, for instance, providing introductory information by using questions and answers·
- *Examples*, for instance, giving clinical examples, carrying out practical exercises, etc·
- *Experience,* for instance reporting experiences, case studies, life histories, etc.

The learning context of "discussion – examples – experiences" is based on J. Dewey's idea of "*learning by doing*[5]". In line with this framework is the concept of "true collaboration" (Posey & Pintz, 2006, p. 681) which necessitates from all actors to creatively explore the subject matter and to reach to a consensus or to find a solution to a problem. Discussing ideas, experiences, knowledge not only engages students in the learning process but it also provides them with the necessary social skills, thus preparing them for their future work (Posey & Pintz, 2006).

According to Mpakirtzis (2005, p. 85) identifying students' needs, as these are experienced and perceived by students through their relationship with themselves and the social and natural environment, which is expressed as emotion, is today's teaching challenge. Acknowledging and understanding students' needs can be useful for the work of educators and health professionals. Such knowledge can be used for developing innovative pedagogical interventions and specialized didactical approaches, in order to provide students with internal and external motives and activate their interests and desires.

CONCLUSION

Nursing has often been depicted as a profession that inherently entails an ethical aspect, that is: the provision of care, sympathy and the therapeutic use of the self for the benefits of others (Brodish, 1982; Mackintosh, 2000). A basic premise of this chapter is that in order to reach quality in healthcare provision there is a need for grounding all relative actions upon ethical values. Education can play a vital role for inflicting to students the ethical dimension of nursing practice, through the aims, the strategies and the specific techniques that it employs.

Such educational strategies might be the application of student-centred methodologies, experiential learning and "learning by doing" techniques. In order to have competent (cognitively and ethically) professionals, it is necessary to act from very early on and engage students in their own education, by actively listening to their needs, perceptions and interests and by developing constructive relationships with them (Ioannidi, 2008, p. 78-90).

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ENDNOTES

1 See also from the Nurse Education and Research field: Koh (2002); McSherry et al. (2008); Williams, Taylor (2008); Vadlamudi et al (2008); Casey (2007); Neville (2004); Stockhausen, Kawashima (2003). Also, for relevant studies in the field of education in general, see: Kiridis et al. (2003); Koulaidis (1996); Flouris, Kalogiannaki (1996); "*We Greeks, We Europeans*…".

2 See: Thorne (2006), who refers to goals, as key concepts, for nurse education in the 21st century.

3 This article refers to such supportive roles through studying certain cases, which agree to nurse education learning environments of different countries.

4 J. Dewey's system in Danassis (1993, p. 97).

5 According to Dedouli (2001, p. 147-148), organizing the learning procedure on "learning by doing" effects on students' active participation in actions such as research, observation, interview, simulation, synthesis, etc. this way experiential learning connects to interactive learning, problem solving and project method.

Chapter 9
The Social Role of Technology in Healthcare Quality Improvement

Anastasia K. Kadda
University of the Peloponnese, Greece

ABSTRACT

The aim of this study is to describe the social role of technology in healthcare quality improvement. Methodologically, the study was based on a review of the relevant literature, Greek and foreign, as well as Internet sources related to the social role of technology in healthcare quality improvement. The main conclusions drawn were the following: a) The development of new technologies in the field of health and their involvement within the social context is today a fast accelerating process; b) The presentday expansion of health-oriented technology is of vital importance because of current trends in the field of healthcare and of the social evolution on healthcare services; c) Information technology is capable of profoundly contributing to the improvement of the quality of health, and thus to the wellbeing of the citizens in a society; d) By the use of health technology, more efficient and productive financial management is achieved with numerous benefits for the economy; e) Electronic health can improve the quality of healthcare thereby facilitating the work of health professionals; f) Greek society is being increasingly influenced by both international and domestic scientific and technological advances in health technology despite the existence of significant legal barriers; g) Current trends in the European Union as far as health technology is concerned are intimately connected with expanding citizen participation in the electronic revolution and their increasing access to the Information Society.

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INTRODUCTION

The rapid development of the Informatics and Communication Technologies, their integration into almost every aspect of everyday life and their widespread diffusion into all sectors of social activity, including the fields of health/healthcare, is today a fast accelerating process. This is observable, to cite but one example, in the intense interest and high levels of involvement manifested by social scientists regarding matters such as science and technology and their relationship with society and all social matters (Agrafiotis, D, 2003). In this context, it is the state's obligation, in the interest of the prudent advancement of the information society, to ensure that this newly developing society will include everyone and will provide all citizens with a high quality of life.

At the continental level, 'electronic' Europe is aiming for accessibility for all European citizens to the benefits of the Information Society with regard to health and healthcare, and this is directly associated with the electronic health initiative for achievement of provision of optimal healthcare quality. Almost all member-states of the European Union have experienced exponential growth in the introduction and increasing implementation of health technologies in recent years, including new medicines and diagnostic tools, telemedicine and surgical equipment. Such innovation provides enormous opportunity for governments, providers and patients to supply and obtain enhanced healthcare services and outcomes.

Today, governments are finding themselves increasingly required to manage scarce resources as strategically as possible, and this may be accomplished by investing in those services that deliver the best health outcomes. This translates as care that is affordable, effective, safe and patient-centred and, in the new century, a range of rapidly proliferating health technologies are offering them the solution. These novel technologies now present governments with unprecedented opportunities to provide high quality and innovative care to meet their population's health needs more effectively while also reining in their exploding healthcare budgets and safeguarding the basic principles of equality, access and choice among all members of society. Moreover, innovation is amply supported via sufficient market access to new treatments (Sorenson, C, Drummond, M, Kanavos, P, 2008).

One of the proposed actions in the framework of electronic Europe is the necessity of using an electronic health card which replaces all papers required until now when healthcare was provided in another member-state, the networks of health information between points of healthcare (hospitals, labs, domiciles) using broadband connectability for this purpose. There also exist pan-European information networks and health services disseminated through the Internet such as information for healthy living and disease prevention, electronic health records, tele-visits, electronic refunds. The accessibility to health information for all citizens using qualitative criteria is also very important (Giberiti, A, 2010, Hristodoulakis, T, 2001).

The present study is based on a review of Greek and foreign literature and of Internet sources and aims to investigate the social role of technology in the improvement of quality of healthcare.

THE SOCIAL NECESSITY OF TECHNOLOGY IN THE IMPROVEMENT OF HEALTHCARE QUALITY

The introduction of the technologies of Informatics and Communication into the sphere of health for the achievement of ever more efficient and high-level healthcare services to be provided to every citizen anywhere and anytime is of the utmost importance in our presentday world. This is on account of the current trends in the field of health and healthcare as well as of the social evolution involving the increased demand of healthcare services such as the following: a) the ongoing transformation of the current nosologic model due

to the increasing prevalence of chronic illnesses (e.g. cardiovascular diseases) and degenerative diseases (e.g. cancer) as a result of our modernday lifestyle habits, including in particular diet, b) the constant appearance of "new" socially derived diseases (e.g. HIV, alcoholism), c) citizens' continuously increasing expectations of better quality of health services and accessibility to them, d) the emergence of a new inexpensive medical technology generating greater possibilities for diagnostic and therapeutic actions, e) the increased interest citizens display in playing a bigger role in the management of their health, this demanding an upgrading of their knowledge concerning relevant issues, f) the increase of the demand of health and social services, g) the increased mobility of patients and health professionals (www.athina.cs.unipi.gr-site).

In addition, problems regarding the health sector such as the rising cost of health services, the paucity of financial resources, the rapid evolution of technology, demographic ageing and other moral and social issues are all intimately interconnected and point to technologically supported healthcare as an essential factor affecting health policy (Agrafiotis, D, 2000).

With the aim of achieving a wholly anthropocentric merging of healthcare services, the technologies of informatics and communication are today being marshalled to bring about such goals such as health promotion and disease prevention, constant augmentation of efficiency and effectiveness in healthcare objectives as regards diagnosis, treatment and rehabilitation, accessibility to every type of medical information and knowledge for everyone, guaranteed access to health services for everyone and improvement of the quality of these services (www.ehealth-congress.com).

In relation to healthcare units, new technology must immediately be adopted in order to ensure quality of healthcare in the three 'dimensions' as set out by John Ovretveit: that is, quality of patient's health/healthcare, quality of health professionals' knowledge/skills, quality of system management (www.agsm.gr).

Concurrently, the introduction, diffusion and use of the new technology within the hospital itself, considerably upgrades its functionality, the new technology providing the following gains: decrease of the period of time of the patient's hospitalization, decrease of the cost of the diagnostic and therapeutic procedure and high-standard quality of health services provided (Agrafiotis, D, 2000).

HEALTH TECHNOLOGY, SOCIETY AND QUALITY OF HEALTHCARE PROVIDED

According to the World Health Organization, the steady development of health technologies is aimed at helping us to solve the numerous health problems of our fellow-citizens and to thereby improve quality of life. They are today a vital element of healthcare systems because, among so much more, they can positively impact disease prevention as well as diagnosis and treatment of patients and can thus contribute to the lessening of disabilities and functional deficiency (www.who.int).

In the domain of health services, information technology can significantly contribute to the improvement of the quality of health and to the wellbeing of the citizens of a society given the fact that these services are considered to be in direct interaction with the social environment since they are affected by social forces and they contribute to social and cultural life. At the same time, a health institution is a place where health services, education and research are developed. It is an indicator of social and cultural processes in a society, a point of interchange where many other activities can intermingle since it can generate new initiatives and new formulas for many aspects of everyday life. A hospital, for instance, is affiliated with education, law, the financial system, etc (Agrafiotis, D, 2000).

In addition, a hospital is a basic constituent of the health system and a sphere for application of health policy; it is therefore nowadays one of the most significant areas where the current achievements of science and technology are being successfully implemented. The introduction, diffusion and use of these innovations are profoundly influencing their organizational and functional structures as well as power relations.

Although difficulties do exist that are associated with implementing information technologies in healthcare institutions, they are certainly not insurmountable. Suggestions are offered as to how managers can adopt information technology systems to improve the delivery of service and thereby enhance the wellbeing of the consumer/patient (Essam, M, Gillian, R, 1998).

The main question is: how can we achieve improvement of the quality of a hospital or of health services in general through the use of new technologies and what precisely are the benefits for society?

This goal can be achieved first and foremost through the application of the system of Total Quality Management given the fact that relevant studies have shown that there is a correlation between successful Total Quality Management and efficacious information systems which fully apply the technology of information. Besides, historically, the applications of Total Quality Management have developed along with the informational systems of the hospitals and have been used in most applications in order to provide administrative support with regard to patient care. Informational systems are indispensable for the improvement of the quality of administrative procedures, for decision making, for matters concerning budgeting and for strategic planning.

Expert systems can also predict the demand for interhospital care, medical services and outpatients' visits if commercially available databases of demographic and of social/financial features of the population of certain areas are used in combination with the historical data concerning the hospital's patients. A study investigating the application of a health management system in Korea demonstrated that the informational system contributed to the decrease of waiting time for patients in a health center and to the simplification of administrative procedures.

Moreover, informational systems can create vital connections with patients in the context of the current increasing trend towards residential healthcare. The University of Wisconsin, for instance, developed a system to enable people suffering from HIV to manage their own care from a house, named a 'workstation'. The results emanating from the use of this system showed that these patients' bills decreased by 30% and that their hospitalizations were shorter: these are definitely positive effects given the fact that health systems must nowadays decrease their costs and increase out-of-hospital care. These patients also felt happier and more comfortable because they were in their own home rather than in healthcare institutions.

It is also interesting to note that, according to research carried out in American hospitals, each type of hospital technology (clinical or information technology) generates different types of quality-related performance (clinical or process), this both directly and indirectly affecting a hospital's financial performance. The study demonstrated that as US hospitals and healthcare organizations become more competitive, wise investments in technology and quality improvement are keys to financial success and survival (Ling, L, Collier, D, 2000).

Moreover, appropriate increases in the use of information technology in healthcare—particularly the introduction of clinical decision support and better linkages in and among systems, resulting in process simplification—can result in substantial enhancement of patient safety. More specifically, the current tendency is to test existing systems to ensure location of errors that could injure patients and, in general, to prevent any possible inaccuracies, to develop systems that communicate with

each other, to use systems in innovative ways, to make existing quality structures meaningful and to improve regulation and remove disincentives for vendors to provide clinical decision support. All of the above can be achieved by implementation of provider order entry systems, i.e. specially computerized prescribing procedures via implementation of bar-coding for medications, blood, devices and patients, as well as utilization of modern electronic systems to communicate key pieces of asynchronous data, such as markedly abnormal laboratory values (Bates, D et al, 2001).

Studies have shown that an electronic medical record that facilitates computerized physician order entry can prevent to a significant degree serious medication errors. Electronic prescribing has been shown to reduce prescription errors and improve compliance with managed care formularies, while point of care decision support tools can furnish providers with alerts for contraindicated medications (Anderson, J, 2007).

Regarding the person/patient or user of health services, the above informational systems have been proven to enhance the quality of care provided while simultaneously saving time and money. These results are observed regardless of their form: e-mail connections, use of electronic health card, patient's involvement in electronic health records and a variety of interactive online self-help networks (Mahmoud, E., G, Rice, 1998).

The use of the electronic health card is particularly capable of improving the quality of healthcare provided and of simplifying the procedures since the use of these smart cards enables the immediate and valid circulation of information between health professionals. In this way we avoid unnecessary repetition of diagnostic exams and prevent the prescription of non-compatible drugs and therapeutic programs. In addition, the storage and timely updating of medical information for each patient increases both the quantity and the quality of available information and, as a result, every insured user of the card enjoys a higher level of quantitative-qualitative patient-centered health services.

What is more, the use of the smart card improves communication between healthcare providers given the fact that it contributes to the exchange of information between the healthcare providers, the insurance organizations, pharmacies and doctors without geographical limitations. Citizens can now enjoy qualitative health services wherever they may be living without unnecessary delays caused by time-consuming bureaucratic procedures.

The decrease of bureaucracy, a serious social phenomenon that has long hampered modern health services, is also ensured and the functional efficiency of insurance organizations and healthcare providers is thereby improved. This is because the electronic availability and processing of the information significantly contributes to cost reduction of request handling and therefore the internal procedures of any healthcare provider become more efficient. The electronic submission of the demands contributes to the decrease of the time required for the completion of the administrative procedures, while it minimizes the possibility of inaccuracies that often ensue from handwritten data entries. Consequently, the prime cost is reduced and more efficient and productive financial management is achieved to the benefit of every provider and of the economy in general.

The electronic health record, the computer storage of information on a person's health, includes demographic features as well as the patient's medical history, risk factors, diagnosis, hospitalizations, operations, medical and pharmaceutical care, lab tests, medical interventions, experts' opinions, medical views and financial data, all this accompanied by text, image and sound.

The electronic health record has many advantages such as secure and direct access to information, to reimbursements and to clinical research support. In addition, it preserves sources and saves effort by eliminating double entries and the con-

stant noting down of a patient's medical history by the doctor: data is stored in one place and is accessible via wireless networks. Furthermore, through the creation of research databases, chronic diseases are better analysed and the patients are empowered through self-management of their electronic health record.

The social utility of the electronic healthcare and treatment record is also based on the fact that it lends itself to both scrutiny and documentation of any communication problems arising between health professionals and health services; it is also a means of analyzing the modes of communication and the values' scale through the functional interdependence of the members of a therapeutic team (Sarris, M, 2001).

Telemedicine is another important application of the new technology in the field of health, which also has extensive social dimensions. Its application meets presentday citizens' abovementioned dual requirement, namely, the trend towards preference for residential care and treatment rather than going to special hospitals, and their demand for better quality of treatment in conjunction with expense reduction. Telemedicine can have the form of a remote examination, of a system of drug prescription, of routine check-ups and residential follow-up, of interactive monitoring of a surgery, of access to telematic networks by healthcare professionals, of installation of networks of transplantation organs and bone marrow banks. It presents several advantages, among which the following: significant decrease of test expenses and of movement and as well as facilitation of management of the healthcare system; lessening of patients' geographical and physical isolation (remote areas, the aged, the disabled); avoidance of the need for repetition of painful examinations, conflicting prescriptions and treatment mistakes; possibility of advice from overseas experts who would otherwise not be accessible (www.medlamp.cs.uoi.gr).

Furthermore, in the framework of the National Health System, the availability and accessibility of services seems to fall off rapidly when there is a great distance between home or place of work and large secondary or tertiary units. There are also difficulties due to the weather and the ability to transport patients. The availability of the units is not the same 24 hours per day and is constrained by many factors such rush hour traffic, congested roads during holiday periods, etc.

Needless to say, time duration of response to any medical demand as well as the appropriateness—from any level of the system—and validity of the response are of vital significance. The advent of telemedicine has abolished the factor of distance between service and client since health expert teams are now enabled to deal with any emergency irrespective of their distance from the point of emergency, with communication time being reduced to a bare minimum. It is thus obvious that telemedicine can effectively contribute to the quality of health services (Karastergiou, H, 2009).

The social role of electronic health via use of current information and communication technologies to wholly satisfy the needs of citizens, patients and health professionals, optimal health being a fundamental requirement, has also been proved by the fact that among the main goals that the European Information Society has set over the past few years was also the improvement of public services and of quality of life without exclusions.

During the past half century, all member-states of the European Union as well as several other countries have increased their technological base for healthcare via knowledge and through investments in equipment, devices and pharmaceuticals. As a result, national healthcare systems have become increasingly advanced as healthcare delivery has introduced a range of technological innovations, such as new medicines and diagnostic tools, telemedicine and surgical equipment.

The introduction of new technologies has brought about remarkable improvements. The numerous recent innovations have resulted in implementation of new therapies with significant benefits for patients, including improved health,

enhanced quality of life and reduced adverse or side effects. Moreover, innovations in clinical practice provide enormous opportunities for physicians and other healthcare providers to improve the effectiveness, safety and quality of treatment. More broadly speaking, technological innovation provides governments with mechanisms to improve the quality and outcomes of national healthcare objectives (Sorenson, C, Drummond, M, Kanavos, P, 2008).

Nevertheless, implementation of information technologies has lagged in most European nations as well as the USA. In 2001, only 29% of primary care physicians in the European Union implemented electronic medical records, while in the USA fewer than 17% of primary care physicians routinely used electronic medical record in their practices. A recent report commissioned by the Health Information Network Europe indicates that hospitals in 15 European nations spend only 1.8% of total revenue on information technology. Only 2.2% of European hospitals have implemented computerized physician order entry systems with clinical decision support compared to 2.5% of American hospitals.

Until recently, information technology products available for healthcare providers were mostly designed for large organizations and were costly. However, recent advances in technology have enabled introduction of information technology applications into the field of primary care in countries with favorable government policies, while financial incentives have followed suit rapidly (Anderson, J, 2007).

According to the abovementioned study (Anderson, J, 2007), physicians as a whole perceive definite potential benefits in implementing information technology, while a recent survey of USA primary care physicians found that almost 75% indicated that these applications could reduce errors, 70% perceived information technology as potentially increasing their productivity and over 60% indicated that information technology tools have the potential to reduce costs and help patients assume more responsibility. Nonetheless, significant barriers impede widescale adoption of information technology, such as lack of access to capital by healthcare providers, complex systems, lack of data standards that permit exchange of clinical data, together with privacy concerns and legal barriers.

The Greek health system is being substantially influenced by international and domestic scientific and technological advances in life and other basic sciences and by technologies that detect and control health risks by preventing, diagnosing and curing illness. Also important are laser technology, blood banks, surgical techniques and alternative therapies (Liaropoulos, L, 1999).

In Greek society, among the problems of the health system (geographical inequalities, over centralization, bureaucratic management, poor incentives in the public sector, open-ended financing, inefficient use of hospital beds and lack of cost-effectiveness) is the fact that there are no specific legal provisions for the control of health technology. Technologies are introduced without standards or formal consideration of needs. Nevertheless, efforts to control health technology advances have been made over the years. For example, in 1997 a law provided for a new government agency responsible for quality control, economic evaluation of health services and the Health Technology Assessment and this last has gained increasing visibility.

The 1997 law, as well as other similar ones, has made possible introduction of the vital elements of evaluation and assessment into health policy formulation while ensuring that cost effectiveness, quality and appropriate use of health technology will receive more attention and will thereby reveal their true value to society (Liaropoulos, L, Kaidelidou, D, 2000).

The social role of technology in healthcare quality improvement has also been proven by two recent European initiatives concerning electronic participation and access to the Information Society: this involves “e-inclusion” (social inclusion)

and "e-accessibility" (electronic accessibility) (www.disabled.gr). The first initiative is related to the procedures necessary for the creation of an Information Society without exclusions so that every person may have the possibility, if he so wishes, to fully participate in the Information Society irrespective of his personal or social deficiencies. This will be not only for purposes of social justice but also for financial reasons such as increase of productivity and reduction of costs incurred by social and financial exclusion. The bridging of the gap in opportunity of accessibility further enables new job openings and services for society.

The second initiative which is called "electronic accessibility" is a means of breaking down the final barriers, opening up possibilities to even the least mobile and able members of our society who have traditionally faced great obstacles when attempting to participate bilaterally in the Information Society and to use products and services related to technology, informatics and communication. It must be noted that people with disabilities constitute 15% of the European population.

The European Union is therefore currently engaged in initiatives to satisfy the demands for models of accessibility to technological applications, the methodology dubbed "planning for all", according to which products and services must be designed in such a way as to be readily accessible to virtually to all European citizens. This comprises schemes and proposals for universal accessibility to the worldwide web, for formulation of criteria of comparative evaluation and monitoring of accessibility and, lastly, for research work specifically aimed at improvement of the quality of health services to be provided on a equal basis for all citizens together with citizen empowerment via electronic participation.

CONCLUSION

The involvement of new technologies in the field of health and in all social contexts is a fast moving development of our modernday world. Possibly one of the most important of these new technologies is the domain of health technology which today is poised to become an indispensable part of any nation's healthcare system, capable of contributing immensely to quality improvements in healthcare institutions. The new technologies are due to bring about a revolution in health and healthcare while guaranteeing their viability in the future. Electronic health and its multiple forms as well as biotechnologies can positively aid in the prevention of diseases in a society as well as to the provision of treatment, while they can additionally support the social trend of the change-over from hospital care to primary care.

To sum up, new technologies in the field of healthcare are proving their capacity to provide better care which is simultaneously more focused on the citizen and which also succeeds in eliminating social inequalities, this particularly in regard to special social groups. It is also able to bring about substantial cost reduction both for the individual and for society as a whole. As fully demonstrated by relevant research, electronic health has proven and is daily proving its unsurpassed efficiency and efficacy within the presentday social and humanistic aspirations for advances both in the quality of healthcare and in the upgrading of social conditions (Giberiti, A, 2007).

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Chapter 10
Intelligent System to Quality Assurance in Drugs Delivery

Antonio J. Jara
University of Murcia (UMU), Spain

Mona Alsaedy
Kingston University London, UK

Alberto F. Alcolea
University of Murcia (UMU), Spain

Miguel Zamora
University of Murcia (UMU), Spain

Antonio F. Gómez-Skarmeta
University of Murcia (UMU), Spain

ABSTRACT

Improving quality assurance and providing effective healthcare are some of the most important aims of information and communication technologies (ICT). This chapter presents a novel solution to improve quality assurance in drugs delivery, i.e., reduce clinical errors caused by drug interaction and dose. For that purpose, we have proposed an innovative system based on Internet of things for the drugs identification. Internet of things (IoT) is one of the latest advances in ICT, providing a global connectivity and management of sensors, devices, users, and information. Our contribution is a solution to examine drug related problems based on IoT technologies, i.e. smart phones and Web, to support ubiquitous access, 6LoWPAN technology to support ubiquitous data collection of patients, sensors and hospitals, and RFID/NFC to support global identification. These technologies offer a wide range of applications in healthcare, which improves the quality of services, reduces mistakes, and even detects health anomalies from vital signs. This chapter presents how IoT technology is applied in a pharmaceutical system to examine drugs in order to detect Adverse Drug Reactions (ADRs), harmful effects of pharmaceutical excipients, allergies, complications and contraindications related with liver and renal defects, and harmful side effects during pregnancy or lactation. Thereby, the system provides an enhanced approach assisting physicians in clinical decisions and drug prescribing.

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The solution presented is based on NFC (Near Field Communication), RFID (Radio Frequency Identification), and barcode identification technologies, which have been integrated in common devices such as smart-phones, PDAs, and PCs. In addition, a remote knowledge-based system based ontologies and rules-engine, has been built to define an intelligent drugs checker, which we have defined as Pharmaceutical Intelligent Information System, where the drug identifies collected from the RFID/NFC tag or barcode is checked, in order to detect whether the identified drug is suitable with respect to the patient's health record.

INTRODUCTION

Severe incidences take place in hospitals worldwide due to clinical errors and negligence are responsible for disabling injuries in about 1 in 25 hospital admissions (Leape, 1991). Most of these injuries are caused by Adverse Drug Reactions (ADR's). These events prolong hospital stay, increase care costs, and nearly double a patient's risk of death (Classen and Pestotnik, 1997; Bates, 1997). About one third of adverse drug events occur during drug administration, when interception is unlikely (Bates, 1995). The confusion caused by expressing the concentrations of drug solutions in different ways is an important cause of dose errors (Rolfe, 1995). Converting between ratios, percentages, international units, moles, micrograms, and milligrams causes substantial difficulty, especially for less experienced physicians. For example, *Epinephrine, lidocaine, heparin,* and *potassium chloride* are frequently associated with drug errors, it may be no coincidence that the strength of these drug solutions are typically expressed in ratios, percentages, international units, and millimoles, respectively. In addition, Adverse Drug Reactions (ADR's) and harmful effects of pharmaceutical excipients are important clinical issues due to the ADR's rate appearance in hospitals. Some studies present ADR's incidence of about 6.7% of severe cases and 0.32% of fatality (Lazarou, 1998, Classen, 1997) of the total cases presented in hospitals worldwide. The ADR's ratio is 8% in Spain (Azumendi, 2009) and 6.5% in the UK (Lammle, 2003). A recent study by the Royal Liverpool University Hospital (UK) shows an example of the ADR's consequences; 80% of the cases required hospital admission with a medium bed stay of eight days and a cost of $847m. The fatality rounded 0.15% of the cases. These problems may be avoided following a deep review of prescribed drugs, interactions and other complications. For this reason, we proposed a drugs checker using Internet of Things and a knowledge-based system to check dose, detect ADR's and drug interactions. Our solution comprises a personal system to check the drug suitability based on mobile devices, such as smart phones, PDAs or laptops. The mobile device identifies the drug by means of NFC (Near Field Communication) or barcode. The compatibility of the drug with the patient profile is checked with the Pharmaceutical Intelligent Information System (PIIS). It detects whether the product is suitable according to allergy profile and medical history of the patient, i.e, Electronic Health Record (EHR). Each time the physician prescribes a new drug to the patient and this drug is added to the patient's history record, the system checks any possible interactions, and warns the doctor of the possibility of alarming interactions.

PIIS is composed of a database, ontology, and a rule-based system. The database is represented in Figure 1, where arrows present the relations and link between the different tables. The content of this database is a detailed drug description, with details such as active ingredients and side effects. The ontology is used to define the patient's profile, including drugs concepts. Finally, it is used a rule-based system to detect allergies and ADR's.

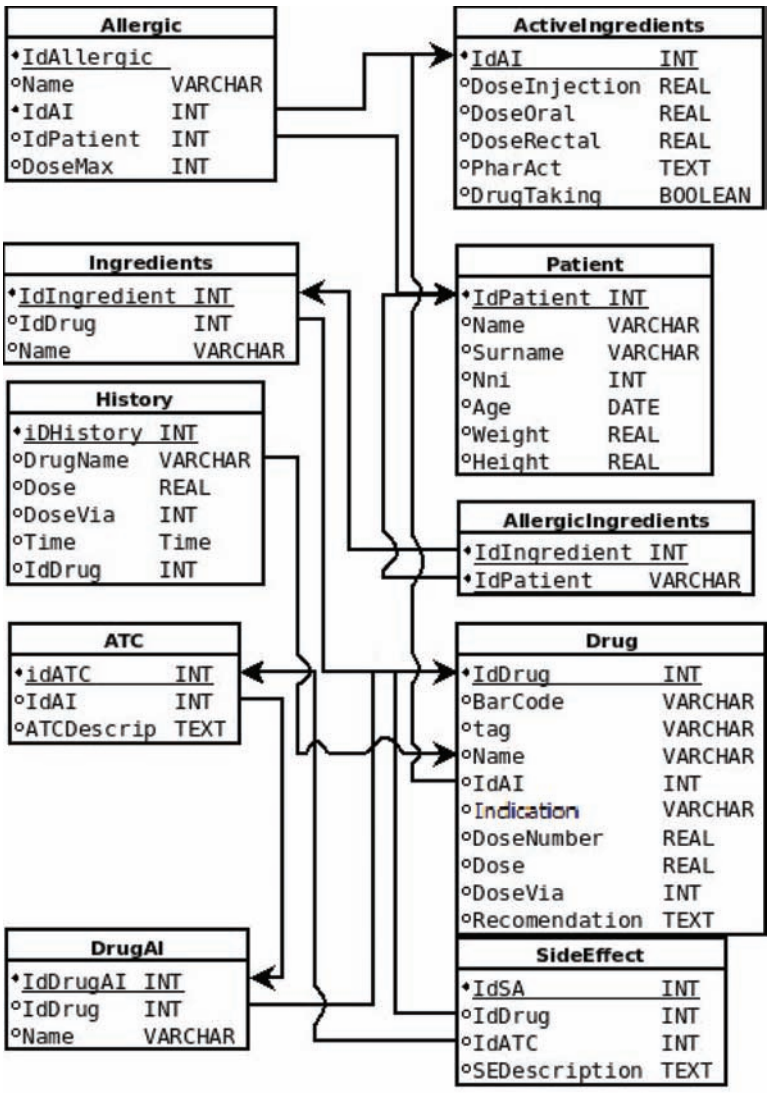
Some initial approaches to the Pharmaceutical Information System can be found in (Tamblyn, 2001; Yamamoto, 1998; Fernandez, 2002). An

approach to deployment of PIIS in Ambient Assisted Living environments can be found in (Alamo, 2008 A similar approach to the Internet of things, also based on RFID tags as a medium to access to de drug identification can be found in (Ting, 2009). Finally, real deployments of this type of solution have been carried out in Japan (Yamamoto, 1998) and Spain (Fernandez, 2002).

The aim of the system proposed in this chapter is to prevent ADR's, such as the previous version of this system (Jara, 2010). In addition, this new version detects active ingredient interactions, renal impairment complications, contraindications in pregnant and lactating women, and problems related with absorption of the drug. The system has been already tested with Non-steroidal anti-inflammatory drug (NSAID's) intolerant patient profile and will be further tested with other drug complications.

The rest of the chapter is organized as follows; presentation of drug related problems are presented by various health services, illustration

Figure 1. Allergies caused by active ingredients / drug sensitivity



of the architecture of the Pharmaceutical Intelligent Information System (PIIS). Furthermore, presentation of different user interfaces and ways to access the drugs ID based on barcode and NFC. Finally, this chapter is concluded and presented the ongoing work.

DRUG INTERACTIONS

Today, drug interactions are a major problem in the pharmaceutical and medical care aspects. The diversity of drugs generates various long and short term complications, such as drug drug interactions and ADR's. Undesirable side effects and drug related complications are in most cases unavoidable, but amendments and cautions are vital, even with commonly used drugs such as over the counter medicines.

A common example in this case would be a person who suffers from depression who may be treated with a Selective Serotonin Reuptake Inhibitor (SSRI), such as *escitalopram* (antidepressant). If the patient also suffers from pain such as headache or joint inflammation disorders, an over the counter NSAID can be bought without a prescription or it can even get prescribed such as *ibuprofen*. NSAID's interacts with *escitalopram* increasing the risk of GI bleeding especially in higher risk patients.

However, various factors may contribute to drug complications such as age, gender, disease state, liver and renal defects. Assessing patient health profile, previously prescribed drugs and monitoring are essential steps for safe prescribing.

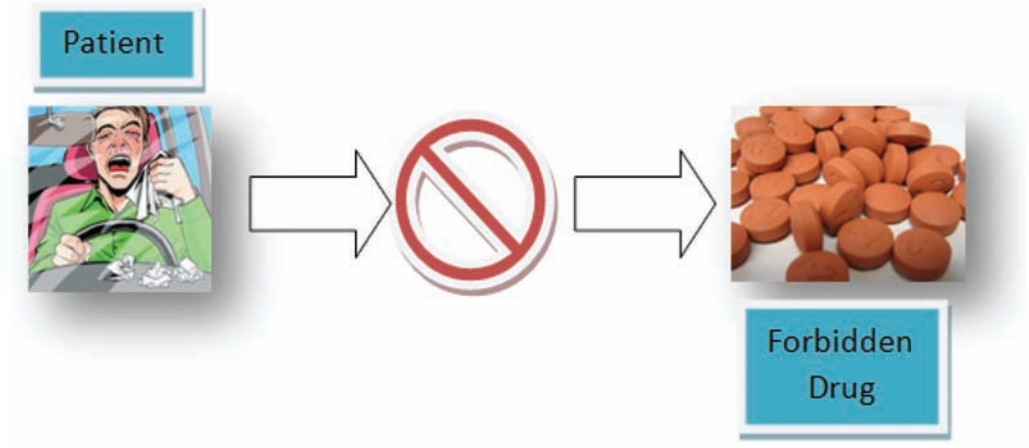
Drug Allergies Caused by the Active Ingredient

Drugs may also generate allergic reactions in hypersensitive patients such as skin rashes and dyspnoea (breathing difficulties) when for instance taking *penicillin*. This intelligent system is capable of alarming the patient and the prescriber of any relevant allergic reaction risks. An example is represented in Figure 2.

Active Ingredient Interactions

This is a drug-drug interaction where the first drug affects the activity of a second drug by either increasing or decreasing its effect. Many active ingredients and excipients may adversely affect the patients through a number of different mechanisms. The effect could be direct or an intrinsic pharmacological effect or pharmacody-

Figure 2. Forbidden active ingredients (allergies)



namic interactions. Whereas the latter mechanism generates an alteration of the second drug's action at the effectors site. The third mechanism of drug interaction is pharmacokinetics interactions where the drug alters the absorption, distribution, metabolism and/or elimination of the second drug.

In some cases these interactions can be toxic and fatal, or just simply produce undesirable side effects that may affect the compliance and the effectiveness of the treatment. The risk can be avoided using such intelligent system that warns of any drug interaction and the effects it may produce and also a proposed solution.

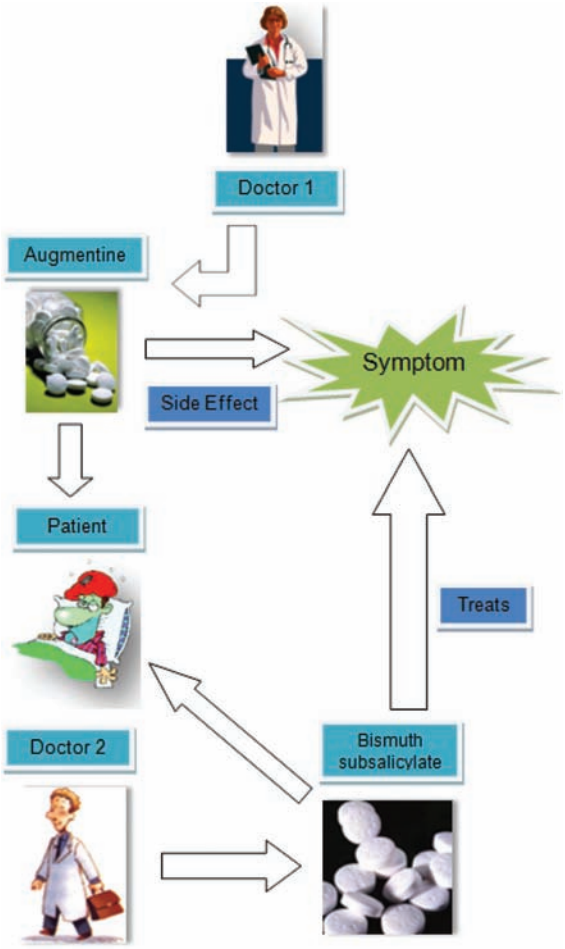
Drug Loops

A frequent problem in hospitals is the drug loops. This problem occurs when administered drugs generate undesirable side effects, which is solved by a second drug rather than changing the first that causes the side effect. As an example, Augmentine is a *Combination of penicillins* belonging to the Anatomical Therapeutic Chemical drug classification (ATC) group, including *beta-lactamase inhibitor. When this drug is* consumed by a patient it may generate side effects such as nausea. Consequently, this system detects other prescribed drugs such as *bismuth subsalicylate* to treat nausea. It advises the healthcare professionals about the possibility that the nausea is caused by *Augmentine*. Thereby, the prescriber may want to change the problematic drug to an alternative. Figure 3 represents a diagram with this type of problem.

Renal Impairment

A number of drugs require dose adjustment according to the renal and liver state especially those drugs that are excreted by the kidney and liver respectively. Some doses require adjustment based on biological tests. Thereby, the prescriber is able to adjust the dose when prescribing drugs simultaneously. An example can be illustrated by

Figure 3. Drug loop



using *Lisinopril*. Dose needs to be calculated in function of the Glomerular Filtration Rate (GFR), which are:

- Maximal initial doses 5 - 10 mg daily if GFR (Glomerular Filtration Rate) is 30 - 80 mL/minute/1.73m^2 (max. 40 mg daily)
- 2.5 - 5 mg daily if eGFR 10 - 30 mL/minute/1.73m^2 (max. 40 mg daily)
- 2.5 mg daily if eGFR less than 10 mL/minute/1.73m^2

Other advanced calculation related to the GFR may also include the patient's gender, weight, height, and lab data such as *createnine* clearance.

Using the above mentioned individualized data may assist the clinicians in their decisions. This may include drugs such as *digoxin, phenytoin* and other drugs that fall into narrow therapeutics index drug category. Hence, this system may provide a tailored dose adjustment advice or a rough guidance to the clinicians.

Pregnancy

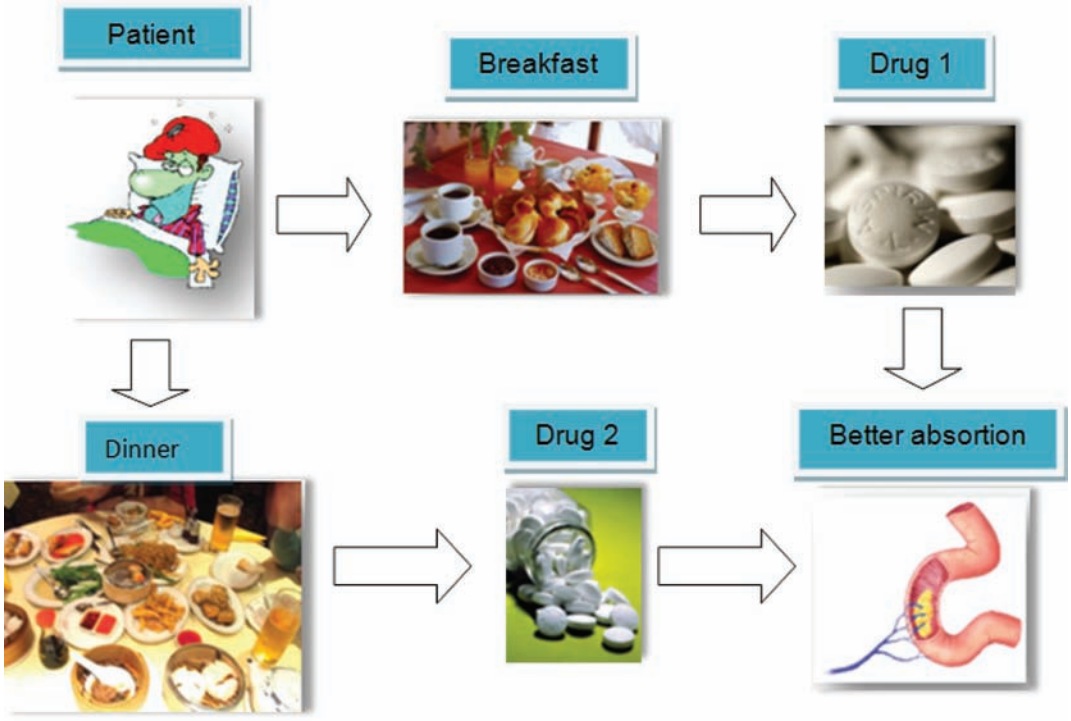
There are some drugs that must be either avoided or controlled during the beginning or during pregnancy and lactation, due to the potential damage produced to the fetus and/or the mother. Examples include:

- **Captopril:** it should be avoided in the first few weeks after delivery, especially in preterm infants, since risk of profound neonatal hypotension.
- **Cilazapril:** it is not recommended. However, alternative treatment options, with better established safety information during breast-feeding.
- **Ramipril:** it is a not recommended. Alternative treatment options, with better established safety information during breast-feeding are available. To be avoided in 2nd and 3rd trimester of pregnancy.
- **Quinapril:** it should be avoided in the first few weeks after delivery, particularly in preterm infants, due to the risk of profound neonatal hypotension.

Optimizing the Absorption Depending on the Time

Another important aspect in pharmaceutical care is drug absorption optimization and correct use. This may mean an enhanced therapeutic effects, or simply reduction of adverse drug reactions either

Figure 4. Absorption schedule



cause by the drugs pharmacological identity or by other interactions. Some avoidable drug interaction in this scenario would be due to ingestion of two drugs at the same time. In some cases, the loss of drug efficacy or loss of the desired absorption profile happens when drugs are administered at the same time. A simple solution is often what it takes to avoid a similar problem. This could be achieved by for example of drug administration at different times. This case is represented in Figure 4.

PHARMACEUTICAL INTELLIGENT INFORMATION SYSTEM

The Pharmaceutical Intelligent Information System (PIIS) is composed by a knowledge-Based System which contains a rule-engine system to detect the possible interactions between prescribed drugs patient, and an ontology where is described the drugs concepts and patients information.

Ontology

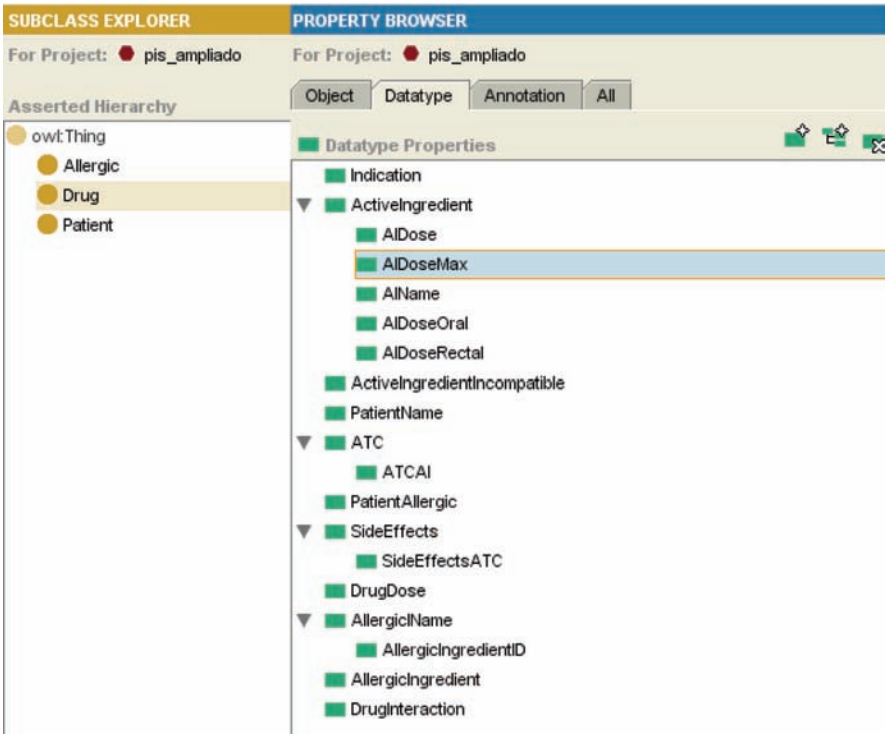
The drugs and patient information is stored in a database which is mapped into the ontology. The ontology is developed based on Protégé (Protégé, 2009), it is represented in the Figure 5.

The fields represented in the Figure 5 are:

- **ATC**: it is the Anatomical, Therapeutic, Chemical drug classification.
- **Dose, DoseNumber and DoseVia:** Information about the dose recommended and via trough which is injected.
- **DrugName:** the name of the drug.
- **RenalInteraction:** information needed in order to detect renal interactions.
- **Indications:** this shows the drug indications. They are useful to detect other types of interactions.

Regarding to the allergies, it can be found:

Figure 5. Protégé ontology concepts and their properties



- **ActiveIngredient:** it is the active ingredient that causes an allergy.
- **AllergicIngredientName:** This indicates an ingredient that causes an allergic.

Examples of values for the ontology concepts described are found in the Table 1. Where are shows some drugs mentioned for the examples from the past section.

Rules-Based System

Once the patient health record has been defined and the drugs information mapped on the ontology, a rule engine system detects drug interactions and allergies. The connection between Protégé and Jess (Jess, 2009) is based on JessTab (Eriksson, 2006). The current state of the rule system has defined rules detect interaction of single or several drugs administered to the patient, allergies caused by certain drugs or excipients and drugs loops. In addition, the system detects other complications taking into account other physiological factors such as the age, the liver and kidney state, the heart and also circumstantial states such as the pregnancy or lactancy.

Some rules of the rules-engine are shows in Table 2. The first rule detects drug interactions, where the main properties used are; active Ingredient and active Ingredient Incompatible. Thereby, it is able to detect when an Active Ingredient is in the list of not compatible Active Ingredients from other drug. The second rule detects patient allergies, the main properties used are Active Ingredient and Patient Allergic Ingredients. Finally, the third rule presented is to detect drug loops, which is based on Side Effects and Indication properties.

In Figure 6 may be found an example of how interact with Jess and Protégé through Java.

Table 1. Some examples of drugs and their features

Drugs	Indication	Possible individual side effects	Specific pregnancy, and lactating	Liver impairment
Captopril	Hypertension, congestive heart failure, myocardial infarction	Tachycardia, serum sickness, weight loss, stomatitis, maculo-papular rash, photosensitivity, flushing and acidosis	Should be avoided within the first few weeks after delivery, particularly in preterm infants, due to the risk of profound neonatal hypotension	
Cilazpril		Dyspnoea and bronchitis	Not recommended, however, alternative treatment options, with better established safety information during breast feeding are available	Monitoring in patients with impaired liver function
Enalapril Maleate	Hypertension; symptomatic heart failure, prevention of symptomatic heat failure in patients with asymptomatic left ventricular disfunction	Dyspnoea, depression, asthenia, arrhythmias, palpitation, flushing, confusion, nervousness, insomnia, vertigo, impotence, muscle cramps, tinnitus, alopecia, rarely gastrointestinal angioedema	Should be avoided within the first few weeks after delivery, particularly in preterm infants, due to the risk of profound neonatal hypotension	Monitoring in patients with impaired liver function
Fosinopril Sodium	Hypertension; congestive heart failure	Chest pain; musculoskeletal pain	Not recommended; alternative treatment options, with better established safety information during breast feeding are available	Monitoring in patients with impaired liver function

Table 2. Jess' rules

Rule	Jess´ Rule
Drug Interaction Rule	**(defrule rule1** **(object (is-a** http://PIIS_ampliado.owl#Drug) **(object ?d1)** **(**http://PIIS_ampliado.owl#**ActiveIngredient ?ai))** **(object (is-a** http://PIIS_ampliado.owl#Drug) **(object ?d2)** **(**http://PIIS_ampliado.owl#**ActiveIngredientIncompatible ?ai))** **(test (= (?ai ?aii)) =>** **(printout t "Drug Interaction found"))**
Allergy Interaction Rule	**(defrule rule2** **(object (is-a** http://PIIS_ampliado.owl#Drug) **(object ?d1)** **(**http://PIIS_ampliado.owl#**ActiveIngredient ?ai))** **(**http://PIIS_ampliado.owl#**PatientAllergic ?pa))** **(test (= (?ai ?pa)) =>** **(printout t "Allergy Interaction found"))**
Drug Loop Rule	**(defrule rule3** **(object (is-a** http://PIIS_ampliado.owl#Drug) **(object ?d1)** **(**http://PIIS_ampliado.owl#**SideEffect ?se))** **(object (is-a** http://PIIS_ampliado.owl#Drug) **(object ?d2)** **(**http://PIIS_ampliado.owl#**Indication ?in))** **(test (= (?se ?in)) =>** **(printout t "Drug Loop found"))**

First, the ontology must be opened. After that, a patient class, two drugs classes and an allergic class are gotten in order to be able to interact to them. The reason of two drugs is because very often the system is going to check the incompatibilities between two drugs. Once the classes are obtained, the instances are updated. The system selects the information either from a sensor or from a database. For example, when a drug tag is read (here, we are using NFC) the information is obtained from the sensor. When is desired to use the patient information, firstly the system has to make a data base select in order to get that information.

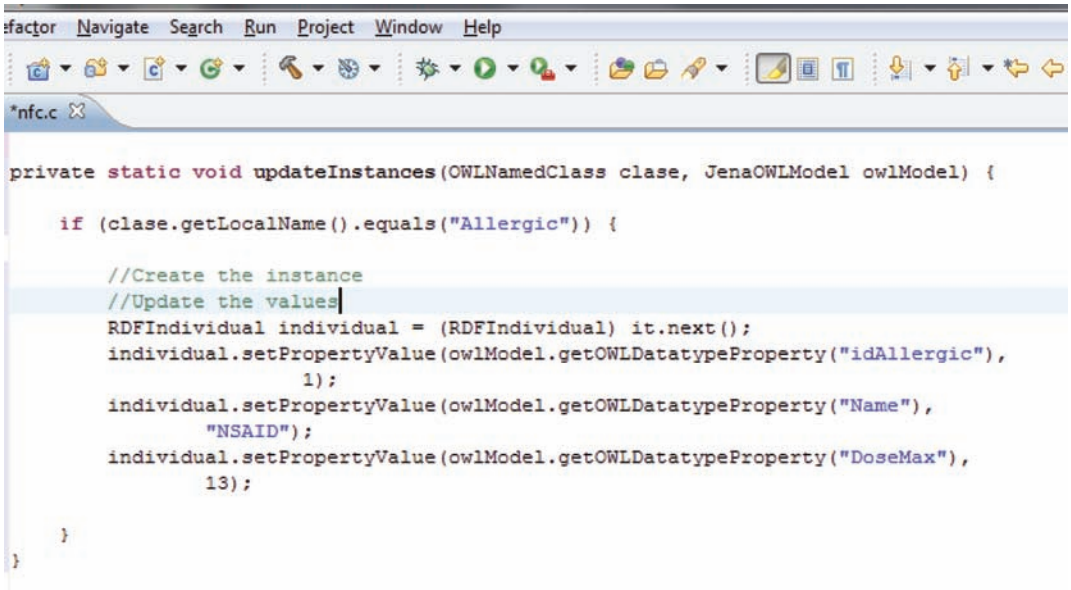
Figure 7 shows an example of the *updateInstance* procedure, where handcode information has been used to define the values of the *allergic* instance.

In Figure 7, the *RDFIndiviual* class is used. Using its method *setPropertyValue*, the values are mapped into the ontology. In the example, it is instantiated an allergic (NSAID in this case).

Figure 6. Java code example

```
//Using protege on Java
//1. Open the ontology
//2. Get the classes; patient, drug1, drug2, allergic
//3. Update the instances. It gets the information from
//   the oracle database and maps it into the ontology.
//4. Finally, run the Rules. The program calls to the
//   file.clp (clips file) and run it.
JenaOWLModel owlModel = openProject();
OWLNamedClass patient = getClass("patient", owlModel);
OWLNamedClass drug1 = getClass("drug", owlModel);
OWLNamedClass drug2 = getClass("drug", owlModel);
OWLNamedClass allergic = getClass("allergic", owlModel);
updateInstances(patient, owlModel);
updateInstances(drug1, owlModel);
updateInstances(drug2, owlModel);
updateInstances(allergic, owlModel);
//Run the Jess Rules
runRules();
```

Figure 7. updateInstance example



DRUGS IDENTIFICATION BASED ON IoT

Radio Frequency Identification (RFID) is one of the basis of IoT and consequently the integration of RFID in smart phones, NFC. For the identification of the drugs and patients, RFID/NFC tags have been considered. The problem is that the devices which integrate NFC are not very extended. Therefore, barcode has also been considered as legacy technology until the NFC is widely extended. In addition, barcode is found in all the drugs from the market. As a result, three different scenarios have been defined to identify drugs, one based on barcode and the rest based on NFC.

Access Based on Barcode Using Smart Phones

Nowadays the most of the smart phones are provided with a camera. Hence, taking in advance this functionality, a solution based on this multimedia resource may be used in order to scan barcodes.

For this project, the smart phone used is Google Nexus One, which is based on Android operative system version 2.2.1 and Zebra Crossing, ZXing library, an open source library used in order to decode multi-format 1D/2D barcode, (Mackintosh, 2009).

Therefore, drugs are identified by reading barcode. Drug ID is sent to the Pharmaceutical Intelligent Information System jointly with the patient profile using Internet connection (e.g. 3G or Wi-Fi). PIIS matches the drug ID with its knowledge-Based system and the patient profile and further sends an informative explanation to the smart phone. This information verifies whether this drug is suitable for the patient with a proposed solution. Figure 8 represents an example based on a NSAID sensitive patient. Left side of the display indicates that the drug is suitable for the patient when it turns green and vice versa when it turns red.

The smart phone application provides extended drugs information with advanced tailored health recommendations, i.e. it is explained why a drug is not compatible with the patient's profile. For example, the top left Figure 9 represents the explanation of *Aspirin* problems with NSAID patient.

Figure 8. Reading barcodes using smart phone

Furthermore, the smart phone solution allows the patients to defines and update their own health profile. The top right of Figure 9 shows the display with the patient profile and it also shows how to update the profile using an intuitive and friendly interface.

Finally, the smart phone application allows to extend existing drug information and to add information to the PIIS database, in order to improve the PIIS knowledge-base. Noting that any information added by the patients is not stored directly into the PIIS, it is saved into a verification subsystem PIIS since it needs to be verified before that can be verified by the system and available for other patients.

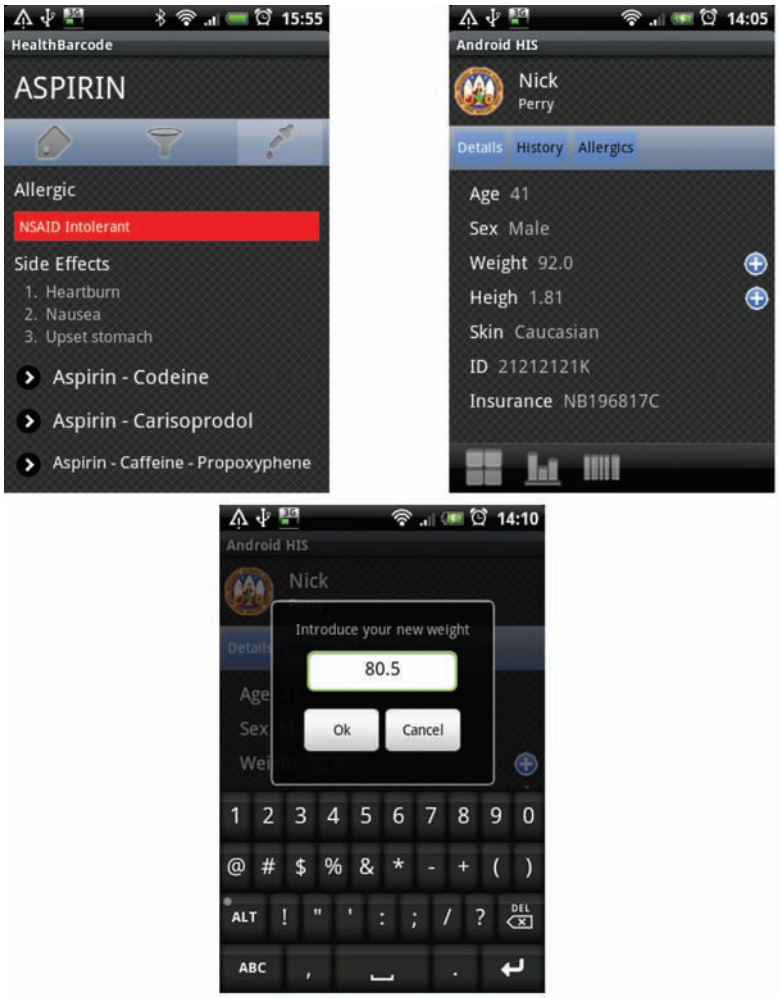
Access Based on NFC Using Pocket PC

The second scenario uses the potential of Internet of things with NFC technology. In this case we have added a RFID tag to each drug box, containing an unique ID to identify each drug without any possible mistake.

NFC solution can be used in smart phones as well, but nowadays are not very extended the models with NFC technology. Meanwhile the NFC test is being carried out using a Pocket PC, which uses the SDID 1010 NFC Card (SDID, 2009).

The process is similar to the barcode, but in this case the Pocket PC is approximated to the

Figure 9. Top left: Drug extended information. Top right: Patient profile. Down: Editing information.



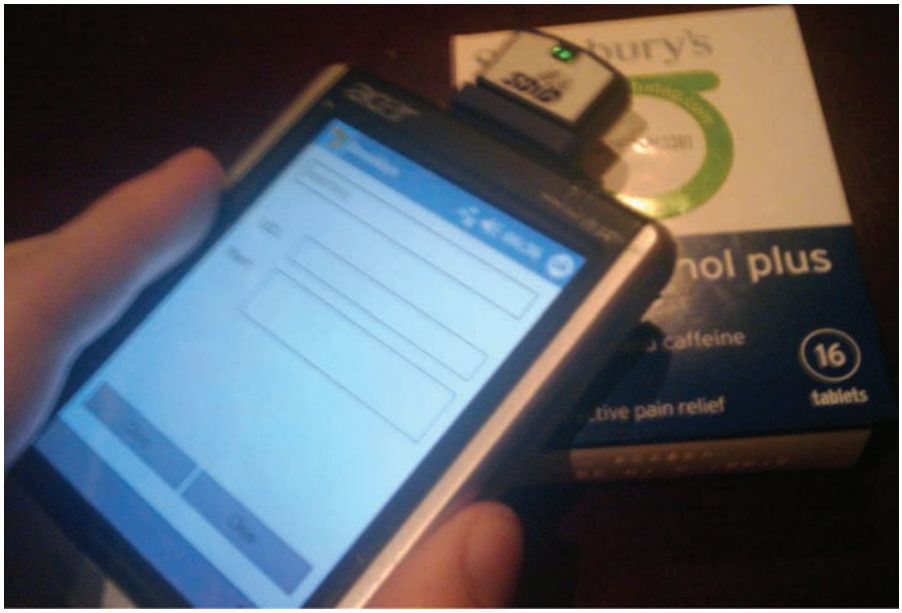
NFC drug tag, which reads the tag and starts the communication with PIIS. Figure 10 represents the Pocket PC reading a drug tag.

Access Based on NFC with USB Reader

Other solutions that were made are oriented to final user or customer so far. This new kind of solution is also based on NFC, but in this occasion using a NFC USB Reader.

This scenario is oriented to pharmacists, the problem found from the pharmacists' point of view is that they do not have access to the patient profile and EHR, therefore they cannot detect the mentioned problems. This problem can be solved by two approaches. On one hand, all the patient information can be stored in the PIIS. Therefore it can be acceded by pharmaceutics solution identifying users by his national insurance number or similar identifications methods. The pharmacist may also be able to confirm the drug scanning it with the NFC Reader. Moreover, the patient information is stored in a RFID smart card such as DESFire. It could be the same that is used in many countries to identify patients, with the difference that this card provides a chip to store the PIIS profile information. In addition, this card offers the appropriate security to manage confidential information (Jara and Zamora, 2009).

Figure 10. Identifying drugs using NFC



The way to proceed is similar to the first case, but in this scenario the pharmacist does not have a web interface to get the patient drug record. The information is obtained from the DESFire card, being only accessible by nominated healthcare professionals as pharmacists and doctors. After the pharmacist had read the NFC DESFire card, they can process it, at the same way that the first solution. Finally, the pharmacist can update the healthcare card with the new drug.

A solution of electronic pharmaceutical card is proposed in (Auzumendi, 2009). An optimized structure in NFC/RFID cards to reduce access latency, optimize capacity, and guarantee integrity has been defined for the pharmaceutical cards (Jara and Alcolea, 2010).

The USB reader solutions is being developed using the ACS 122 reader from Touchatag (Touchatag, 2009) and the libNFC library (LibNFC, 2009). Figure 11 shows how to read drug information using a laptop and the USB reader.

The information is stored in the patient's card and is showed in Figure 12, where the mentioned optimizations are applied.

CONCLUSION AND FUTURE RESEARCH DIRECTIONS

This chapter has presented a solution to improve quality assurance in drugs delivery; this is reaching reducing clinical errors caused by drug interaction and dose. For that purpose, we have proposed an innovative system based on Internet of things for the drugs identification and a remote knowledge-based system based ontologies and rules-engine, to define an intelligent drugs checker, which we have defined as Pharmaceutical Intelligent Information System.

Drugs are identified by means of an interchangeable mobile device, such as PDAs, laptops and smart phones, where is being considered the incipient NFC technology. This solution also offers support for a legacy identification solution based on barcode, in order to offer the solution before that NFC technology is widely implemented in new devices, and barcode is substituted by RFID/NFC tags.

Once the drug has been identified, this information is sent to the Pharmaceutical Intelligent Information System, where decomposed active ingredients are matched with the patient's allergy

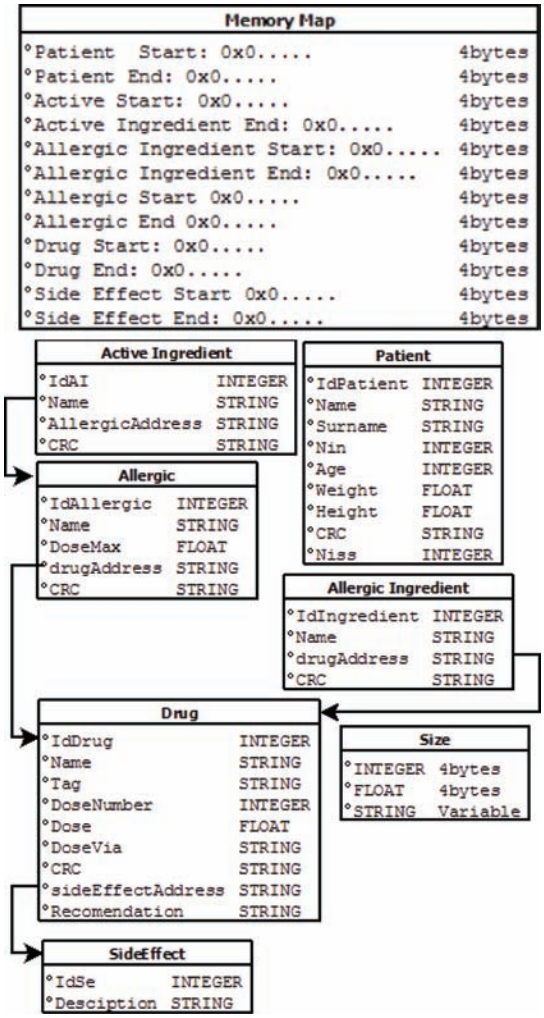
Figure 11. Identifying drugs using a NFC USB reader

profile and Electronic Health Record, in order to detect potential reactions.

A patient, with an NSAID's sensitivity profile, has been considered to test the system under real conditions. The drug database is synchronized with the Spanish Pharmaceutical Association database (PortalFarma), to embrace the whole set of current and future drugs and to keep it up to date.

Ongoing work is going to be, further development of the solution with additional drug interactions, an evaluation of the system is going to be carried out in UK chemists. Furthermore, the rules-engine system is going to be specialized to consider more specific cases from real life. Finally, since NFC microSD card is not being developed by the market right now, we are building a novel Future Internet device, in order to support this solution, this is going to be released Q1 2011.

Figure 12. Patient information records with all the structure optimizations presented in (Jara and Alcolea, 2010).



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Chapter 11
Quality in Telemedicine Services

Eleni Christodoulou
University of Peloponnese, Greece

Sofia Zyga
University of Peloponnese, Greece

Maria Athanasopoulou
University of Athens, Greece

ABSTRACT

Telemedicine refers to the delivery and provision of health care and consultative services to individual patients and the transmission of information related to care, over distance, using telecommunications technologies.Quality of service is a critical determinant of telemedicine performance. In addition, it can also be a critical factor in determining long-term viability of a telemedicine service provider. Although the term "quality assurance" was, at first, the main term for the experts to deal with, concepts such as "total quality management" and "continuous quality improvement" have come forth and are often used interchangeably. It is crystal clear that telemedicine services should meet the international quality requirements in order to accomplish quality assurance in healthcare provision. Technology advances have brought forward new and evolved services and technical infrastructure that promote and enhance quality healthcare services, such as telepresence and wearable technology. Nevertheless, there are several obstacles in telemedicine performance that need to be resolved.

INTRODUCTION

More and more attention has been given to telemedicine because of the progress that has been achieved in the telecommunications and information technologies. The facility and accessibility to the information is vital for health services in order to succeed in the current competitive environment of health care management.

According to the American Telemedicine Association (ATA), telemedicine is the use of medical information exchanged from one site to another via electronic communications to improve patients' health status (ATA, 2007). The term telemedicine has evolved into telehealth, often considered to have a broader scope towards health promotion and disease prevention (Koch, 2006).

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Telemedicine, in the simplest terms, is an expression that defines the delivery and provision of health care and consultative services to individual patients and the transmission of information related to care, over distance, using telecommunications technologies.

Even so, telemedicine is not merely a simple combination of health care and technology. Telemedicine is also not a service that is processed only at a certain time. Instead, it can be defined as a value-added health delivery system that is formed and delivered through the telemedicine system.

Healthcare services have moral, ethical, social and legal reasons to provide acceptable and guaranteed quality. Quality of service is a critical determinant of telemedicine performance. In addition, it can also be a critical factor in determining long-term viability of a telemedicine service provider. Therefore, assessing the telemedicine user's needs are key factors to the success of modern telemedicine.

QUALITY CONCEPTUAL APPROACHES

Defining quality is a matter of several parameters and according to Thomas Pyzdek (1990:Chapter 1), even the quality experts do not agree on a consistent definition.

According to Dr Joseph Juran, the concept of quality revolves around the concept of "fitness for use". Philip Crosby defines quality in terms of performance that produces "zero defects", whereas Dr. W. Edward Deming defines quality as a "never-ending cycle of continuous improvement" (Crosby, 1994Crosby, 1996 Deming, 2000).

According to a general definition, quality refers to the degree to which health care services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge.

Although the term "quality assurance" was at first the main term for the experts to deal with, concepts "such as "total quality management" and "continuous quality improvement", have come forth.

Quality assurance refers to a program for the systematic monitoring and evaluation of the various aspects of a project, service, or facility to ensure that standards of quality are being met. It merely concentrates on identifying poor providers rather than defective processes.

On the other hand, *Total Quality Management* (TQM) refers to a structured organizational process for involving personnel in planning and executing a continuous flow of improvements to provide quality health care that meets or exceed expectations.

Continuous Quality Improvement (CQI) refers to programs designed for clinical settings and encompasses quality improvement efforts and philosophies.

These two terms are often used interchangeably (McLaughlin et Kaluzny, 2006).

Evaluating quality is of key interest to many groups and individuals related to healthcare services. In an attempt to categorize the healthcare users, we would form two groups:

The first group is the one of the *internal users*, which includes the board of directors, individual physicians, clinicians, healthcare managers and employees, while the second is that of the *external users*, meaning the patients and their care givers, private and public purchasers/ payer groups, academic institutions/ researchers and the media.

All of these individuals need to measure or evaluate quality for different reasons. For example, in telemedicine research, the number of controlled variables directly depends on the scientific background of the team carrying out the study, i.e. telecomm variables for engineers, clinical variables for doctors and so on.

ENSURING QUALITY

The quality of services is always determined by certain attributes that they have or should have. The most important attributes health services should have, are accessibility and availability, usage facility, public's acceptance and all these always in relation to their cost.

An international communication code, or else a cognitive map, has been invented for ensuring quality. Always having the citizens and their well-being as the common threshold, the International Standardization Organization has composed product and services standards, known as ISO 9000 (-1-2-3-4), the equivalent standards of EN 2900 (-1-2-3-4) used in The European Union and the standards of ANSI used in the USA.

Hence, it is crystal clear that telemedicine services should meet the international quality requirements in order to accomplish quality assurance in healthcare provision.

Some of the factors that contribute in quality assurance are:

- "Quality costs", which encompasses the costs involved in making mistakes, in not doing things "right the first time," etc. (Antony, 2002).
- Performance improvement
- Services in a cost-effective and simple manner to meet the needs and expectations of the user while driving down quality costs
- Satisfying and exceeding user requirements and expectations.

The dimensions of quality healthcare services can be specified to the following:

- Tangibility, which includes physical facilities, tools, or equipment and appearance of personnel.
- Reliability is the ability to perform the expected service dependably and accurately.
- Responsiveness means the willingness to provide prompt service and help customers.
- Assurance is courtesy and knowledge of service providers and their ability to inspire and build confidence.
- Empathy is the providing of caring and individual attention to customers.

ENHANCING QUALITY

Apart from the already widely applied telemedicine methods, technology advances have brought forward new and evolved services and technical infrastructure that promote and enhance quality healthcare services.

- **Video conferencing systems**
  In the homes
  - of children with life-limiting conditions
  - after serious surgeries
  - of chronically ill patients,

to facilitate their care and support. This allows medical consultants to talk via video link with patients and their families. Patients have face-to-face discussions in the comfort of their own home reducing the need for regular hospital visits and allowing patients and their families to spend more time together.

In Multi-Disciplinary Teams; specialists and general practitioners examine patients and discuss treatment options together even if they are in different places or even countries. It is this collaborative environment that can improve patient care, cut down on traveling time and costs, thereby freeing hours for ongoing work and eventually provide high quality of care.

In virtually anywhere in the world, by breaking down geographical barriers and provide specialized medical care. Specialists' knowledge is more accessible. Patients can have a diagnostic

examination within hours or days without having to travel to a specialist center, saving both time and money.

- **Telepresence Systems:** it is about an innovative patient care delivery concept which combines state-of-the-art video, audio and call center technology with medical information, with the aim at creating a virtual face-to-face experience for patients and caregivers who may be miles apart. It provides access to medical diagnostic equipment, such as stethoscopes, otoscopes and vital signs monitors (http://www.vcinsight.com/default.asp?artID=4850).
- **Wearable Technology:** sensors and monitoring devices that can unobtrusively monitor the health and well-being of individuals (directly and/or remotely) (Sungmee et Jayaraman, 2003).
- **Medical Interpreter Services:** language barriers compromise patients understanding of treatment advice and their disease, increase the risk of complications, and make it harder for patients to explain their symptoms. Therefore, it is vital for healthcare systems to overcome the language barriers and have access to readily-available trained medical interpreter services for the provision of quality telemedicine services, especially in multi-cultural societies.
- **Multiplexing Technology:** This allows the transmission and reception of data simultaneously.

TELEMEDICINE ISSUES: OBSTRUCTING QUALITY

Telemedicine's growth and good medical practice, however successful, could be slowed down by several obstacles, having to do not only with the technical part but also with the human factor.

- The rapid advance of information and telecommunications technologies, move the key hardware and software components of telemedicine applications from state of the art to outmoded.
- The cost of the technical infrastructure can sometimes be prohibitively expensive.
- The technical infrastructure can also be complex, ambiguous and not user-friendly.
- There is an acute scarcity of trained manpower, especially in the developing world.
- Continuous employee education is a necessity.
- The lack of experience and research makes the telemedicine users reluctant.
- The language barriers can lead serious misunderstanding.
- There are legal, ethical and regulatory issues to be resolved.

These issues and many others must be taken into consideration in strategy planning of quality telemedicine services.

CONCLUSION

If there is one trait common to people throughout the world, it is the desire to keep one's health. The valuable tool called telemedicine will surely play a key role in global healthcare in the new millennium.

The systematic growth of Telemedicine constitutes a continuous challenge, in which knowledge, modern planning and programmed actions are essential. This modern planning is a responsibility and obligation of all relevant institutions (telecommunications, health institutions), which will contribute to the wide implementation of effective and efficient telemedicine networks.

The rise of the interest in quality of telemedicine services among all the individuals involved is nothing short of remarkable. Quality of service is a

critical determinant of telemedicine performance. It can also be a decisive factor in determining long-term viability of a telemedicine service provider. The modern electronic communication and information technology systems are the means that would allow health professionals and health services to deliver quality patient care.

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Chapter 12
Quality Based on a Spatial SERVQUAL Model in Healthcare

Stelios Zimeras
University of the Aegean, Greece

ABSTRACT

The main purpose of this work is to represent an alternative effective model for measuring the quality of healthcare (SERVQUAL) considering the geographical location of the under examination healthcare sectors. Based on that consideration, a new modeling is taking place introducing a spatial interaction between neighboring regions (spatial-SERVQUAL model). New directions are analyzed implementing specific questionnaires taking advantage of the spatial information given by the evaluation of the model. Moreover the role of spatial information in the health sector in relation to, for example, local health improvement performance management, is analyzed to support needs assessment and resource targeting as one of the principal action points in healthcare policies. Finally, the benefits of the GIS systems are illustrated, combined with the spatial assumptions, introducing a real-time health and disease monitoring tool to identify significant health trends in real-time data streams and geographic information systems.

INTRODUCTION

User satisfaction and/or service quality (SERVQUAL) constitute essential components of healthcare. Users determine the strategy for quality management in healthcare services. Methodological issues concerning service quality measurements of healthcare have been discussed in the international literature for many years and have been the subject of topical studies throughout the world.

Worldwide, systems to measure service quality have been developed which have subsequently been modified according to the particular case and adopted by major organizations. With reference to the nature and structure of the service provided, the public sector is conducting user-satisfaction measurement research in competitive environ-

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ments in order to develop prior principal and support services with the aim of improving the relationship between the provider and the user. Satisfaction with service quality depends on a large number of dimensions - both tangible and intangible attributes of the product-service offer. The impact of intangible dimensions on consumer satisfaction is of particular interest at this point. To explain the importance of adopting common semantics when developing health geo- information services that span administrative boundaries, geographical information for the evaluation of the healthcare sectors are considering. Geography plays a major role in understanding the dynamics of health, and the causes and spread of disease.The classic public health emphasizes the importance of geographic location (environment or space where we live) in health and disease. Today's health planners aim at developing health policy and services that address geographical and social inequalities in health, and therefore should benefit from evidence-based approaches that can be used to investigate spatial aspects of health policy and practice, and evaluate geographical equity (or inequity) in health service provision. It is therefore crucial to develop an alternative modeling approach where spatial analysis techniques and geographical locations are combined suggesting a more effective model for the evaluation of the customer satisfaction in healthcare. In this work the benefits of the geographical information are analyzed considering specific tasks (directions) inside the SERVQUAL model.

In more complex spatial models it would also be possible to include a spatial density of the location of the healthcare sectors, thus allowing for distinguishing between more rural and urban areas. This would translate into a more sophisticated approach of the availability factor. However, it is expected that such sophisticated models are more appropriate to address issues such as location planning and management policies. Finally because there is a close relationship between spatial analysis and GIS systems, in this work the benefits of these systems in healthcare decisions making policies are presented taking advantage the information from the evaluation process.

Motivation

User satisfaction and/or service quality (SERVQUAL) constitute essential components of healthcare (Donabedian, 1988). Users determine the strategy for quality management in healthcare services (Hasin et. al., 2001). Methodological issues concerning service quality measurements of healthcare have been discussed in the international literature (Lin and Kelly, 1995) for many years and have been the subject of topical studies throughout the world (Ovretveit, 2000). In Greece, significant efforts have been made to develop user satisfaction models (Grigoroudis and Siskos, 2002; Athanasopoulos et. al., 2001) as well as to assess user satisfaction in healthcare services (Angelopoulou et.al 1998; Merkouris et. al., 1999; Moumtzoglou et. al., 2000; Camilleri and O'Callaghan, 1998). The fact is that "user satisfaction" and/or "service quality" are complex phenomena involving intricate operations such as the measurement of quality in healthcare services, currently under examination, their perceived "value", and the social image of the organization. Geography plays a major role in understanding the dynamics of health, and the causes and spread of disease.The classic public health emphasizes the importance of geographic location (environment or space where we live) in health and disease. It is therefore crucial to develop an alternative modeling approach where spatial analysis techniques and geographical locations are combined suggesting a more effective model for the evaluation of the customer satisfaction in healthcare.

Definition of Quality in Healthcare

Worldwide, systems to measure service quality have been developed which have subsequently been modified according to the particular case and adopted by major organizations. With reference to the nature and structure of the service provided, the public sector is conducting user-satisfaction measurement research in competitive environments in order to develop prior principal and support services with the aim of improving the relationship between the provider and the user. The final objective lies in increasing the market share of the organization. This observation also refers to the close relation between quality management and the marketing policy. With regard to healthcare services, a range of actions within the framework of a "special Marketing" can be introduced, which apart from taking into account the user satisfaction measurement will also aim at meeting user expectations. Quality measurement objectives in the Public Sector differ from the ones in the Private Sector as illustrated by the following statements (Robinson, 1999; Teas, 1993): A) Questions regarding service pricing rarely arise, whereas data concerning the user's perceived "value" of the service provided are taken into consideration (Juran, 1988). B) Often questions asked refer to the Public dimension of the Healthcare System, which must function in keeping with the concept of public interest, safeguarding its basic principles such as equal treatment, protection and safety, totality as well as accessibility to health services. **C)** The "user expectations" parameter assumes major importance for user "acceptance" of the services provided and also for the final "value" (Parasuraman et. al., 1988) attributed to the public system of healthcare services. The trilogy for quality management based on Juran (Parasuraman et. al., 1995), is consisting of Quality Planning, Quality Control and Quality Improvement. Quality planning involves identification of customers and their needs, and development of products to satisfy customer needs, and processes to produce those product attributes. Quality control involves evaluation of the gap between actual and targeted quality performances and the actions to fill the gap. Quality improvement deals with development of infrastructure, identification of improvement goals, establishment of project teams and allocation of resources to implement quality improvement projects.

The approach based on user satisfaction, considers the expectation measurement as well as on disconfirmation of user expectations. In this case, the established model is the SERVQUAL "Service Quality" model developed by Parasuraman et al. (Parasuraman et. al., 1985; 1988; 1995) Moreover, within this approach Service Quality (SQ) is measured by comparing Perceptions "P" with Expectations "E" and is defined as the difference between perceptions and expectations (SQ=P-E). Regardless of the numerous distinct views voiced within the framework of this specific approach, it is agreed that the user perceives "high" quality when perceived performance exceeds his or her expectations. Another broadly known methodology relating to the expectation disconfirmation approach lies in the model developed by Oliver (Oliver, 1996) (expectation disconfirmation model).

SERVICE QUALITY IN HEALTHCARE

Service quality is relating to meets customers satisfaction needs, leading to the investigation of preserved service quality in order to understand customers. Parasuraman et. al. (Parasuraman et. al., 1985; 1988; 1995) looks at preserved quality as the difference between customers' expectation and their perceptions of the actual service received. Customer satisfaction is an individual emotional response to the evaluation of an object (or a service) (Woodruff, 1997).

It is difficult to measure service quality due to fewer tangible cues available when customers purchase services (Parasuraman et. al., 1988),

fewer search properties but higher in experience and credence properties (Parasuraman et. al., 1988), as compared to services. There are a number of different definitions as to what is meant by service quality. One that is commonly used defines service quality as the extent to which a service meets customers' needs or expectations. Service quality can thus be defined as the difference between customer expectations of service and perceived service. If expectations are greater than performance, then perceived quality is less than satisfactory and hence customer dissatisfaction occurs.

Although the SERVQUAL model (figure 1) has raised debates about dimensionality, the need to measure expectations, the reliability and validity of difference-score formulation, and the interpretation of expectations, it have been the predominant method used to measure consumers' perceptions of service quality. It represents a multi-item scale that can be used for measuring expectations and perceptions of service quality- as perceived among consumers. The SERVQUAL model, a 22-item scale has five generic dimensions or factors and is stated as follows: (1) Tangibles, (2) Reliability, (3) Responsiveness, (4) Assurance, (5) Empathy (Figure 1). When referring to surveys related to healthcare institutions the dimensions of service quality can be adjusted as follows (Parasuraman et. al., 1988, 1995):

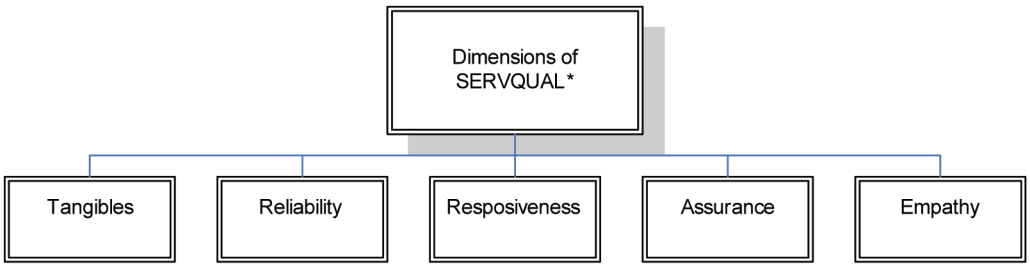
1. **Reliability:** ability to perform the service dependably and accurately
2. **Tangibles:** appearance of physical facilities and provision of appropriate equipment
3. **Responsiveness:** willingness to help customers
4. **Assurance:** the knowledge of employees and their ability to inspire trust and confidence.
5. **Empathy:** the caring individualized attention the firm provides to its customers.

It is important to note that without adequate information on both the quality of services expected and perceptions of services received then feedback from customer surveys can be highly misleading from both a policy and an operational perspective. To improve quality services to these customers we must first of all understand their needs. In order to understand their needs, we must in turn understand the quality attributes embraced by the customers. People perceive quality differently.

When analyzing the data gathered most of the surveys are based on the 'gap theory', that is, the difference between clients' expectations about performance of a service and their assessment of the actual performance of service. For example 'gap theory' in healthcare context has been used to develop a number of questions in order to compare what customers 'look for' (expect) and what they 'experience' from doctors, equipments, healthcare services e.t.c.

It is important to note, that the SERVQUAL is designed to investigate the abovementioned gap; nevertheless it is important to recognize the existence of four other gaps (Figure 2):

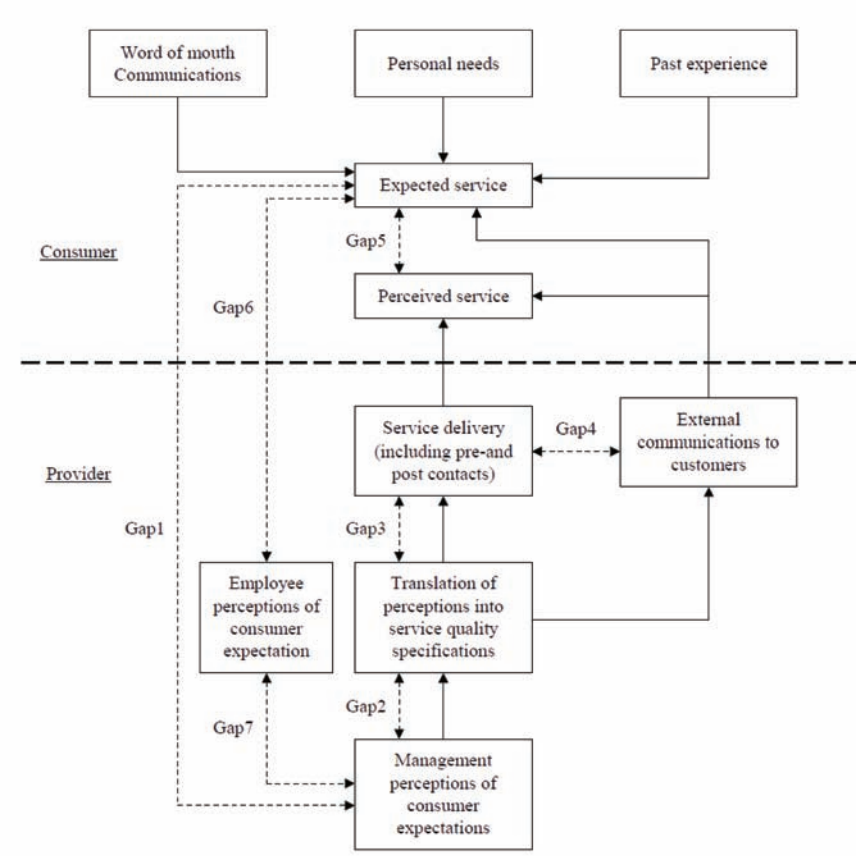
Figure 1. SERVQUAL's five dimensions



- **The understanding gap:** the difference between what consumers expect of a service and what management perceives consumers to expect.
- **The design gap:** the difference between the management perceives and consumers expect and the quality specifications set for service delivery.
- **The delivery gap:** the difference between the quality specifications set for service delivery and the actual quality of that service delivery.
- **The communications gap:** the difference between the actual quality of service delivery and the quality of that service delivery as described in the 'firm's' external communications.

When gathering the data from the questionnaires, in the case study of the survey, there may be a 'mismatch' between customer's expectations and their perceived quality. The analysis of the gathered data may be descriptive as well as inferential. A researcher assessed the scale's reliability by calculating the Cronbach alpha; for analyzing the service quality, the mean and the standard deviation scores for each of the items were calculated for the perception level (P) and the expectation levels (E). The mean perception scores were compared to the mean expectation scores for the various customer requirements and the design characteristics, so as to identify the Gap scores (P-E=Gap). The underlying dimensionality was tested through an exploratory factor analysis conducted on each of the correlation matrices of the percep-

Figure 2. Model of service quality gaps (Parasuraman et al., 1985)



tion, expectation, and Gap scores. Thereafter the QDF[1] matrix was used, wherein the respondent was required to specify the relationship between the customer requirements and design characteristics. Finally, the quality function deployment technique along with correlation analysis was used to identify the minimum set of designs elements (synonymous to the quality components) able to cover the customer requirements.

Data Analysis Procedures

Some researchers, in order to test whether the initial conceptual framework was optimal, they use two types of factor analyses, namely exploratory factor analysis (EFA) and confirmatory factor analysis (CFA), followed by reliability analysis and qualitative analysis. Confirmatory factor analysis is first performed to evaluate the validity of the original SERVQUAL conceptual framework. Exploratory factor analysis is then performed for determining the number and structure of the dimensions that are underlying the data. Then the resulted dimensions from the exploratory factor analysis are then confirmed again by using the confirmatory factor analysis and reliability analysis. The derived dimensions and items are then compared with the findings of qualitative analysis for its validity and usefulness.

Due to the large number of variables, the use of statistical methodologies comprising multivariate analysis techniques (Mardia et. al., 1979) may elucidate the variables of overriding importance for the complex problems under investigation and depict correlations. Multivariate statistics facilitate the analysis of complex sets of data. Multivariate techniques are recommended when there are many independent and possible dependent variables, which are correlated to each other to varying degrees. It reduces attribute space from a larger number of variables to a smaller number of factors. In many scientific fields, particularly behavioral and social sciences, variables cannot be measured directly. Such variables, called latent variables, can be measured by other 'quantifiable' variables, which reflect the underlying variables of interest. Factor analysis attempts to explain the association between variable values in terms of the underlying factors, which are not directly observable.

Principal components analysis (PCA) is a statistical technique applied to a single set of variables to discover which sets of indicators in the set form coherent subsets that are relatively independent of one another. PCA is used as a tool to reduce a large set of variables to a more meaningful structure. The main purpose of PCA is to reduce the dimensionality from p to d, where $d<p$, while at the same time accounting for as much as the variation in the original data as possible. With PCA, we transform the data to a new set of coordinates that are a linear combination of the original variables. The observations in the new principal component space are uncorrelated. The idea behind this procedure is that we can gain information and understand better the structure of the data in the new space.

The factor analysis model extracts only that proportion of variance, which is due to the common factors and shared by several items. The proportion of variance of a particular item that is due to common factors (shared with other items) is called communality. The proportion of variance that is unique to each item is then the respective item's total variance minus the communality. The first principal component is the combination of variables that explains the greatest amount of variation.

PCA is often used as a pattern recognition technique. The procedure involves a correlation operator for the grid point errors over time. In order to investigate the spatial significance of the error, a correlation matrix $C(x_i, x_j)$ of the errors for each grid point is constructed that contains the spatial relationship of the errors over time. The matrix $C(x_i, x_j)$ is decomposed into its eigenvalues and eigenvectors in two parts. The first employs the reduction technique to reduce the matrix to a

symmetric tridiagonal matrix. The second employs a way to find the eigenvalues λ_j and eigenvectors e_j of the tridiagonal matrix. These eigenvectors are constructed in order of decreasing variance reflecting the spatial relationship of events in time.

Also cluster analysis could be considered in cases we investigate the effectiveness of the characteristics into spatial regions. Using this technique, we could justify which regions are important so we could direct our analysis to these areas. Cluster analysis is a technique used to classify objects into relatively homogeneous groups called clusters. Objects in each cluster tend to be similar to each other and dissimilar objects in the other clusters (Malhotra and Birks, 2003). A very useful graph in displaying clustering results is the dendrogram. The dendrogram is read from left to right. It shows where the clusters are joined together as well as the distance at which the clusters are jointed. It is very useful to decide the appropriate number of clusters.

Definition of Spatial SERVQUAL Model in Healthcare

Quality is defined as conformance to requirements (Crosby, 1979). This definition of quality implies that the term is related with the definition of fundamental needs, desires or requirements of individual customers. Also quality could be defined as the ability to get the desired services from the chosen provider at the right price (satisfaction). Hemon (2002) implies that quality is in close relation between customers and the organization and between expectations for excellent services and perceptions of service delivered. In process, quality satisfaction is the intensity of various emotions tied to specific requirements during a period. Based on that statement, quality satisfaction is promptly related to demographical characteristics and geographical requirements of the particular regions, that the services are taking place. Geographical analysis implies calculation of specific weights based on expectation and perception for the customers needs. General speaking geography plays an important role for understanding specific needs and desires considering various customers groups. Also it could be used as a powerful tool for developing health policies and services considering geographical locations. For that reason, an alternative model must be considered (spatial SERVQUAL model) that should try to search and use effective information in everyday decision-making processes for the public sectors in order to minimize investment of efforts and funds in areas where there is solid evidence of no effect, or evidence of harm, or of poor cost-effectiveness. Evidence-based approaches can also highlight areas where the evidence may be less than reliable, requiring further assessment before expending large funds and efforts (Boulos, 2004).

Developing a mathematical model for customer satisfaction lets define healthcare provision during a period t with a variable x_t and customer experience which is represented by a certain level of satisfaction with variable s_t. Based on the spatial analysis of the model a weight variable w_t^r must be calculated including a wide area of characteristics for every region r. As a pilot analysis, it could be considered the answers' results based on the 5 dimension. These two models could be represented by the following equations $s_t = w_t^r f(x_t)$ and $x_{t+1} = w_{t+1}^r g(s_t)$.

The equally-weighted model (identical weight for all service dimensions and characteristics) is expressed by the following ratios:[11]

$$SQ_{kj}^r = \frac{\sum_{j=1}^{n_j} P_{ij}^r - E_{ij}^r}{n_j^r} \;\&\; OSQ^r = \frac{\sum_{k=1}^{N}\sum_{j=1}^{5} SQ_{jk}^r}{5 \cdot N}$$

where, SQ_j^r stands for Service Quality of the dimension j for every respondent (k=1,…,N) for region r, E_{ij}^r stands for the Expected Quality for characteristic i of dimension j of the service for

region r, P_{ij}^{r} stands for Perceived Quality for characteristic i of dimension j of the service for region r, n_j^r stands for the number of characteristics i of dimension j of the service for region r. Service Quality is estimated for every dimension, taking the SQ_j main value for all respondents (k=1…N). The Overall Service Quality (OSQ) is estimated by the main value of the five dimensions.

The weights employed in SERVQUAL models arise either by introducing a third section of questions for the estimation of weight by the users themselves or by the personnel in the organization through preliminary empirical studies *(structured survey or interview)* or through a combination of the above, in keeping with the following ratio:

$$OSQ_t^r = \sum_{1}^{N} \mathbf{W} \cdot \left(\mathbf{P} - \mathbf{E}\right)$$

W is the weight array assigned to quality dimensions and/or characteristic estimated by the user or otherwise, calculated by the variables w_t^r which includes characteristics for every region r at time t.

GIS are potentially powerful resources for community health for many reasons including their ability to integrate data from disparate sources to produce new information, and their inherent visualization (mapping) functions, which can promote creative problem solving and sound decisions with lasting, positive impacts on people's lives. Most traditional analysis of spatial data in healthcare is the choropleth maps, which are characteristics spatial incidences (or events), are illustrated on these maps as polygons. Each of these polygons represents a numerical identification of the represented map characteristic in every region. Finally spatial statistical analysis techniques like kriging or kernel method could be considered to estimate the spatial densities of the regions.

Spatial Statistics Techniques

In the case of a point pattern, we are concerned with the *intensity*. *First order intensity* refers to the mean number of points per unit area. *Second order intensity* refers to the spatial relationship/dependence among the number of events in different areas of the region. The most basic is a simple point estimate which counts the number of points and divides by the total area. Units will be points/events per area so the ground scale becomes important. Other methods give local estimates and hence the intensity is likely to vary across the region.

First-order properties are described in terms of intensity $\lambda(\mathbf{s})$, of the process, which is the mean number of events per unit at the point **s.** This is defined as $\lambda(s) = \lim_{ds\to 0}\left\{\frac{E\left(N\left(ds\right)\right)}{\left|ds\right|}\right\}$, where d(**s**) is a small region around the point **s**, E(.) is the expected value, and N(d(**s**)) is the number of events in the small region (Cressie, 1993; Diggle, 2003).

The three most common techniques for estimating the first-order intensity λ(s) are: 1. the Basic estimator, which assumes λ(s) = λ for some constant λ; 2. the Binning estimator, which is simply a smoothed two-dimensional histogram; and 3. the Kernel estimator that employs methods from kernel density estimation to obtain an estimate of λs). Estimators of intensity over a study region A are given by:

$$Basic: \hat{\lambda} = \frac{n}{|A|}$$

$$Binning: \hat{\lambda}(s) = \sum_{j=1}^{bins} k\left(\frac{s - s_j}{b}\right)\sum_{j=1}^{n}\frac{I\left(s_i \in ds_j\right)}{\left|ds_j\right|}$$

$$Kernel: \hat{\lambda}_b(s) = \frac{1}{p_b(s)}\sum_{i=1}^{n} k\left(\frac{s - s_j}{b}\right)$$

I(·) is an indicator function, $|ds_j|$ the size of the bin centered at s_j, κ(·) is a kernel function, and b is an bandwidth satisfying standard conditions (Diggle, 2003), b is a bandwidth, and p_b is an edge-correction factor given by $p_b(s) = \int_A \frac{1}{b^2} k\left(\frac{s-u}{b}\right) du$, s∈A. [6] provides details on the choice of κ(·) and suggestions for selecting the bandwidth b. A recommended choice for the bandwidth is $h=0.68n^{-0.2}$ (Diggle, 2003).

The choice of the bandwidth (d) is purely subjective. It is up to the researcher to investigate the results using several different bandwidths and selecting the one that best represents the pattern. However, it is important to remember that when d is large, the kernel estimator produces a smooth estimate of the density function (i.e. small variance, large bias). If d is small, the kernel estimator produces a rough estimate of the density function (i.e. large variance, small bias). Considering the results, it is clear that the data are clusters (introducing a cluster pattern) with two strong pattern concentrations.

Various techniques have been suggested to investigate the nature and extent of spatial correlation between demographic variables. These techniques constitute what is now called exploratory spatial data analysis (ESDA) by reference to exploratory data analysis (EDA) proposed by Tukey in the late 1970s. These techniques include visual and quantitative methods to summarize the spatial properties of a variable, to describe its specific patterns in space, spot extreme values or outliers, and to identify specific geographical subsets. The availability of data in a GIS (geographic information system) format allows the systematic spatial exploration of the data.

The general idea behind these techniques is the examination of the nature of spatial variation (referring as spatial auto-correlation) between values of the same variable at different spatial locations (referring as neighborhood observations). Once the concept of "neighboring observations" is defined, the correlation between neighbors may be compared to the general variance of the sample in the same way as in ordinary correlation analysis. The resulting measure of spatial autocorrelation is a first indication of the spatialized nature of the phenomenon studied: this correlation may be non-existent, low or strong according to the variables used.

Moran introduced in 1950 the first measure of spatial autocorrelation in order to study stochastic phenomena, which are distributed in space in two or more dimensions. Moran's index has been subsequently used in almost all studies employing spatial autocorrelation. Moran's I is used to estimate the strength of this correlation between observations as a function of the distance separating them (correlograms). Like a correlation coefficient the values of Moran's I range from +1 (meaning strong positive spatial autocorrelation) to 0 (meaning a random pattern) and to –1 (indicating strong negative spatial autocorrelation); negative autocorrelation is extremely unusual in social sciences. Values near +1 indicate similar values tend to cluster; values near -1 indicate dissimilar values tend to cluster; values near -1/(n-1) (which goes to 0 as n gets large) indicate values tend to be randomly scattered. Since spatial data are easily mapped, it is thus only natural that techniques have been developed for generating and mapping local counterparts to many global measurements.

The definition of Moran's I (Anselin, 1995) for a spatial proximity matrix w_{ij} for a variable y_i at location *i* is defined below as

$$I = \frac{n\sum_{i=1}^{n}\sum_{j=1}^{n} w_{ij}(y_i-\bar{y})(y_j-\bar{y})}{\left(\sum_{i=1}^{n}(y_i-\bar{y})^2\right)\left(\sum_{i\neq j}\sum w_{ij}\right)} = \frac{\sum_{i\neq j} w_{ij}(y_i-\bar{y})(y_j-\bar{y})}{n\sigma^2(y)}$$

where $\sigma^2(y)$ is the sample variance. Usually, the proximity matrix w_{ij} is everywhere 0 except for contiguous locations *i* and *j* where it takes the value 1. Based on the proximity matrix computation for different distances could be take place. This provides a complete correlogram of spatial autocorrelation by distance class and the impact of distance on the strength of spatial autocorrelation for each variable can be examined. Note that values at neighboring lags of a correlogram are highly correlated, since the correlation at larger lags is in part a function of correlations at smaller lags.

CONCLUSION

The main purpose of this work was to present an alternative effective model for measuring the quality of healthcare (SERVQUAL) considering the geographical location of the under examination healthcare sectors. Based on that consideration a new modeling is taking place introducing a spatial interaction between neighboring regions (spatial-SERVQUAL model). New directions are analyzed implementing specific questionnaires taking advantage of the spatial information given by the evaluation of the model. A general framework for modeling customer satisfaction based on that spatial model is illustrated considering healthcare provision and customer experience. Finally, spatial statistical techniques like kernel, kriging, multivariate analysis and cluster analysis are proposed for the evaluation of the geographical results.

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ENDNOTE

[1] The QDF matrix was framed with rows representing customer requirements'-'what'- and the columns representing the 'design characteristics'-'how'. Customers were asked to rate on a five-point scale, the level of importance they assigned to the different customer requirements. They were also asked to give their perception of the relationship between items of each row and the items of each column.

Chapter 13
Bayesian Ontologies in Spatial Integrating Medical Information Systems

Stelios Zimeras
University of the Aegean, Greece

ABSTRACT

Geographical Information Systems (GIS) play a major role in all areas of health research, especially for the understanding of spatial variations concerning disease monitoring. The information produced by the spatial analysis can be modelled and displayed using maps. Spatial analysis (as an alternative statistical technique) may be used in order to suggest health patterns for describing the spreading of various diseases. Areas where GIS can be of benefit include the point mapping of patients and aggregated analyses within different geographical areas. The incorporation of GIS sections in Healthcare Information Systems aims towards the efficient and automated follow-up of prevalence of various diseases in diverse geographic regions. A very important feature of the current system is the integration of queries for the extraction of specific information regarding the above parameters. The queries have been developed through the ontologies of the system. Each ontology refers to each of the correlations that are being explored. The appropriate ontology design techniques have been used to assure the validity of the query output. This work describes the methodological approach for the development of a real time electronic health record, for the statistical analysis of geographic information and graphical representation for disease monitoring. Uncertainty of the ontology system may be achieved by proposing Bayesian type statistical techniques like Bayesian network and Markov logic. Implementation of the proposed techniques will be illustrated considering real accident data.

INTRODUCTION

Geography plays an important role with regard to the understanding of various causes in health dynamics. The trinity of public health that is constituted by the individual, means and the environment underlines the importance of geography (environment where we live) as far as health and illnesses are concerned. The main application in the territorial database systems is GIS. Geographic Information systems (GIS) are constituted by hardware, software data and people, aiming to

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collect, store, analyze and handle information that refer to particular geographic regions (Harris, 1989); Harris and Batty, 1993).

GIS technology and its applications is a powerful tool for community health for many reasons. Among these, the possibility of incorporating data from different sources, in order to gain new information, is included. This is made possible by making use of the possibilities given by presenting these elements (mapping), which can lead to the efficient analysis of health problems and support decision-making, for the improvement of healthcare provision. GIS provides the possibility of finding regions with an increased frequency of diseases and inverse health conditions and it examines the territorial relation between the prevalence of diseases and information that is shown through the territorial GIS systems. Geographic Information Systems (GIS) can be used for territorial analysis, quantified representation and the analysis of territorial elements (Klosterman, 1997). By selecting the appropriate populations needed for the analysis, it uses the attributes of these individuals in order to acquire the population size, the composition and the characteristics as well as their geographic spread patterns (Klosterman, 1993). The data allow the classifications in table according to the data attributes and the demographic analysis that uses statistical techniques (Chou, 1997; DeMers, 2000). There have been developed many clinical GIS systems worldwide. (Odero et. al., 2007) describes an electronic system of monitoring accidents. Also, the study of (Williams et. al., 2003) should be mentioned, in which Geographic Information Systems were used in order to analyze and describe the geographic distribution of incidents of burns in children aged between 0-14 years. The follow-up of accident rates, as well as of mapping and territorial statistical analysis allows primarily for the monitoring of accidents and for the identification of small geographic regions with high frequency of accidents. Finally, Brownstein et al (2005) examined issues of personal data protection and secrecy of information for GIS systems (Wu et. al., 2006).

In our country, the adoption of Electronic Health Record is yet to be incorporated in the healthcare system, and the same is valid for monitoring and data analysis tools. The development and utilization of effective and real time efficient electronic health records combined with spatial databases can be used for the improvement of the health status and the follow-up of related health parameters. The incorporation of GIS sections in Healthcare Information Systems aims towards the efficient and automated follow-up of prevalence of various diseases in diverse geographic regions (Zimeras et. al., 2009a; 2009b).

A very important feature of the current system is the integration of queries for the extraction of specific information regarding the above parameters. The "correlation" queries are the basis for the creation of a spatial statistical analysis of selected parameters so that the user of the system can draw conclusions regarding the correlation between the selected factors that want to be analysed. The queries have been developed through the ontologies of the system. Each ontology refers to each of the correlations that are being explored. The appropriate ontology design techniques have been used to assure the validity of the query output. Ontology is an explicit, formal representation of the entities and relationships that can exist in a domain of application. Ontologies are formal representations of knowledge about a given domain, typically expressed in a manner that can be processed by machines. Specifically, an ontology explicitly represents the types of entities that can exist in the domain, the properties these entities can have, the relationships they can have to one another, the roles they can play with respect to one another, how they are decomposed into parts, and the events and processes in which entities can participate.

A major shortcoming of ontologies is their uncertainty to represent incomplete data. Under this condition, implementation of probability ontologies is in big demand. Some tasks like learning and mapping ontologies already automatically produce uncertain ontologies. In most of the cases,

this uncertainty is represented as a probability. Uncertainty becomes more prevalent in concept mapping between two ontologies where it is often the case that a concept defined in one ontology can only find partial matches to one or more concepts in another ontology. Probabilistic ontologies expand standard ontologies by providing logically constructs for representing statistical regularities and probabilistic interrelationships in a domain of application based on Bayesian networks.

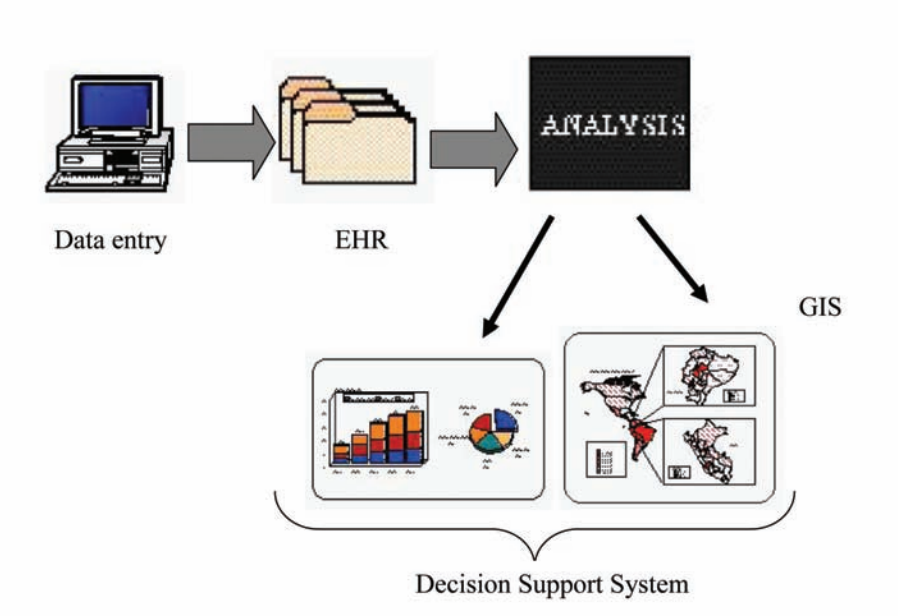
In this approach, the source and target ontologies are first translated into Bayesian networks (BN); the concept mapping between the two ontologies are treated as evidential reasoning between the two translated BNs. Probabilities needed for constructing conditional probability tables (CPT) during translation.

Another solution could be the Markov logic where: (1) If the ontologies are combined with some kind of uncertainty information, like probabilities, Markov Logic can be used to reasoning about this information; (2) If the ontology contains individuals, those individuals can be used to automatically learn the uncertainty of the ontology;

Markov Logic combines first-order logic and probabilistic graphical models (Markov networks) in the same representation. The main idea behind Markov Logic is that, unlike first-order logic, a world that violates a formula is not invalid, but only less probable. This is done by attaching weights to first-order logic formulas: the higher the weight, the bigger is the difference between a world that satisfies the formula and one that does not, other things been equal. These sets of weighted formulas are called Markov Logic Networks (MLNs).

This work describes the methodological approach for the development of a real time electronic health record, for the statistical analysis of geographic information solving the uncertainty of the ontology system by proposing Bayesian type statistical techniques like Bayesian network. Implementation of the proposed techniques would be illustrated considering real accident data.

Figure 1. Typical example of the development of a GIS EHR system



GIS PROTOTYPE SYSTEM

System Analysis

The development of an electronic health record with data on injured patients is being described. This is an example of following-up a series of health parameters for the production of statistical information by performing direct data analysis. Territorial (GIS) databases are proposed to be used in order to support the system described herein. The main characteristic of this category of databases is the management of large collections of relatively simple geometric objects. In specific, the methodology is based on the development of a pseudo-GIS Electronic Patient Record (Zimeras et. al., 2009a). The proposed model for the identification of the source of disease spread is indicated in Figure 1.

The functions provided are: the Electronic Health Record, the graphical presentation of the collected data through a series of forms which provide access to the diagrams that were described above and the dynamic graphical presentation of certain health and demographic parameters in a map of a Greek island. This map presents the results of the above mentioned parameters in relation to the various regions of the island (Figure 2).

A health information system for the epidemiological study takes into account specific parameters that are related with accident types. All fields that have been included into the system, either health or patient related, have been standardized and given a specific range of values to select from. The health and other related fields of the database have been structured in a way that ensures that every time a new entry is added, the system labels this entry with an appropriate numeric code that will be used for the statistical functions of the system.

Every database table that is being used as a reference table to give values to other tables contains a non auto-number primary key (identifier) that has been set by the database designer. The key values of these fields are identical to the desired coding of the values that will be used for the statistical analysis. Database normalisation is based keeping always in mind the main objective of data utilization.

Database Analysis

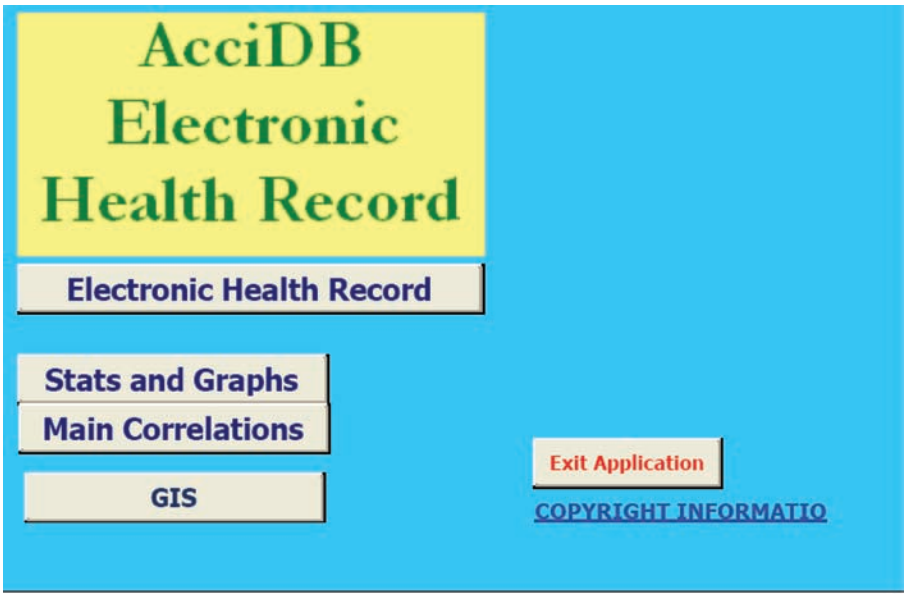
A database is an integrated collection of logically related records or files consolidated into a common pool that provides data for one or more multiple uses. A database system can be thought of as a computerized record-keeping system. Such a system involves the data itself (stored in the database), hardware, software, and-most important-users.

A Database Management System (DBMS) is a set of computer programs that controls the creation, maintenance, and the use of the database in a computer platform and its end users. The DBMS accepts requests for data from an application program and instructs the operating system to transfer the appropriate data. The queries and responses must be submitted and received according to a format that conforms to one or more applicable protocols. The DBMS has the ability to transform the data from the internal schema to external views, based on the queries

Relational databases became popular because they stripped away the machine-specific storage mechanics of the older models so developers no longer needed to worry about how the data was stored and how to retrieve it; they could focus on the data itself and concentrate on building functionality-rich applications.

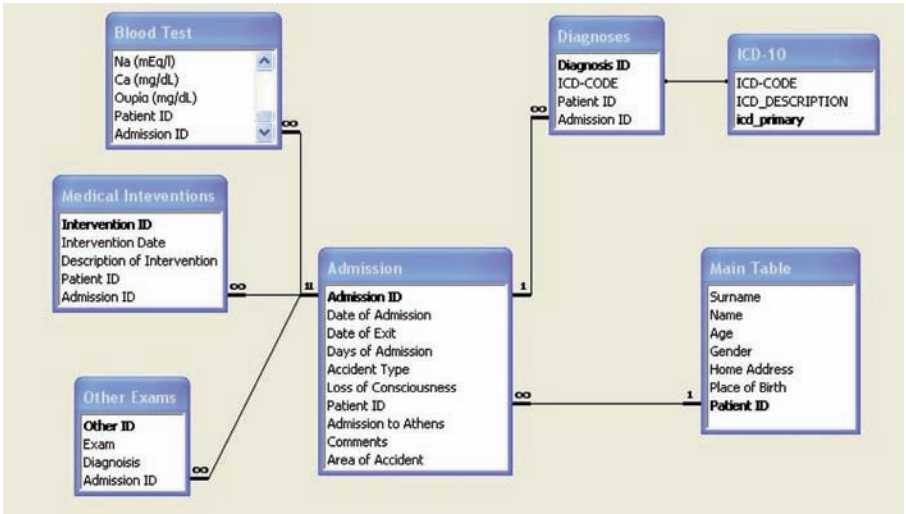
Our interest is directed to the Relational Model which is based on a formal theory called the relational model, according to which data is represented as rows in tables (interpreted as true propositions), and operators are provided that directly support the process of inferring additional true propositions from the given ones (Figure 3). Relational DataBase Model is a database system

Figure 2. Main form of the pseudo-GIS Electronic Health Record (Zimeras et. al. 2009a, 2009b)



made up of files with data elements in 2d array (rows and columns). This DataBase system has the capability to recombine data elements from different relations resulting in a great flexibility of data usage. The relational model contains the following components: collection of objects or relations set of operations to act on the relations and data integrity for accuracy and consistency. Relations play a major impact in relational database. A formal definition of relation is in (Codd, 1970): Given a collection of sets D_1, D_2, …, D_n, *R* is a relation on these sets if it is a set of ordered n-objects $\{d_1, d_2, \ldots, d_n\}$ with $d_i \in D_i$. The sets D_i $(1<i<n)$ are called domains of *R* and n is the degree of *R*. The element d_i is the attributes of the relation. The database application that has been

Figure 3. Relational model DB (Zimeras et. al. 2009a, 2009b)



used for the development of the EHR build based on Microsoft Access 2007, which is considered as an all in one solution for Database and User Interface environment development.

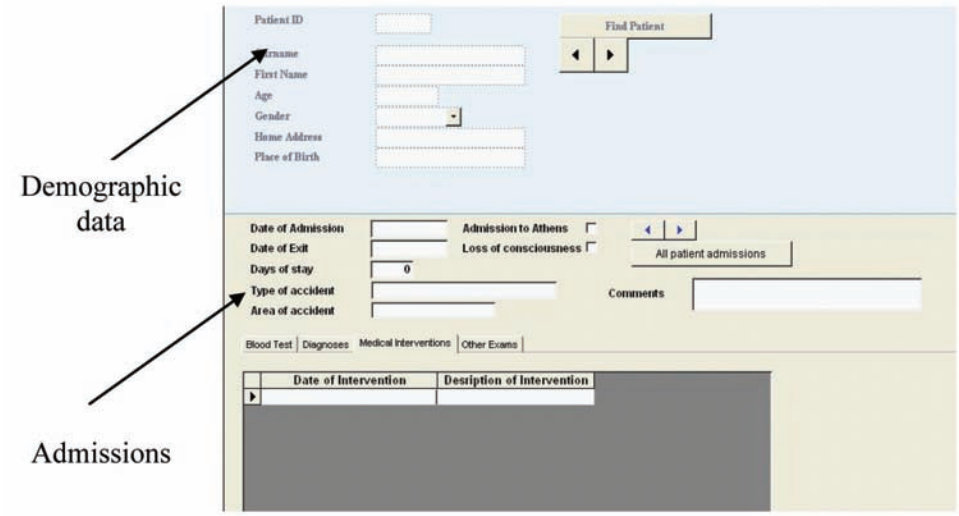
Electronic Health Record Analysis

Electronic Health Record (EHR) systems need to offer a flexible framework for recording the consultation process, and accommodate the individuality of the clinician as well as the patient (Lodwick, 1988). To share and use data from multiple institutions, data must be built upon common words (data elements and terminology), structures, and organizations (interoperability).

The system includes electronically maintained information about an individual's lifetime health condition and health care. Development of fully functional interoperable EHR system remains a major challenge. Recent research has proposed prototype service-oriented architecture (SOA) models for EHR in various contexts including clinical decision support, collaborative medical (mammogram) image analysis, and health clinic setting. Included in this information are patient demographics, progress notes, problems, medications, vital signs, past medical history, immunizations, laboratory data and radiology reports. At any point in time a patient's health record provides the information basis against which new findings are interpreted (Lodwick, 1988). The system has the ability to generate a complete record of a clinical patient encounter, as well as supporting other care-related activities directly or indirectly via interface - including evidence-based decision support, quality management, and outcomes reporting".

The sections of information have been spilt into 3 Categories: the Demographics information of the patients, which are *Name, Surname, Age, Gender, Home Address,* and *Place of Birth*. The second section involves the health record, namely *Medical Interventions, Blood Test, Diagnosis* and *Other Examinations*. The sub sections include a list of Diagnostics which receive values from an additional table with the International Classification of Diseases ver.10 (ICD10), the Blood Test with the fields *Date of Exam* as well as an

Figure 4. The electronic health record form (Zimeras et al. 2009a, 2009b)



additional 17 fields of relevant blood test results (i.e. *Ht, Blood Sugar, K, Na,* etc) and the Medical Interventions with the fields: *Date of Intervention, Description of Intervention*. Finally, the third section includes the Admissions which are the *Date of Admission, Date of Exit, Days of Stay, Accident Type, Place of Accident, Loss of Consciousness (Yes-No), Comments* (Figure 4).

The database collects health information as well as information of other characteristics that are not explicitly connected or might have correlation with health data fields. The collected data will be utilized for this purpose. Implementation of the system was applied based on 90 patients where for the most of them have been declared the types of accidents and for 34 cases the geographical position of the accident type. The fields of interest that have been selected for further exploration consist of the following pairs: Age-Days of Admission; Diagnosis-Type of Accident; Blood test results-Age. The exploration of a possible correlation between the above parameters may give an answer to the following key questions:

- Does the age of an injured patient affect the days he/she stays in the hospital? Do specific types of accidents cause specific health problems?
- Does the patient age affect specific blood test values?
- Is there any correlation between specific types of accidents and admission days?
- Is the hospital unable to cure patients injured by specific types of accidents leading to the need for the admission to a larger hospital in city of Athens?

BAYESIAN NETWORKS: ONTOLOGIES

There are many ways of representing and dealing with uncertainty. In this paper, we restrict our attention to approaches that use probabilistic methods for representing uncertain information. We focus on these approaches, because we believe that probabilistic methods are a natural choice for representing the kinds of uncertainty we often find out.

Bayesian Networks (BNs) is one of the best understood models for representing the joint probability distribution of a domain of interest is the model of Bayesian Networks (BNs) (Jensen, 2001). Mittal and Kassim (2007) and Pourret et al. (2008) provide an extensive list of areas where BNs have been applied. A BN is a graphical representation of probability distributions. It consists of two components. The first is a directed acyclic graph in which each node represents a random variable, while the set of arcs connecting pairs of nodes represents certain conditional independence properties. This component captures the *structure* of the probability distribution. The second component is a collection of *parameters* that describe the conditional probability of each variable given its parent in the graph. Together, these two components represent a probability distribution (Pearl, 1987). Each random variable has an associated conditional probability distribution, normally represented as a table (conditional probability table, CPT). These CPTs capture the conditional probability of the random variable given its parents in the graph. The task of building a BN can be decomposed into several subtasks: (1) identify the variables of interest; (2) specify the values these variables can take; (3) define the relations between the variables; (4) assign a conditional probability distribution.

In general, the joint probability distribution of variables (X_1, X_2, ……., X_n) in a BN is computed using these local distributions. In general,

$$P(X_1, X_2, ..., X_n) = \prod_{i=1}^{n} P(X_i \mid Parents(X_i))$$

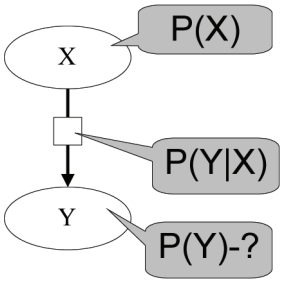
where parents(X_i) represents the set of parents of variable X_i (Figure 5) with

$$P(Y = y_j) = \sum_i P(X = x_i) \cdot P(Y = y_j \mid X = x_i)$$

Ontologies have become ubiquitous in current generation information systems. An ontology is an explicit, formal representation of the entities and relationships that can exist in a domain of application. Bayesian ontologies are used to describe knowledge about a domain with its associated uncertainty

A probabilistic ontology (Costa 2005) is an explicit, formal knowledge representation that expresses knowledge about a domain of application. A more formal, algebraic, approach, identifies an ontology as a pair (S, A_i), where S is the vocabulary (or signature) of the ontology (being modelled by some mathematical structure, such as a lattice or an unstructured set) and A is the set of ontological axioms, which specify the intended interpretation of the vocabulary in a given domain

Figure 5. Example of a simple Bayesian network (Terziyan, 2003)



of discourse (Kalfoglou and Schorlemmer 2003). Considering the relational model of the DB (Figure 3), the BN model could be introduced as (Figure 5) where A: Admission; B: Blood Test; C: Medical Interventions; D: Other Exams; F:Main Table; G1: Diagnosis and G2: ICD-10.

Calculation of the appropriate probabilities could be calculated based on the particular query combination combined with the Bayesian network. In case we investigate the query Age vs Days of Admission then based on the Figure 6 nodes A vs F must be considered. So the P(A|F) could be calculated by: P(A|F) = {p_1(A|F), p_2(A|F), …, p_r(A|F)} where is the conditional dependence random variable between A and F with r possible values.

Define as A ={a_1, a_2, ..., a_n} the predictive attribute with *n* values and B ={b_1, b_2, ..., b_q} the contextual attribute with *q* values then in case we introduce another parameter (for example C: Medical Interventions), then is we goal is to estimate the probability P(A = Days of Admission) then we calculate the probability for the conditional dependence:

Then we estimate the following joint probability:

$$P(A = a_j, B = b_i,$$
$$P(A \mid B) = p_k(A \mid B)) = p_k(A = a_j \mid B = b_i) \cdot P(P(A \mid B) =$$
$$p_k(A \mid B)) \cdot P(B = b_i)$$

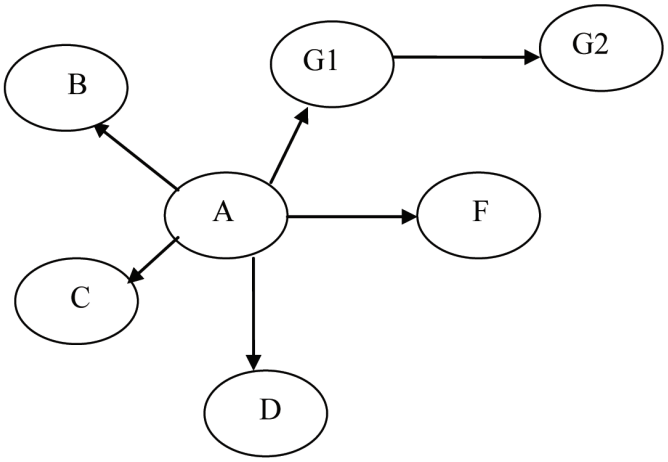
with after substitutions becomes

$$P(A = a_j, B = b_i,$$
$$P(A \mid B) = p_k(A \mid B)) = p_k(A = a_j \mid B = b_i) \cdot$$
$$P(X = b_i) \cdot \sum_{m=1}^{q} \{P(C = c_m) \cdot P[P(A \mid B) = p_k(A \mid B) \mid C = c_m]\}$$

Applying marginalization we obtain:

$$P(A = a_j) = \sum_{k=1}^{r} \sum_{i=1}^{n} \{p_k(A = a_j \mid B = b_i) \cdot P(B = b_i) \times$$
$$\sum_{m=1}^{q} [P(C = c_m) \cdot P(P(A \mid B) = p_k(A \mid B) \mid C = c_m)]\}$$

Figure 6. Bayesian network for DB



or in more compact form the general calculation scheme will be as follows:

$$P(A) = \sum_{\forall P(A|B)} \sum_{\forall B} \{P(B) \cdot P(A \mid B) \cdot \sum_{\forall C} [P(C) \cdot P(P(A \mid B) \mid C)]\}$$

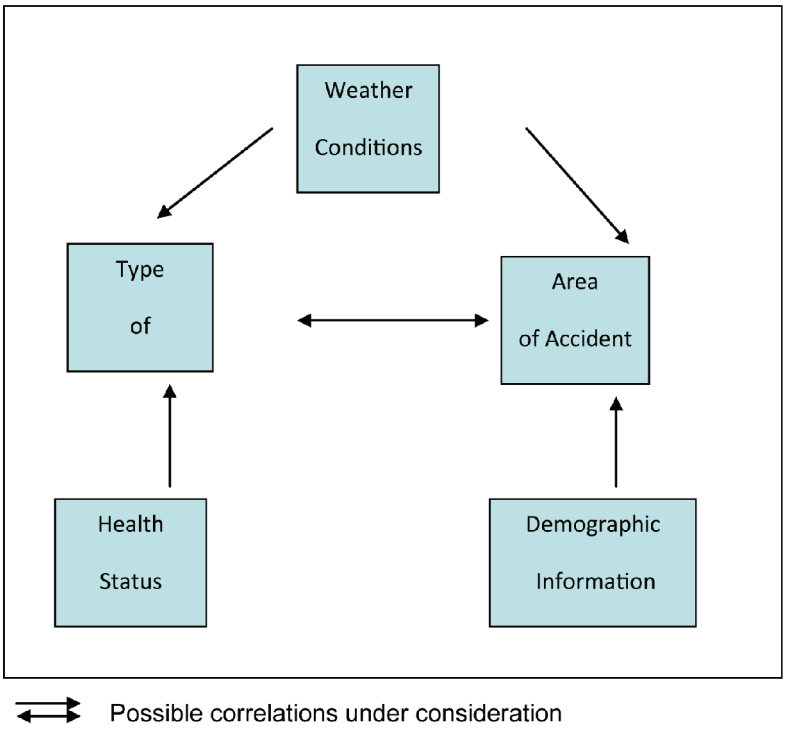
GIS System

Essential information that should be collected by such a system is the geographic area of the accident, season and hour of the accident, weather conditions at the time of the accident, demographic information, and other health problems that may be related to the accident.

All these require need to be taken into account by the GIS application in order to make the information easy understood, interpreted and promptly utilised. Until recently, doctors and public health professionals measured health strictly in terms of indicators of ill-health such as morbidity and mortality. Using these systems, the main task is to examine the distribution of disease and death at various geographic scales, in an attempt to determine whether the presence or absence of particular illness is associated with some factor(s) in the social or physical environment. In the case of infectious diseases, there is an additional dimension of examining the spread of disease through space over a given period of time. Although disease mapping can be relatively straightforward, the interpretation of spatially referenced disease data can sometimes be challenging. Regardless of the difficulties in data acquisition, map representation, scale, statistical analysis, and the interpretation and utilization of results, the study of disease distribution may well be the most challenging and fascinating research area.

In general, the objectives of GIS are the management (acquisition, storage and maintenance), analysis (statistical and spatial modelling), and display (graphics and mapping) of geographic data. GIS is a valuable tool to assist in health research, in health education and in the planning, monitoring and evaluation of health programmes and health systems. An alternative is the use of dynamic graphics such the association of a map with a histogram of case occurrences. Selecting the upper tail of the histogram automatically highlights the corresponding cases on the map thus allowing the identification of regions by high incidence of a specific disease.

Figure 7. Schema of possible correlations under consideration



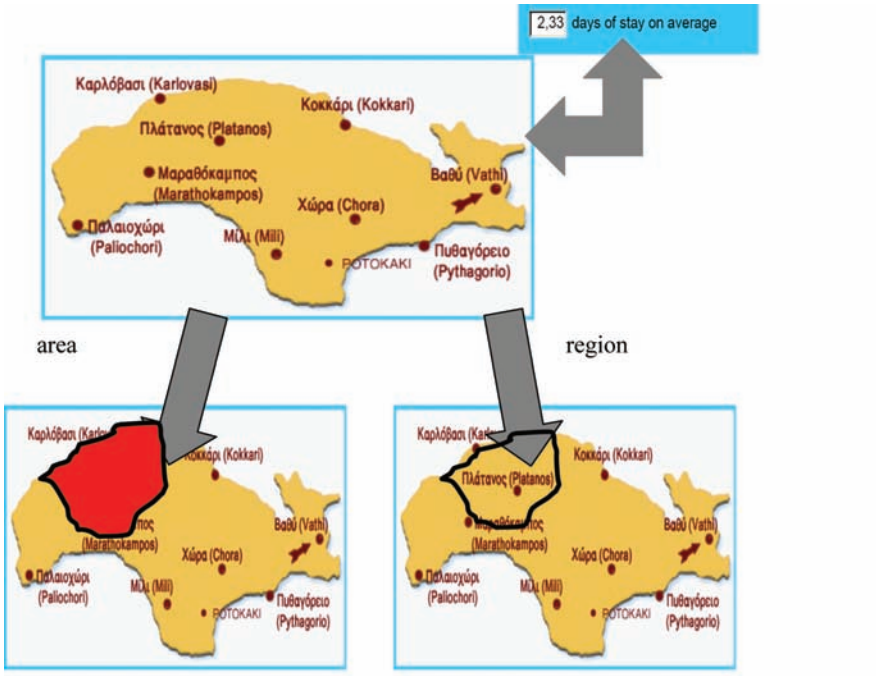
The geographic distribution of accidents can contribute to the identification of regions in which there is a high risk for such events, in order to support the construction or reinforcement of infrastructures (ie road construction, labelling etc) of public and local services. The aim is to prevent accidents by identifying geographic regions with high percentages of accidents.

The design of such an Information System takes into consideration the territorial information which is collected in the spatial database in order to investigate parameters which are potentially related with the accidents. Descriptive statistical analysis can be performed by such system with the support of a robust database structure design, in order to obtain an initial view of the frequency of occurrence of accidents. The data could potentially be used in cross-correlation techniques. Proposed parameters for further investigation constitute the: (1) relationships of accidents to demographic characteristics. (2) the relationship of the region of accident with the type of the accident. (3) the relationship of the health status to the type of accident (Figure 7).

An important characteristic of the proposed scheme is the utilization of the above mentioned parameters for the cross-correlation and investigation of possible relationships. The proposed structure can be used by researchers in the area of health, in order to test hypotheses and draw initial conclusions based on the analysis, while further analysis of data with the utilisation of specialised statistical software can be realised to obtain more sophisticated results.

The above sections of information are organized into the Information system accordingly. The manipulation of the collected information includes descriptive statistics (mean, mode, standard deviation) and non-parametric statistical test (Pearson chi-squared). These tests have been

Figure 8. GIS system



incorporated into the system through an integral user interface. The correlation of the specified variables is performed in database-level by the transformation of the various correlation test functions into SQL code. SQL stands for Structured Query Language, which is a standard interactive and programming language for instituting interactive communication with databases through a Database Management System (DB MS). Using SQL, one can retrieve data from a database, create a database and database objects, add data, modify data, and perform more complex functions. The SQL system is acceptable by database programmers, thus minimizing compatibility issues and enabling the easy exchange of information among different systems. The correlation of the specified variables is performed in database level by the transformation of the various correlation test function into SQL). The diagrams are being updated automatically after each modification of the patient data. The diagrams appear in Visual Basic forms, which can be accessed through the unified Electronic Health Record environment

The GIS related directly to the database system, such as enter and edit data and update information in the existing database. Data storage implies storage of both maps and data. Data are usually stored in a relational database system contained within the GIS and data by a spreadsheet or query-driven user interface. Geographical Information Systems (GIS) can be used to undertake spatial analysis, quantitative representation and modelling of spatial data; making them fit for population analyses which uses attribute data about humans in order to get the size of population, its composition, characteristics, and how they are and will be spatially distributed. Data are normally collected at the point level (individuals) but it is always available spatial entities (e.g. administrative units) to allow tabulations according to various data characteristics and demographic analysis using statistical techniques. GIS, with its spatially referenced data and spatial analysis tools can provide solutions

to display 2D or 3D spatial data. The interactive maps have been created by using links behind each map area [13]. Each link enables the execution of a specific query that refers to the selected area (region or area) (Figure 8).

CONCLUSION

In this research paper an Electronic Health Record (EHR) is developed with the use of spatial databases and GIS techniques for the registration of epidemiological data. A real time efficient EHR environment is analysed, providing statistical analysis and graphical representation of disease monitoring. Moreover the whole analysis is describing an electronic system of monitoring accidents, as well as the methodological approach for the development of a real time electronic health record.

Ontology explicitly represents the types of knowledge consider the relationships between various queries. Uncertainty is appeared when incomplete data are presented. Probabilistic ontologies expand standard ontologies by providing logically constructs for representing statistical regularities and probabilistic interrelationships in a domain of application based on Bayesian networks

This work describes the methodological approach for the development of a real time electronic health record, for the statistical analysis of geographic information introducing and analysing Bayesian Networks. For that purpose, the conditional probabilities of the DB ontologies are computed based on particular examples. Implementation of the proposed techniques is illustrated considering real accident data

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Chapter 14
Improving Quality of Care through Risk Management Knowledge Transfer and Quality Assurance in Nursing Care

Kleopatra Alamantariotou
University Hospital of Larissa, Greece

ABSTRACT

The purpose of this chapter is to provide innovative knowledge and creative ideas of improving quality of care and to explore how risk management and Knowledge transfer and quality assurance can improve health care. Under careful consideration, our purpose is to summarize which factors improve and promote the quality of care and which factors diminish quality. There are forms of ongoing efforts to make performance better. Quality improvement must be a long-term, continuous effort, reducing errors and providing a safe trust environment for health professionals and patients. After reading this chapter, you should know the answer to these questions: What role can risk management and knowledge transfer play in quality of care? How can risk management and knowledge transfer work together? What are the factors that improve risk management and quality assurance in health care? How does knowledge transfer support, inform, and improve care?

INTRODUCTION

Health care quality is difficult to sum up as there are a combination of many factors like technical professional performance and every individual components of good quality of care (Moss, 1995). In this chapter we will discuss the importance of risk management knowledge transfer and quality assurance in quality care. Starting by definition risk management is about reducing the errors that are costly in terms of damage, discomfort or distress to an individual as result to limit financial loss to an organization. The outcome of effective risk management is, the provision safe patient care during their episode of illness or treatment and the provision of a safe trust environment for patients, staff and the public.

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In order to improve the quality of care we need to use risk management programmes, therefore involve all aspects of work, production in health care that promote the excellence in quality care (Moss, 1995; FSRH, 2010).

Moreover Knowledge transfer is an important element close to risk management. in order to diminish the errors the organization or researchers and individuals in health care make and to increase the need to transfer the right Knowledge with the best practices.

On the other hand Knowledge transfer and Knowledge into action can promote quality of care by using the best tools evidence based and decision making practises (Tetroe, 2008).

Quality assurance provides the mechanisms to effectively monitor patient care provided by health care professionals using cost-effective resources.

Nursing programmes of quality assurance are concerned with the quantitative assessment of nursing care as measured by proven standards of nursing practice. In addition, they motivate practitioners in nursing to strive for excellence in delivering quality care and to be more open and flexible in experimenting with innovative ways to change outmoded systems (Current Nursing, 2010).

The use of risk management, knowledge transfer and quality assurance can clearly promote the quality of care in a positive creative way.

QUALITY OF CARE CONCEPTS

Defining quality is not an easy process. The expense of quality is an interactive process between customer and provider.

1. Quality is defined as the extent of resemblance between the purpose of healthcare and the truly granted care.
2. In an economic dimension quality is the extent of accomplished relief case with a justified use of means and services.
3. Government and those who pay of the care will see quality as a weighing out between results and costs to fulfill certain expectations in health care (Current Nursing, 2010).

Definition of Quality

The British Standards Institute defines Quality as "the totality of features or characteristics of a product or services that bears on its ability to satisfy a given needs." It can be translate into "quality is that which gives complete customer satisfaction.

Dimensions and Elements of Quality

Sridhor approaches these dimensions similarly but describes them as elements of quality. He sets out the following elements (Sridhor, 1998):

- **Appropriateness:** the service or procedure is what the population or individual actually needs.
- **Equity:** Evidence of bias.
- **Accessibility:** services are not compromised by undue limits of time and distance.
- **Effectiveness:** Staffing levels and skills, Equipment and if services are achieving the intended benefits for the individual and for the population.
- **Acceptability:** services are provided such as to satisfy the work expectations of patients, providers and the community.
- **Efficiency:** resources are not wasted on patient or one services to the determent of another (Current Nursing, 2010).

THE ROLE OF RISK MANAGEMENT AND KNOWLEDGE TRANSFER IN HEALTH QUALITY

Risk Management is an approach to improving the quality and safety of health care by identifying circumstances that put patients and staff at risk and acting to prevent or control those risks.

Definitions of Risk Management may vary, but the most commonly accepted comprehensive definition is provided by the joint Australia/New Zealand standard (AS/NZS 4360:19991): "…… a logical and systematic method of establishing the context, identifying, analyzing, evaluating, treating, monitoring and the communication of risks associated with any activity, function or process in a way that will enable organizations to minimize losses and maximize opportunities. Risk Management is as much about identifying opportunities as avoiding or mitigating losses." These processes have now been transfer into the Standards for Better Health. The standards are the main guide for continuous improvement in quality. They provide the framework for the provision and assessment of health care in terms of safety, patient focus, clinical and cost effectiveness, governance,, accessible and responsive care, environment and amenities and public health (FSRH, 2010).

Risk Management Strategy should be read in conjunction with all other Trust key documents, policies and procedures, having relevance to the management of risk, such as:

- Health and Safety Policy
- Fire Policy
- Infection Control Policies
- Incident Reporting Policy
- Complaints Procedure
- Claims and Legal Advice Policy
- Manual Handling Policy
- Information Governance Policy
- Record Keeping Policy
- Lone Working Policy

An effective Risk Management System is the systematic application of management policies, procedures and practices to the tasks of establishing the context of, identifying, analyzing, evaluating, treating, monitoring and communicating risk. A Typical Risk Management Process includes:

- Communication and Consultation
- Monitor and Review
- Establish Context
- Identify Risks
- Assess Risks
- Analyze Risks
- Evaluate and Rank Risks
- Treat Risks
- Register Risks.

Risk management and programmes involves all aspects of work, production with the organization and in health care this includes looking beyond clinical care. A risk management progammes is essential to be free of mistakes. The role of risk management is a responsibility and an everyday process for quality improvement and to succeed quality of care. Some of key roles in clinical function is to assist the lead of care and manage the health service in relation to the day to day co-ordination of delivery of health care.

Is important in order to improve quality to ensure the relevant guidelines and policies are effectively and efficiently implemented. Clinical risk is interdependent on other types of risk, i.e. operational and financial and should not be viewed in isolation. There may be resource implications to minimizing or eliminating risk. The management of clinical risk is the responsibility of all staff and should be part of a quality improvement programme. All staff must accept the management of risk as one of their fundamental duties. Individual roles and responsibilities should be clearly.

Another factor is the maximum use of all resources can maximize the quality of care. The establishment of sound communications and consults with others in a multidisciplinary team can ensure that services works and in the best interests of the client within the context of risk management. The promotion of quality of care can succeed only if the risk manager is aware of obligations in regard to health and safety management.

Also infection control ensure the highest standards in all clinical areas for the promotion of safety health. Through education and development the role of risk management is to assist service and development and continues professional development. Through evaluation of bad practices can promote the importance of best practices and tools in quality health care. In addition through service development risk management can assist to quality of care with research and audits projects within the unit in order to provide optimum care and services. In summary Every Hospital organization trust should have an ongoing risk management process of identifying, assessing and prioritizing risks with the objective of preventing avoidable risks and managing and controlling those risks that remain (FSRH, 2010).

Similarly Knowledge Transfer and Knowledge translation can promote quality in care. Practices underpinned by research based evidence based and theory are essential. Education and continues development in health care producing new evidence and tools in knowledge and better practices (Norris, D. et al, 2003).

Knowledge translation is a dynamic process that include synthetic, dissemination, exchange and ethically sound application of knowledge to improve the health, as result to provide more effective health services and products that strengths the health care system. This process takes place within a complex system of interaction between researchers knowledge users risk managers and policy makers (Tetroe, 2008).

Policy makers have become more and more active in decision making in health care and evidence based practice. It is true in order to put knowledge into action it is necessary to start a process of knowledge transfer. This is concerned with the flow by which tacit knowledge is transmitted among people, that implies a subsequent level of absorption In academic area knowledge transfer goes from universities to a greater community of users(like public). This process aimed. at increasing, cultural educational and social benefits for society. different tools in health can be used to create knowledge integration. Some of the well structure as academic public or industry collaboration other like spontaneous interaction can promote circulation of ideas for further innovation in quality of care (Urso J. et al, 2009).

Staff and organizations can learn and evaluate from risk management the incidence that occurs in hospitals settings and then through a knowledge transfer plan could translate the best practices and tools in order to avoid unhealthy or dangerous practices.

The process by when knowledge is transferred can be divide into six stages: generation, transform, diffusion, reception, adoption and utilization. These stages are not linear from one stage to another, and are necessary to go from an initial idea to its application in real world. Openness' to knowledge is much easier when users need it (Roling Niels G., 1992).

Clinical research and knowledge transfer is consistently producing new findings that may contribute to effective and efficient patient care. The findings of that research will not change the population outcomes unless health services and health care professionals adopt them in practice (Tetroe, 2008). Consistent evidence of failure to translate research findings or hospital errors. As result 30-45% patients do not get treatments of proven effectiveness. And also 20-25% Patients get care that is not needed or is harmful(McGlynn et al, 2003).

Many evidence based innovations fail to produce results because their implementation is untested, unsuitable or incomplete. Knowledge transfer has to be the bridge between risk management and between discovery and impact in evidence based practice.

Nearly knowledge users and researchers work together to shape the research questions. Move the research into practice and identify errors that reduce quality in health care settings (Tetroe, 2008).

Knowledge and understanding of managerial responsibilities and knowledge of current safety agenda guide to quality in health care. The partnership of risk management process and knowledge transfer can promote educational sessions with practitioners, policy makers health professionals for the successful purpose of quality. Another way to increase the quality of care is to engage knowledge users in developing dissemination and implementation plan. Also would be most beneficial of evaluating the implementation process and outcomes and to work under a conceptual framework of quality improvement.

In addition early involvement of decision makers, risk managers in the design and implementation of research as result in defining both scientific and utilizing objectives for research projects (Keown et al, 2008).

Another way to increase quality of care in frequent face to face contacts between know ledge transfer researchers policy makers and risk managers, to enable the ongoing sharing of relevant research results. Knowledge transfer professional and risk managers need to be part of a network to maximize their effectiveness. Knowledge brokers and risk managers needs to be evaluated to determine its effectiveness.

It is worth noting as well that producing evidence based summaries of available literature on relevant topics about incidence and quality of care for distribution to local policy makers who do not have the resources to undertake reviews to support decision making and improve the quality of care.

Research organizations need to offer training sessions and continues professional development for staff health professionals and researchers to enable then to better understand how government works policies how to identify dissemination activities in quality of health care and how risk management and knowledge transfer can change the practice for effective care (Pyra, 2003).

Concepts of Quality Assurance

Quality assurance originated in manufacturing industry. The idea was "to ensure that the product consistently achieved customer satisfaction". In particular Quality assurance is a dynamic process through which nurses assume accountability for quality of care they provide. It is a guarantee to the society that services provided by nurses are being regulated by members of profession. Quality assurance is a judgment concerning the process of care, based on the extent to which that cares contributes to valued outcomes. Quality assurance as the monitoring of the activities of client care to determine the degree of excellence attained to the implementation of the activities. Quality assurance is the defining of nursing practice through well written nursing standards and the use of those standards as a basis for evaluation on improvement of client care (Nursing Management, 2010).

Factors that Influence Risk Management Knowledge Transfer and Quality Assurance in Health Care Quality

There are many factors influence the risk management knowledge transfer and quality assurance in health care. For instance some of them can described below.

- The philosophical values and beliefs of the institution, or organization or individuals.
- The goals and objectives of the health care delivery system
- The ways of problem solving
- The policy development implementation and practices.
- The available resources in terms of money and materials
- The disease pattern, and health facilities available
- The good organization structure and function

- The staff activities
- The culture and background of the people
- Technology developments
- The luck of good quality staff
- Luck of security of staff
- The lack of continuing professional development and strategic Management.
- The luck of knowledge and skills.
- The luck of continuing structure functional performance and implementation.
- The luck of knowledge and learning from failures and moving from low quality to high quality organization.
- Knowledge and demonstrated application of Knowledge Management theories, practices and goals.
- Knowledge and demonstrated applications of planning, modeling concepts, and program and project theories principles and practices.

Also the knowledge of clinical operations environments and implications for application of Knowledge management and transfer implications. Effective communication with strong organizational skills, clients focused orientation and commitment in order to provide quality services. Moreover leadership skills including demonstrated ability and comfort with decision making responsibilities, coaching and teaching, and the ability to inspire and build confidence in others. Also the ability of staff or managers to manage change and create innovative solutions for complex and diverse issues can promote healthy effective care.

There are also some characteristics of total quality management that are close to risk management and improves of care according to Moss and Garside (1995) are below.

- Making customers needs a priority for everyone.
- Examining the process of production rather than individuals performance for explanations of flaws of poor quality.
- Using measurements in order to understand how to improve quality.
- Removing barriers between staff and promoting effective teamwork.
- Promote training for everyone and continues professional development.
- Understanding the quality improvement in risk management.
- Involving the whole workforce in the task of improving quality.

Some key ideas of improvement of quality care are the importance of improvement knowledge and skills are below:

- Managing the human dimensions of change.
- Building and nurturing an improvement culture.
- Working with groups, evaluating improvement and leading improvement.
- Moreover involving patients and care givers is essential. Process mapping, analysis and redesign.
- The measurement of improvement working in systems redesigning roles and use technology to improve service (Institute for innovation and improvement, 2010).

Further evidence for improving quality in health is Change Leadership. In details the ability to initiate, implement and support new or modified approaches, practices and processes in the organization. Recognizes need for change across service areas and set priorities accordingly. Engages key stakeholders in change process, understands the impact on both internal and external stakeholders and manages risks and benefits accordingly. Continually analyses the change process and makes necessary adjustments to maximize effectiveness. Utilization of formal planning and implementation processes based on sound evidence and experience with the principles of change management.

Moreover the subjects of Relationship Management improve quality through interaction and collaboration with internal and external partners and participants, influences the direction of operations and service delivery for the Leading Practices & Innovation Division. Creates and builds commitment to the vision for Leading Practices & Innovation through effective communication strategies. Moreover serves as a role model in the development of an organizational culture and promotion of the Division's vision, ensuring support of the vision, mission and goals of Health Services.

Therefore the main results ensures and the importance of strong working relationships and synergies across operational areas and portfolios to support system-wide learnings and evidence (Institution for innovation and improvement knowledge and skills, 2005).

Recent evidence prove that Operational Leadership, Improves quality of care, and provides leadership through effective communication, ethical decision making. Also and provides leadership through commitment to achieving established goals and objectives, ensuring program delivery measurements and standards are enforced. Identifies challenges and opportunities and provides leadership to support the development of innovative and strategic approaches in ways that enable the achievement of operational goals and strategic vision. Fosters a highly effective workplace with strong commitment to quality and process improvement and patient care focus (Institute of innovation and improvement, 2005).

CONCLUSION

To ensure quality in care there is a combination of many factors. Some of these factors that we indicate is a combination of risk management knowledge transfer and quality assurance programmes. To ensure quality nursing care within the contemporary health care system, mechanisms for monitoring and evaluating care are under scrutiny. As the level of knowledge increases for a profession, the demand for accountability for its services likewise increases. Quality assurance programme will helps to improve the quality of nursing care and professional development. Individuals within the profession must assume responsibility for their professional actions and be answerable to the recipients for their care. As profession become more interdependent, it appears that the power base will become more balanced, allowing individual practitioners to demonstrate their expertise.

Risk Management has to work close to knowledge transfer and quality assurance in order to promote an environment that fosters improvement and a culture of safety for staff and patients. Is essential to commits to continually improving health and safety performance through promotion of culture supporting hazard assessment, risk management, incident identification, reporting and correction and compliance with applicable regulations, policies and safe work practices.

In conclusion Knowledge Transfer similarly needs to implement the best practices according to evidence based, to evaluate with risk management and quality assurance the best methods that promote quality in health and improvement. Knowledge transfer methods and skills that diminish errors in health environment can successful increase the quality of care. Good quality care is more or simply care free of mistakes. No single technology that purpose to improve the quality of care can encompass the many dimensions of quality (Moss, 1995).

While approaches to quality improvement depend on the situation criteria guidelines can be helpful: Quality improvement must not be a fad; it must be a long- term continuous efforts. While top-management commitment is of vital importance, everybody in an organization, from top to bottom, must be committed to quality. Quality control should be done at crucial steps in the operations process.

There are ideas and suggestions for quality improvement can come from many, often unexpected, sources. Most quality problems require the cooperation and coordination of many functional departments, production design testing, engineering, manufacturing, marketing. A quality improvement plan is not enough. Provision must make for its implementation. Quality improvement can collaborate with risk management process knowledge transfer quality assurance and continuously to develop every process in every part of an organization, with intent of meeting and exceeding customer expectations and outcomes. The successful story in that process is the establishment of standards of practice where can provide a guide to knowledge, skills and Judgment attitudes that are needed to practice safely.

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KEY TERMS AND DEFINITIONS

Risk Management: Is about reducing the likelihood errors. Its particularly aims are to reduce errors that are costly in terms of damage, discomfort, disability, or distress to an individual and to limit financial loss to an organization.

Quality Assurance: A means for monitoring and maintaining the goodness of a service, product, or process.

Knowledge Transfer: Primarily about making users aware about knowledge and facilitating their use of it in order to improve health and health care systems.

Event: A hazard which materializes.

Risk: The likelihood of occurrence and severity of consequence of an event occurring. Other words, such as probability, or impact are sometimes used instead.

Risk Assessment: The systematic process for prioritizing risks on the basis of a combination of the severity of consequence and likelihood of occurrence.

Risk Management: The systematic process for identifying, assessing, mitigating and reviewing risk.

Incident: Any event which results, or might have resulted, in injury or abuse to any staff, patients, visitors, external contractors, students, volunteers or other person or loss of or damage to property or equipment.

Chapter 15
Role of Information Technology in Healthcare Quality Assessment

Stamatia Ilioudi
University of Peloponnese, Greece

Athina Lazakidou
University of Peloponnese, Greece

Maria Tsironi
University of Peloponnese, Greece

ABSTRACT

Quantifying and improving the quality of health care is an increasingly important goal in medicine. Because quality of life is difficult to define and even more difficult to measure - particularly with physically and mentally vulnerable people - outcomes from nursing in continuing care are not easily articulated. The focus of the nursing assessment tool is therefore on increasing quality of life, rather than perceiving health gain simply as increased longevity. Assessment is considered to be the first step in the process of individualized nursing care. It provides information that is critical to the development of a plan of action that enhances personal health status. It also decreases the potential for, or the severity of, chronic conditions and helps the individual to gain control over their health through self-care. In this chapter the authors try to describe how important is the role of information and especially of the Information Technology in healthcare quality assessment.

INTRODUCTION

Everyone needs to be well informed and concerned about the quality of care. Everyone means patients and their families, consumer agents and advocates, health professionals, administrators of health plans and facilities, purchasers of health care services, and policymakers at all levels.

Computer-based patient record (CPR) technology is essential for health care. This role begins in the care process as the CPR provides patient information when needed to support clinical decisions and continues as a key information source for

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quality review and improvement. It can be linked to clinical-practice guidelines, clinical alerts, and up-to-date research findings to help patients and clinicians in making choices. The desire to improve the quality and usefulness of health care data is shared by patients, practitioners, administrators, researchers, and policymakers.

Quality assessment and improvement are knowledge-driven enterprises. We know far more today than in the past. Yet we still do not know enough about what works in medicine and health care, for what conditions, under what circumstances, and at what cost to improve the quality of health care to the greatest extent possible. Effectively functioning markets require that patients, employers, and other consumers have good information for decision making, including knowledge about the performance of health plans and the efficacy, effectiveness, and cost-effectiveness of health services, both new and established.

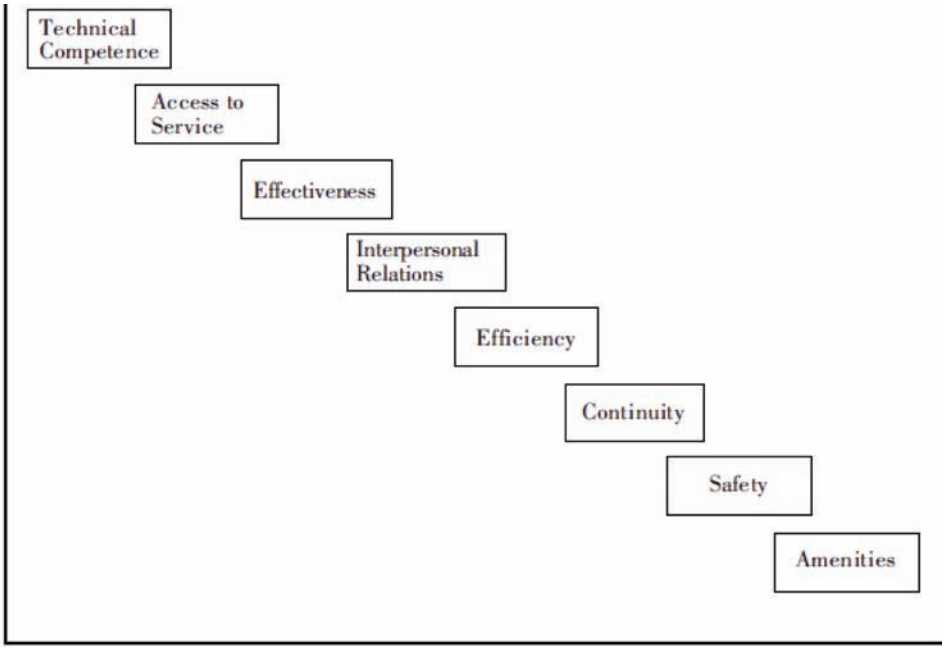
Health-services researchers, government agencies, health plans, purchaser coalitions, and others have done much to improve ways of measuring health outcomes, comparing the outcomes of different health care practices, evaluating the performance of health care providers and practitioners, and developing credible and useful guidance for patients and clinicians in making medical decisions.

Dimensions of Quality

Quality is a comprehensive and multifaceted concept. Experts generally recognize several distinct dimensions of quality that vary in importance depending on the context in which a Quality Assessment effort takes place. Quality Assessment activities may address one or more dimensions, such as technical competence, access to services, effectiveness, interpersonal relations, efficiency, continuity, safety, and amenities (see Figure 1).

These dimensions of quality are a useful framework that helps health teams to define and analyze their problems and to measure the extent to which they are meeting program standards. The

Figure 1. Dimensions of Quality (Quality Assurance Project, n.d.)



eight dimensions discussed in detail in this section have been developed from the technical literature on quality, and synthesize ideas from various Quality Assessment experts. These dimensions of quality are as appropriate for clinical care as for management services that support service delivery.

Challenges to Data Quality

Identifying appropriate patients in whom to apply performance measures is complicated by limitations in current information technologies. Patients with conditions for which hospitalization is usually required can be found in hospital administrative records. However, administrative sources of data lack important clinical elements and can be inaccurate with respect to the principal diagnosis for which a patient was treated. In a patient for whom quality of care will be judged, the latter problem may require confirmation of the diagnosis through additional parameters. The limitations of administrative records exist because the original collection of data was for a purpose other than assessment of healthcare quality.

Retrospective chart abstraction can often further clarify important patient characteristics, but the recording of such data by healthcare providers may be incomplete. Even when the data are available, inaccuracies can occur in documentation or abstraction. Prospective data collection has the potential to provide the most useful information when the data are specifically defined and collected for quality-assessment purposes.

Prospective data collection also permits acquisition of data directly from patients or physicians and allows assessment of variables such as health status. Unfortunately, in the absence of electronic medical records, prospective data collection is expensive and requires substantial organization to be incorporated into routine patient care.

Collection of outcome data adds another level of complexity and expense. Although deaths can be tracked through administrative sources such as the National Death Index (there is a substantial time lag), most other outcomes require the tracking of individual patients over time. Some patients will be lost to follow-up, and their characteristics and outcomes may differ substantially from those for whom data are available. Many desired outcomes, such as health status and readmission, require collection of data directly from patients, and inaccurate telephone numbers, addresses, and lack of patient cooperation with follow-up efforts may limit efforts to collect this information (AHA, 2000).

Perspectives on the Meaning of Quality

The definitions and dimensions outlined above constitute a broad conceptual framework that includes almost every aspect of the health system performance. All these dimensions come into play as clients, health providers, and health care managers try to define quality of care from their unique perspectives (Quality Assurance Project, n.d.). What does quality of health care mean for the communities and clients that depend on it, the clinicians who provide it, and the managers and administrators who oversee it?

The Client

For the clients and communities served by health care facilities, quality care meets their perceived needs, and is delivered courteously and on time. In sum, the client wants services that effectively relieve symptoms and prevent illness. The client's perspective is very important because satisfied clients often are more likely to comply with treatment and to continue to use primary health services. Thus, the dimensions of quality that relate to client satisfaction affect the health and well-being of the community. Patients and communities often focus on effectiveness, accessibility, interpersonal relations, continuity, and amenities as the most important dimensions

of quality. However, it is important to note that communities do not always fully understand their health service needs--especially for preventive services--and cannot adequately assess technical competence. Health providers must learn about their community's health status and health service needs, educate the community about basic health services, and involve it in defining how care is to be most effectively delivered. Which decisions should be made by health professionals and which should be made by the community? Where does the technical domain begin and end? This is a subjective and value-laden area that requires an ongoing dialogue between health workers and the community. Answering these questions requires a relationship of trust and two-way communication between the parties.

The Health Service Provider

From the provider's perspective, quality care implies that he or she has the skills, resources, and conditions necessary to improve the health status of the patient and the community, according to current technical standards and available resources. The provider's commitment and motivation depend on the ability to carry out his or her duties in an ideal or optimal way. Providers tend to focus on technical competence, effectiveness, and safety. Key questions for

providers may be: How many patients are providers expected to see per hour? What laboratory services are available to them, and how accurate, efficient, and reliable are they? What referral systems are in place when specialty services or higher technologies are needed? Are the physical working conditions adequate and sanitary, ensuring the privacy of patients and a professional environment? Does the pharmacy have a reliable supply of all the needed medicines? Are there opportunities for continuing medical education? Just as the health care system must respond to the patients. perspectives and demands, it must also respond to the needs and requirements of the health care provider. In this sense, health care providers can be thought of as the health care system's internal clients. They need and expect effective and efficient technical, administrative, and support services in providing high-quality care.

The Health Care Manager

Quality care requires that managers are rarely involved in delivering patient care, although the quality of patient care is central to everything they do. The varied demands of supervision and financial and logistics management present many unexpected challenges and crises. This can leave a manager without a clear sense of priorities or purpose. Focusing on the various dimensions of quality can help to set administrative priorities. Health care managers must provide for the needs and demands of both providers and patients. Also, they must be responsible stewards of the resources entrusted to them by the government, private entities, and the community. Health care managers must consider the needs of multiple clients in addressing questions about resource allocation, fee schedules, staffing patterns, and management practices. The multidimensional concept of quality presented here is particularly helpful to managers who tend to feel that access, effectiveness, technical competence, and efficiency are the most important dimensions of quality (Quality Assurance Project, n.d.).

Conceptual Framework for Evaluating Healthcare Quality

Obviously, obtaining accurate insight into healthcare quality is difficult, yet important. Consequently, there is a great need for a framework of organizing and presenting data—its meaning and limitations—to providers, payers, and the public. Some organizations are working to resolve this problem, but more research is needed to learn how to summarize and display the results and uncertainties of healthcare-quality assessment.

Beginning with the seminal work of Donabedian (1988), healthcare quality has been separated into 3 components:

- Structure
- Process
- Outcomes

Structure refers to the components of the healthcare system: personnel training and skills, adequacy of equipment resources (both diagnostic and therapeutic), and organizational systems to efficiently mobilize these resources for optimal patient care.

Process refers to the use of appropriate diagnostic and therapeutic modalities for individual patients. To facilitate the interpretability of process assessments, "ideal" patient subsets—those without contraindications for therapy—are often used as the denominator, and those who received appropriate treatments are reported as the numerator.

The term "outcomes" refers to the consequences of treatment and can represent markers of disease progression (mortality, readmission, etc), health status (symptoms, functioning, and quality of life), and/or cost.

Healthcare Quality Assessment and Improvement Strategies

It is of paramount importance to recognise that the quality of healthcare cannot be measured in a single dimension but it is a complex of more than one. Thus, quality is a multi-faceted concept which is difficult to define. Apart from that, there is a simple definition which refers that quality means: "Doing the right thing right, right away" (Deming & Edwards, 2000). However, in healthcare, it has been suggested that quality should be understood as a concept with multiple stakeholder interpretations – with all the difficulties this implies (Leatherman & Sutherland, 2004). At a first glance, quality reflects the extent to which a healthcare service or product produces a desired outcome (Runciman et al, 2007).

Recent studies have shown that experts recognize several distinct aspects which represent a basis in order to define and measure quality of healthcare system.

At a more detailed level, researchers from the technical literature on quality provide the following nine dimensions that synthesize and define the quality of healthcare services:

- **Effective:** It characterizes the degree to which the desired results are produced The Institute of Medicine in the United States defines effectiveness as "providing services based on scientific knowledge to all who could benefit, and refraining from providing services to those not likely to benefit" (Institute for Healthcare Improvement, 2003).
- **Appropriate:** Appropriateness refers that care giving is patient-centred. This entails that healthcare provided with respect and compassion to patients, which means "care, intervention or action provided is relevant to the client's needs and based on established standards" (NHPC, 2001).
- **Safe:** Patient safety means do no harm. This ensures safety for all patients, in all processes, all the time in order to avoid adverse incidents. Patient safety is "the avoidance or reduction to acceptable limits of actual or potential harm from health care management or the environment in which health care is delivered " (NHPC, 2001).
- **Responsive:** This aspect serves three goals: physical infrastructure, comforts and choices for patients. Moreover, physical infrastructure typically refers to the technical structures that support a responsive healthcare system. By the term comforts we focus on the physical appearance of the facility, cleanliness, comfort, quality of amenities, and other aspects that are

important to clients, since it is client oriented ability. In addition, a responsiveness healthcare system has to establish patients' participation in choices of provider, insurance plan or treatment.

- **Accessible:** Access to services is the ability of people to obtain healthcare services without geographic, economic, social, cultural barriers anytime.
- **Continuous:** It obtains continuity of services and without delay. This is the ability to deliver uninterrupted care or service by the same healthcare provider over the time when it is appropriate and communication between providers as well.
- **Efficient:** Efficiency measures the degree which achieves desired results with most co-effective use of resources.
- **Interpersonal Relations:** Patients have to trust, respect and communicate with health providers.
- **Technical Performance:** The degree to which the tasks carried out by health workers and facilities meet expectations of technical quality (i.e., adhere to standards)

Undoubtedly, the assessment and improvement of healthcare quality is a major function in any health care system around the world. A quality improvement (QI) strategy refers as an intervention aimed at making great advances in quality healthcare services.

Nine types of Quality Improvement strategies are outlined and considered of paramount importance in order to improve and assess the healthcare quality (Shojania et al, 2004):

- **Provider Reminder Systems:** The investigators defined a reminder system as any patient- or clinical encounter-specific information, which provided verbally, in writing, or by computer, to prompt a clinician to recall information, or intended to prompt consideration of a specific process of care. The reminder also may include information prompting the clinician to follow evidence-based care recommendations.
- **Facilitated Relay of Clinical Data to Providers:** It is used to describe the transfer of clinical information collected directly from patients and relayed to the provider, in instances where the data are not generally collected during a patient visit, or using some format other than the existing local medical record system.
- **Audit and Feedback:** Researchers defined audit and feedback as any summary of clinical performance for health care providers or institutions, which performed for a specific period of time and reported either publicly or confidentially to the clinician or institution.
- **Provider Education:** This term is used to describe a variety of interventions including educational workshops, meetings, lectures (in person or computer-based), educational outreach visits. The same term is also used to describe the distribution of educational materials (electronically published or printed clinical practice guidelines and audio-visual materials).
- **Patient Education:** This strategy is centered on in-person patient education, either individually or as part of a group or community, and through the introduction of print or audio-visual educational materials.
- **Promotion of Self-Management:** This aspect includes the distribution of materials (i.e., devices for blood pressure or glucose self-monitoring) or access to a resource that enhances the patients' ability to manage their condition, the communication of useful clinical data to the patient, or follow-up phone calls from the provider to the patient, with recommended adjustments to care.

- **Patient Reminders:** It is used to define any effort directed by providers toward patients that encourages them to keep appointments or adhere to other aspects of the self-management of their condition.
- **Organizational Change:** This strategy includes a series of sub-strategies which entails organizational change in communications between distant health professionals (i.e., telemedicine), disease management, and treatment team by adding new members or revising the roles of existing team members, and changes in medical records systems as well.
- **Financial, Regulatory or Legislative Incentives:** This strategy includes any intervention having features such as positive or negative financial incentives directed at providers or patients.

The Role of the Internet in Improving Healthcare Quality

The Internet is an electronic highway connecting millions of computers all over the world and millions of individual subscribers coordinated through a complex association of government agencies, and regional and state networks (European Committee for Standardization, n.d.). Through the use of the Internet various parties have access to worldwide information resources. The district goal in adapting these technologies is the improvement in the delivery and quality of care to patients. Much of the current literature indicates that the benefits resulting from Internet use will outweigh the costs.

Nowadays, more and more healthcare organizations are developing their own Extranet and Intranet, and utilizing them along with the Internet, to create seamless access to data across numerous separate and different systems to enhance patient care (Kerwin, 2002).

An Intranet is a private computer network which can be created specifically for the patients of a healthcare organization in order the administrators and physicians to access pertinent information in documents, such as patient records and clinical reports, In addition, patients may obtain access to member policies and individually tailored healthcare information via an Intranet (Emerman, 2001). Moreover, an Intranet may be used as well as a patient-education tool, which provides current health news and information to patients.

An extranet is a computer network which allows controlled access from the outside for specific business or educational purposes. Extranets can improve the quality, access, and delivery of care by streamlining and enhancing the flow of information among hospitals, health plans, physicians, insurance companies, and employers (Massachusetts Health Data Consortium, 1999).

It is common knowledge that Internet applications for healthcare organizations are numerous. First of all *e-mail* presents an attractive alternative to more traditional modes of communication for several reasons. E-mail systems are highly compatible across platforms, resulting in seamless communication between operating systems. Its enormous reach and asynchronous nature are especially attractive to healthcare providers whose busy schedules make it difficult to connect over the phone.

Internet technologies have become useful tools for medical practice. Healthcare providers can search the medical literature and find both synoptic and full-text medical journal content (Harrington, 1993). Furthermore, more organizations have developed *web sites* in the past several years to augment the services they provide. A practice web site may assist in the delivery of services because it has the ability to reach increasing numbers of consumers with relative ease and little cost (Thompson & Howard, 2000).

The Internet also allows visual contact. For example, the healthcare providers can easy communicate each other through *videoconference*. Due to the rapidly declining costs of technology, electronic communication tools are booming. Fast internet access, coupled with cheap web cameras

is already creating opportunities for *online videoconferencing* through telephones and computers in health sector (Glowniak, 1995). For example, online consultations through videoconference given to healthcare providers could reduce costs of medical treatment and improve quality of services.

Both videoconference and web sites allow credible healthcare content to be provided online and information to be shared between health providers and patients in a convenient format, which may strengthen their relationships.

Information technology is now improving patient care decisions through the use of *Medical Knowledge Management (KM), Decision Support Systems (DSS), and Computer-Assisted Management Protocols*, thus allowing all healthcare providers to make better informed decisions affecting their patients' care. Medical knowledge management gives providers easy access to relevant, reliable information in an effective and efficient way through various programs, rather than necessitating that practitioners locate the latest information through hard copy journals or other time consuming resources (Badenoch & Tomlin, 2004).

Electronic prescribing systems can provide computer-based support for the creation, transmission, dispensing, and monitoring of pharmacological therapies. A study by the Institute of Medicine in 1999 estimated that up to 98,000 patients die every year as a result of preventable medical errors (Hatfield, 2001). Illegible writing on prescriptions, frequently leads to medical errors. Electronic drug prescriptions via Web-based applications have the potential to greatly reduce this type of errors. Many stakeholders, including practising clinicians, hospitals and other health care providers, pharmacies, health plans, pharmacy benefit management organizations, employers and other health care purchasers, and most importantly, patients benefit from the quality, safety and efficiency improvements that an electronic prescribing system brings.

Communication and Information Technologies in Medical Education

One of the problems with communication and information technology in Medical Education is that medical students have to learn on their own without any interaction with teachers and other students. This entails that the students must learn how to use the web effectively, and manage the plethora of information that it can provide.

Many institutions have developed virtual campuses on the web (panel), which gradually taking over traditional paper-based administrative functions and the dissemination of teaching and learning material, in order to obtain the effective usage of Communication and Information Technologies. As an example, the GKT (Guy's, King's, & St Thomas' School of Medicine) Virtual Campus (http://www.kcl.ac.uk/gktvc) was developed as an all-inclusive joint venture. In this virtual environment the students interact and collaborate by academic and administrative staff, easily whether they are on campus, at home, on placements, or on electives abroad.

Another, virtual campus Rhineland-Palatinate (VCRP) is jointly held by the universities of applied sciences of Rhineland-Palatinate (http://www.vcrp.de). The VCRP (23) does not comprehend itself as an independent university which claims substitution for other universities. On the contrary, it aims to provide support for the implementation of new media into the universities. The VCRP pursues two directions of e-learning: the support of teaching activities of universities as a main task and the extension of teaching activities in the fields of continuing education.

Moreover, at the University of Sydney, Australia (http://www.gmp.usyd.edu.au) in medical school, the problem-based learning programme is supported by mechanisms that guide students to patients' data, including all clinical investigations (text and images), related lectures and practical classes, and laboratory resources (such as im-

ages from anatomy, histology, and pathology). Preliminary assessments of sites such as these show that they are very much appreciated by users and encourage students to adapt resources to their own needs, and tentatively suggest that knowledge is improved with repeated visits (Turchin & Lehmen, 1999); (Yolton & deCalesta, 2000); (Fresnel et al, 1998).

Nowadays, more and more students contribute to online conference and discussion groups facilitated by a tutor, in order to fulfil a predefined task (Pychyl et al, 1999). This approach is flexible, easily and save time. Comparing to traditional tutorials, online contact can assist less forthcoming students by fostering vicarious learning (Lee et al, 1999). This type of learning characterized as student centred.

A board of experts located at the Regional University Computing Centre in Kaiserslautern is responsible for the maintenance of the technical platform of the VCRP. In the course of a public private partnership, Sun Microsystems sponsored a redundant server system to store and distribute the net-based teaching content of all universities of Rhineland-Palatinate. The locations of the universities are interconnected by an ATM-based net (rlp-net). A wireless local area network (W-LAN) is under construction. In 2002, the learning environment 'WebCT' has been established at the VCRP to test this learning platform for providing learning content and for performing administrative tasks with the aim of implementing e-learning in existing syllabi and to create new study programmes (Efferth, 2002).

Among other disciplines, the VCRP has a large number of medical online learning topics. The VCRP medical database comprises more than 600 links to educational web pages out of 28 different medical areas. The students find virtual lectures and seminars, scripts, image collections, tutorials, animations and simulations, movie clips, search engines, virtual libraries, and discussion platforms.

IMPACT OF INFORMATION TECHNOLOGY ON HUMAN RESOURCES IN HEALTHCARE

Incorporation of advances in Information and Communication Technology (ICT) into the workplace has had a major impact in human resource utilization in sectors of the economy where it has occurred in a substantial manner, such as manufacturing and financial services. While many benefits of ICT have been realized in healthcare, the full impact of its benefits will only be realized if it is incorporated in a systematic form, rather than in the current patchy and uneven manner seen around the province and across the country to date.

Furthermore, many governments are dedicating significant financial resources in aligning some of these ventures, such as electronic medical records and digital, filmless imaging systems. However, there is significantly more to be done as healthcare finally embraces information technology on human resources in healthcare but do not discuss the necessary component of planning for infrastructure, which has to go hand in hand with this conversation.

Advances in technology impact on healthcare human resources on three separate thrusts:

1. Changes in efficiencies brought about at local institutions leading to the removal of some positions and creation of others.
2. Changes in types of medical care provided, such as gene therapy and minimally invasive interventions, which will have an impact of shifting of medical healthcare professionals from one subspecialty to others.
3. Decentralization of this healthcare delivery system, which will shift significant resources from tertiary care facilities to primary and community care institutions and home healthcare.

Most healthcare institutions have already realized efficiencies by incorporating aspects of ICT, such as institutional electronic health records and Picture Archiving and Communication Systems (PACS) for digital imaging. However, in many institutions ICT is still in its rudimentary stages. Only partial electronic health records exist, and most institutions still rely heavily on paper reports and charts. During the next decade, we will undoubtedly see the development and deployment of comprehensive electronic health information systems incorporating all aspects of in-hospital and out-of-hospital care.

These systems will allow health providers at all levels to access information quickly and share information, improving both patient care outcomes and operational efficiency. Health information technologies that we can expect to see implemented over the next two decades include the following (Anvari, 2007):

- Electronic lifetime health records will store data from many sources (e.g., text and voice notes, medical images, laboratory values) and be accessible from any locale, providing seamless care for patients and reducing expensive duplication.
- PACS (Picture Archiving and Communication Systems) will capture, store and provide access to diagnostic images (x-ray films, magnetic resonance images, computed tomography scans) from any location.
- RFID (Radio Frequency Identification) Systems will use radio waves to wirelessly track hospital patients, and microchips to carry information on medications, laboratory tests, imaging studies and medical devices.
- Automated systems will track and manage inventories of pharmaceuticals and other medical and general hospital supplies.
- Decision support systems will give healthcare providers real-time advice on diagnosis and treatment options based on continuously updated information.

Making Existing Technology Safer in Healthcare

Technology, equipment and medical devices are vital for effective healthcare throughout the world but are associated with risks. These risks include device failure, inappropriate use, insufficient user-training and inadequate inspection and maintenance. Further risks within the developing world include challenging conditions of temperature and humidity, poor infrastructure, poorly trained service providers, limited resources and supervision, and inappropriately complex equipment being supplied without backup training for its use or maintenance (Newton, 2010).

Evaluating, certifying and monitoring the quality of the provision of healthcare services using agreed standards is an excellent method of improving the safety of healthcare technology, particularly when it prompts change, subsequent reappraisal and a culture of continuous improvement, problem solving and critical self-examination. Quality assurance and improvement are achieved by ensuring standards of governance, using performance measures or indicators to measure an organization's performance and encouraging the use of guidelines. Accreditation skeptics cite an increased workload, particularly for hospital middle management, a lack of consistency and significant cost. With reference to technology, however, accreditation can encourage training and continued professional development, improve audit and catalyze change to equipment and estates (Newton, 2010).

Vital for the provision of safe technology are maintenance programs and consideration of intelligent redesign to reduce the risks that are contrib-

uted by the end user. Furthermore, there needs to be adequate training and education programs for healthcare professionals. Common standards for accreditation and quality assurance schemes will also improve safety. In the developing world, it is essential that all these safety mechanisms and solutions be affordable, appropriate and, above all, able to be realized.

CONCLUSION

Health care is characterized by complex and interrelated processes. Often, these processes do not work well, with unnecessary duplication of tasks, high number of (computer-based) tools to be used in one process leading to media breaks and transcription of data, high efforts for coordination of tasks between professional groups, high efforts to search for required patient information, and limited usability and functionality of used tools. These problems can lead to severe disruption of workflow in a healthcare institution and to low stakeholder satisfaction both with regard to the overall organization and the Information Technology tools used. This can contribute to IT failures and IT boycott. All of this indicates that in the healthcare area the stakeholder view of the processes is particularly important. Measuring quality can also help organizations monitor their progress toward public health goals and become more accountable to both the populations they serve and policy makers.

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About the Contributors

Athina Lazakidou currently works at the University of Peloponnese, Department of Nursing in Greece as Lecturer in Health Informatics. She worked as a Visiting Lecturer at the Department of Computer Science at the University of Cyprus (2000-2002) and at the Department of Nursing at the University of Athens (2002-2007). She did her undergraduate studies at the Athens University of Economics and Business (Greece) and received her BSc in Computer Science in 1996. In 2000, she received her Ph.D. in Medical Informatics from the Department of Medical Informatics, University Hospital Benjamin Franklin at the Free University of Berlin, Germany. She is also an internationally known expert in the field of computer applications in health care and biomedicine, with six books and numerous papers to her credit. She was also Editor of the "Handbook of Research on Informatics in Healthcare and Biomedicine" and "Handbook of Research on Distributed Medical Informatics and E-Health," the best authoritative reference sources for information on the newest trends and breakthroughs in computer applications applied to health care and biomedicine. Her research interests include health informatics, e-Learning in medicine, software engineering, graphical user interfaces, (bio)medical databases, clinical decision support systems, hospital and clinical information systems, electronic medical record systems, telematics, and other web-based applications in health care and biomedicine.

Andriani Daskalaki is a Dentist and Bioinformatician. She presently works in the field of molecular medicine and bioinformatics at the Max Planck Institute for Molecular Genetics in Berlin. She completed her PhD in 2002 on the applications of photodynamic therapy in the area of oral medicine from the Free University of Berlin. She received a two-year DAAD scholarship for her research in the field of PDT. Dr. Daskalaki received a MS in Medical Informatics from TFH Berlin with her work in "Development of a Documentation Software for Robot-Assisted Intraoral Operations" and a MS degree in bioinformatics with her work in "Variance Analysis of Multifactor Models in Gene Expression Experiments with Application to the Identification of Genetic Markers for Hypertension." She received a poster prize for her participation in the International Photodynamic Association Meeting in Nantes. She is the editor of the "Handbook of Research on Systems Biology Applications in Medicine" and has presented many oral presentations at national and international meetings. She is a founding member and committee member of the Greek Dental Laser Association. Her research interest areas include systems biology, PDT, and laser applications in dentistry.

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Maria Adamaki is a Molecular Biologist currently occupied at the oncology lab of Aghia Sofia Children's Hospital. She possesses a BSc (Hons) in Molecular Biology, an MSc in Molecular Medicine, and has scientific research experience in inherited blood disorders and in children's leukemias. Her current work is focused on investigating the expression and analysis of certain genes in childhood leukemias.

Kleopatra Alamantariotou is an external Researcher at the University of Peloponnese Greece and UK in informatics research and has done many collaborations in book chapters' publication. Kleopatra Alamantariotou is currently undergoing PhD studies in innovation science, and she is chairing European cooperation research project (COST IS0907) as a knowledge transfer professional. and other various collaboration in innovation management in EUROPE and worldwide. During 2010-2011, she has been working in quality and improvement department in collaboration with university hospitals in Greece, and European Department of Health. She has worked as a health policy and guidelines producer in American Hospital Dubai UAE the period 2008-2009. Also she worked in United Kingdom, as a midwife and researcher in strategic management in IT IM in Chelsea Westminister Hospital London for 2.5 years (2005-2007). In 2007, she received her Msc in midwifery from the department of Health Science, Middlesex University London, and in 2008 she received her second master Msc. in Health Informatics from City University, London England. Kleopatra Alamantariotou obtained her degree in Midwifery (Bsc) from Technological University of Athens in 2004. She wrote book chapters on quality of health information on internet, in places such as Handbook of Research on Distributed Medical Informatics and E-health. She has memebership in the Higher Education Academy in United Kingdom and global forum of health in World Health Organization and Knowledge transfer. Her research interests include health informatics, e-learning, virtual communities, innovation science and entrepreneurship, innovation in health social innovation technology knowledge transfer, midwifery, and global health.

Alberto F. Alcolea-Ayala received the B.S. degrees in Computer Science from the University of Murcia (UMU), Murcia, Spain, in 2010 and currently doing an Msc Degree in Networks and Telematics. He is with the Department of Information and Communication Engineering, UMU, since 2009, where he works on several projects related with the RFID applications in healthcare. He is also working on a security research project from the European 7th Framework Program on the Ceuti City Council Hall.

Mona Alsaedy received B.S.C (Honours degree) in Pharmacy from Kingston University and St George's University of London (SGUL), London, UK, 2010. Currently undertaking Undergraduate Master in Pharmacy in Kingston University, London, UK, 2010-2011. The Master project involves modification of natural polymer for pharmaceutical nanoparticle drug delivery through the Central Nervous System. Since 2009, she has been cooperating with the Department of Information and Communication Engineering, University of Murcia, Spain, in developing a pharmaceutical information system to detect advanced drug allergies, interactions, and drug related complications.

Kristine Al Zoukra was born on the 8th of July in 1981 in Schwedt/O., Germany. She received her Diploma in 2006 in Biomathematics at the University of Greifswald, where she also was undertaking research from 2006 to 2010 towards a PhD. Her research interest lie in several fields of numerical mathematics including high dimensional optimization problems arising in ordinary differential equations in physiology. She is currently working as scientific research assistant in the angiology group at Free University Berlin.

Maria Athanasopoulou is a Registered Nurse of the University of Athens. She has been practicing nursing since 1995 in public hospitals. She has worked for different units, such as kidney dialysis, emergency room, pathological and surgical department. In 1997 she attained official speciality exercising pathological nursing. During her postgraduate (MSc) and PhD course in community nursing, her focus was on transcultural and family nursing. Her presentations are centered on transcultural nursing with regards to family nursing. In the meantime she leads and trains students of University Athens department of nursing in their clinical exercise in hospitals of Athens. She has attended and participated in various congresses and seminars in Greece and European countries.

Stefan Bernhard was born on the 8th of December, 1975, in Darmstadt, Germany. He received his B.Sc. (Hons) Degree in Applied Physics in 2001 at the University of Central Lancashire in Preston, United Kingdom and his MSc and PhD in Physics in 2002 and 2006, (summa cum laude) respectively, at the Georg-August-University, Göttingen, Germany. Since 2006 he has been employed as a postdoctoral research fellow at the Institute of Mathematics and Computer Sciences at Free University Berlin. His research interests include molecular systems and statistical inverse problems in cardiovascular physiology. He is now head of the angiology group within the innovation laboratory Mathematics for Diagnosis (M4D).

Sharmila Chatterjee, MBBS, is currently studying for her MPH (Community Oriented Primary Care) at George Washington University, Washington DC, USA. Dr. Chatterjee completed her DNB clinical training in Family Medicine from AMRI, Kolkata, India in 2009. She is the author of several scientific articles in major peer-reviewed journals and presented several papers in international conferences. She has been in medical gastroenterology practice for several years prior to this. Her major work and interests include clinical gastroenterology, clinical trial management, epidemiological research in severe sepsis, clinical research, regulatory affairs, and bioethics.

Amit Chattopadhyay, PhD, FFPH-RCPUK, MPH, CPH, MDS, BDS, PGDHHM, PGDMLS, DipGlobalEthics, GCertID, GCertPHO, DipJourn, DcFM, MSASMS, MACE, is an Epidemiologist at the ARCAB, Gaithersburg, MD, USA. He is also employed with NIH-NIDCR. He is a Diplomate of the American National Board of Public Health Examiners and a Diplomate of the American Board of Dental Public Health. He has a broad variety of research interests and scientific publications as author /editor of several books and book chapters and as principal author in peer-reviewed journals of repute encompassing epidemiology; applied biostatistics; pathology; HIV/AIDS; cancer-precancer; clinical trials; and health economics. Alongside those areas, he is currently developing research in *in-silico* simulation of biological processes, disease etiology and outcomes; life-like cellular automaton; application of imputation models in public health and dentistry; ethics and philosophy.

Eleni Christodoulou has worked as a Registered Nurse in a Pathology Department of a general hospital for ten years. She is also a graduate of the Department of Primary Education of the University of Athens. She has a Postgraduate Degree in Health Management, and she is a PhD student in the Department of Nursing at the University of Peloponnese. For the last four years, she has been working as a vocational non-compulsory nursing school teacher and as a nurse educator. Her professional and research interests focus on nursing education and new technologies at the disposal of the nursing and

medical science. She has attended and participated in various congresses and seminars and she has also been a Member of the Scientific and Organizing Committee of several Scientific Nursing Congresses.

Evangelia Loukidou received her Bachelor in Psychology from Aristotle University of Thessaloniki. She has continued her studies at Warwick University-UK from which she has received her Master's Degree in Organizational Studies. In 2008 she was awarded the Doctorate degree in Organizational Behaviour from Loughborough University. She has then followed a career in research by working as a project manager for the Ministries of Health and Education, for various European-funded programs related to the broader sector of mental health. She is currently working as a psychologist at a public school for children with special needs. She has a satisfactory number of publications at world conferences and academic journals.

Antonio F. Gómez-Skarmeta received the M.S. degree in Computer Science from the University of Granada and B.S. (Hons.) and the PhD degrees in Computer Science from the University of Murcia Spain. Since 1993 he is Professor at the same department and University. Antonio F. Gómez-Skarmeta has work on different research projects in the national and international area, like Euro6IX, 6Power, Positif, Seinit, Deserec, Enable, and Daidalos. His main interested is in the integration of security services at different layers like networking, management, and web services. He is Associate Editor of the IEEE SMC-Part B and reviewer of several international journals. He has published over 90 international papers and is a member of several program committees.

Suying Huang received her B.Sc. degree from Jiangxi University of Traditional Chinese Medicine in 1977. She has engaged in clinical, teaching, and research work for 31 years. She is currently a Chief Physician and the head of out-patient department in Shanghai Literature Institute of Traditional Chinese Medicine. Academically, she is a successor of Mr. Xiaosun Cai, a famous gynecologist of Traditional Chinese Medicine, and has been the eighth generation of Cai's Gynecology. Huang has published five books such as "Keys for monitoring the menstrual cycle in gynecology," and more than 30 papers. Clinically, she is an expert at the treatment of different gynecological diseases.

Stamatia Ilioudi graduated from the Department of Informatics, University of Piraeus, Greece. She is currently a Postgraduate student in Information Systems at the Athens University of Economics and Business. Her research interests include electronic communication systems, information systems, e-business, e-health systems, applications in health organizations, and other areas. Currently, she is PhD student at the Department of Nursing at the University of Peloponnese.

Vassiliki Ioannidi works currently as Teaching Staff in "*Special Education: Juvenile delinquency-Pedagogic approach*" at the Department of Nursing at the University of Peloponnese in Greece. She studied at the Faculty of Greek Literature, School of Philosophy at the National and Kapodistrian University of Athens, in Greece. She received her PhD in Philosophy from the Department of Pedagogic, Faculty of Philosophy-Pedagogic-Psychology, School of Philosophy at the National and Kapodistrian University of Athens, Greece. During and after her doctoral studies, she participated in research at the University of Athens, as well as in various scientific collaborations. She taught as a postgraduate scholar of the Greek State Scholarship's Foundation and as a Research Fellow-Lecturer in the Depart-

ment of Pedagogic, Faculty of Philosophy-Pedagogic-Psychology of the University of Athens, graduate and postgraduate. She also taught, as a Research Fellow-Lecturer in the Department of Philology and in the Department of History, Archaeology and Cultural Resources Management of the University of Peloponnese (Kalamata), Hellas. Today, apart from being a member of the Teaching Staff in Special Education at the Department of Nursing (Sparta) of the University of Peloponnese, she is also scientific associate at the interdisciplinary Postgraduate Course "Museum Studies" between the Department of History-Archaeology and the Department of Geology of the University of Athens, in collaboration with the Department of Conservation of Antiquities and Works of Art of the Technological Educational Institution of Athens, Hellas. Her scientific interests focus on the introduction of innovative pedagogical approaches, in strengthening the link between theory and practice in Special Education, in developing specialised pedagogical approaches, methodologies, and didactical interventions, and also didactical exploitation of new technologies in Special Education. She is a member of various scientific and humanitarian organizations.

Antonio J. Jara-Valera received the B.S. (Hons. - valedictorian) degrees in Computer Science from the University of Murcia (UMU), Murcia, Spain, in 2007, and M.S. Computer Science degree from the University of Murcia (UMU), Murcia, Spain, in 2009, where his Master Thesis was about "Internet of things in clinical environments." He earned a second M.S. Computer Science degree about advanced networks and artificial intelligence from the University of Murcia (UMU), Murcia, Spain, in 2010, where his Master Thesis was about "Mobility protocols for 6LoWPAN." He has been with the Department of Information and Communication Engineering, UMU, since 2007, where he is working on several projects related to the ZigBee/6LoWPAN and RFID applications in Intelligent Transport Systems (ITS), home automation, and mainly healthcare. He is especially focused on security and mobility for Future Internet and its applications in healthcare, which is the topic of his PhD. He has published over 20 international papers.

Anastasia Kadda is a Sociologist specialized in the sociology of health, illness, and medicine with a historical perspective. She is also specialized in health units´ management and in educational science. Her publications focus upon the following issues: the role of wider social environment in health-illness, social dimension of health and illness, the role of sociologists in health promotion, stress management, diseases and society in a historical perspective, relation between health-medicine and society, the social role of medical manuscripts, and social utility of personalized e-health services. She has been a teaching staff (P.D. 407/80) in the Department of Nursing of the University of Peloponnesus for two years and teaching staff in the Master's course Intercultural Nursing in the Department of Nursing of the National and Kapodistrian University of Athens as well. She is a scientific collaborator, Lecturer on Sociology of Health in the Department of Nursing B, and in the Department of Health Visiting of the Educational Institute of Athens. She also works in the Department of Health in the Prefecture of Athens.

Eleftheria Koultouki was born in Greece. She studied Medicine in the Aristotle University of Thessaloniki, Greece, and specialized in Pediatrics in Hospitals in the United Kingdom and in Greece; qualified in 2003. Since then she works in the Hematology / Oncology Unit of the 1st Department of Pediatrics of the University of Athens. Currently working on her PhD thesis, she is involved in cell biology studies, flow cytometry, and microarray techniques.

George I. Lambrou is a biologist who was born in Greece. He received his biology training at the Philipps-Universität, Marburg Germany (Diplom). He has specialized in developmental biology and neurochemistry. His main research interests include tumor cell proliferation dynamics, in particular non-linear and chaotic models, as well as pharmacogenomics with emphasis on the glucocorticoid receptor NFκB signal transduction pathway and resistance to glucocorticoid-induced apoptosis. He is also occupied with modeling methodologies and computational biology approaches, along with high-throughput methodologies, such as microarrays.

Guo-Zheng Li is Professor with Department of Control Science & Engineering at Tongji University, China, Committee Member of CAAI Machine Learning Society and CCF Artificial Intelligence & Pattern Recognition Society, Fellow of The International Society of Intelligent Biological Medicine (ISIBM). He obtained his PhD degree from Shanghai Jiao Tong University in 2004. Dr. Li is interested in machine learning and medical informatics. He is Principle Investigator of projects under grants of Natural Science Foundation of China. Li has published 50+ refereed papers in several professional journals and conferences and three chapters. He is Associate Editor and/or Editor on board of 8 international journals, such as IJDMB and IJMLC, Program co-Chair of IJCBS 2009, ITCM 2010. He has obtained Best paper awards at PRICAI 2006, ICAI 2007 and BIBE 2007, and Academic Service Award by ISIBM at IJCBS 2009.

Khushdeep Malhotra, MPH, BDS, is a Resident in the Dental Public Health Residency Program at NIH-NIDCR. Dr. Malhotra was trained as a Dentist in Bangalore, India, and went on to obtain her Master's degree in Public Health (major Health Education) from Western Kentucky University (WKU), KY, USA. She has worked extensively with vulnerable youth in Kentucky and Indiana, in the area of health education. She has presented results of her work at several conferences. Currently, she is investigating socio- behavioral factors and their link with dental care utilization and delivery.

Maria Moschovi is an Assistant Professor of Pediatric Hematology and Oncology at the Medical School of the Kapodestrian University of Athens and the Director of the Hematology / Oncology Unit of Aghia Sofia Children's Hospital. She has extensive knowledge and experience in the field of medical oncology and is currently responsible for the running of various laboratory-based scientific research projects. Her main scientific interests lie in the investigation of metabolic and genetic changes that contribute to the development of childhood cancers, aiming for a more targeted therapeutic outcome in the clinical practice.

Svetoslav Nikolov received a M.Sc. in Mechanical Engineering from the Technical University Sofia (Bulgaria) in 1994, and a PhD in Biomechanics from the Institute of Mechanics and Biomechanics (IMech), Bulgarian Academy of Sciences in 1999. During 1999, he was a researcher at IMech. Since 2003, he has been a tenured Associate Professor position at the Sofia University, Biological Department, and since 2005 the same position at the IMech. His research interests are in application of methods of nonlinear dynamics and systems theory to systems biology.

Robert Penchovsky is an Assistant Professor of Genetics at Sofia University "St. Kliment Ohridski," Bulgaria, where he obtained his Master's degree in biochemistry and molecular genetics, and his Associate's degree in applied computer sciences. Robert is a Reserve Military Officer of the Bulgarian Army.

He earned his Doctoral degree in Genetics from Cologne University, Germany while researching in the fields of microfluidics and nanobiotechnology for Fraunhofer Gesellschaft at Schloss Birlinghoven in Sankt Augustin (near Bonn), Germany. Robert did his postdoctoral study in the fields of computational and RNA synthetic biology at Yale University, New Haven, CT, USA.

Alessandro Prigione is a post-doctoral Scientist at the Max-Planck Institute for Molecular Genetics in Berlin, Germany. He received his M.D. (*summa cum laude)* at the University of Milan, Italy in 2002 and subsequently graduated from the International PhD Program in Molecular Medicine at the Vita-Salute San Raffaele University in Milan, Italy in 2008. Before joining Max-Planck for his current position, Dr. Prigione studied Parkinson's disease at the University of Milan-Bicocca (Italy), basic mitochondrial biology and dysfunctions at the University of California at Davis (USA), and stem cell biology and cellular reprogramming at the San Raffaele Scientific Institute (Italy). His current research focuses on the interrelation of mitochondrial biology and "stemness," as well as mitochondrial modifications occurring in human embryonic stem cells and upon cellular reprogramming.

Christof Schütte was born in Warburg, Germany in 1966 and received the PhD degree in Mathematics in 1994 at Free University Berlin. After research from 1994 to 1998 as Research Assistant at Free University Berlin he has held the position Head of the department "Numerical Analysis and Modeling" at Zuse Institute Berlin from 1998 to 1999. In 1999 he received his habilitation in mathematics at the Free University Berlin, where he since 2000 holds a position as full Professor and Head of BioComputing Group. Since 2008 he is Co-Chair of the DFG Research Center MATHEON. In the same year he won the IBM Research Prize (SUR-Grant).

Antonios K. Travlos is an Assistant Professor at the Department of Sport Management at the University of Peloponnese in Sparta Lakonias, Greece. He teaches courses in sport psychology, motor behavior, statistics, organizational behavior, and organizational psychology. He received his Master of Arts from McGill University in 1988 and his doctorate from the University of Alberta in 1993. His areas of research interests include the role of physical activation in mental functioning; scheduling of practice; aggression and violence in sports; bullying in school and sport environment; ethics at work, job satisfaction, and job analysis. He has published more than 15 refereed journal articles, 3 books, and has made over 150 national and international conference presentations. Dr. Travlos has work for many years counseling coaches, referees, and athletes from youth sports through professional levels.

Maria Tsironi was born in Athens in 1968. She has studied medicine at the Medical School of the University of Athens and graduated in 1992. She was then specialized in internal medicine at the first Department of Internal Medicine, Laikon Hospital, University of Athens. She had obtained her PhD degree in 2002, working on the myocardial perfusion of sickle thalassemic patients. Since then, she was working as a Consultant of internal medicine, especially with hemoglobinopathy patients, till 2008. She was elected as an Assistant Professor of internal medicine at the Department of Nursing, University of Peloponnese. Her current scientific interests are focused on myocardial dysfunction in hemoglobinopathies, endothelium disorders in hemoglobinopathy patients, and the effects of exercise on the cardiovascular system, focusing on healthy ultramarathon runners.

Sofia Vasileiadou is a Nurse (RN) working for the General University Hospital "Attikon" in Athens, Greece. She has a MA in "Theory, Practice and Evaluation" from the department of Philosophy, Pedagogy and Psychology, University of Athens. She is involved in nurse education and she is mostly interested in special needs and learning disabilities nursing. She has participated in Greek and international conferences.

Julio Vera holds a degree in Applied Physics from the University of La Laguna (1999, Spain). His Ph. was in Biochemistry and Molecular Biology (2005, University of La Laguna). He is currently leading the FORSYS Junior Research Group on Systems Biology for Cancer and Aging (CALSYS, www.sbi.uni-rostock.de/calsys), associated to the group of Systems Biology & Bioinformatics at the University of Rostock, Germany. His expertise is in data-based mathematical modelling of metabolic and cell signalling pathways, predictive simulations, and development of mathematical methods and software tools for the analysis of biological systems.

Olaf Wolkenhauer earned his first degree in control engineering, followed by a PhD at the UMIST in Manchester UK, a joint position with the Department of Biomolecular Sciences. Since 2005 he occupies the Chair in Systems Biology & Bioinformatics at the University of Rostock, Germany (www.sbi.uni-rostock.de). His research interests are in systems biology and bioinformatics, focusing on dynamical inter- and intra-cellular processes.

Liaoyu Xu received his Master's degree from Shanghai University of Chinese Medicine in 2003. Now he is Chief Information Officer of Shanghai Literature Institute of Traditional Chinese Medicine. He is interested in information technology for Chinese Medicine, including database and data mining.

Mingyu You received her PhD degree from Zhejiang University in 2007. She is currently Assistant Professor in the Department of Control Science & Engineering, Tongji University, China. Her research interest is bioinformatics, artificial intelligence in medicine, intelligent human-machine interaction, and pattern recognition.

Miguel A. Zamora-Izquierdo received the M.S. degree in automation and electronics and the PhD degree in computer science from the University of Murcia, Spain, in 1997 and 2003, respectively. Since 1999, he has been an Associate Professor with the Department of Information and Communication Engineering, UMU, where he works on several projects related to the remote monitoring and control, and is especially focused on sensors system and embedded system.

Stelios Zimeras was born in Piraeus, Greece. He received his B.Sc. in Statistics from University of Piraeus, Depart. of Statistics and Insurance Sciences, and his her Doctor of Philosophy Degree in Statistics from University of Leeds, U.K. Research productivity is summarized in various articles (monographs or in collaboration with other researchers) in International Journals, International Conference Proceedings, International Book Series and International Book Chapters. Since 2008 he is Assistant Professor at the University of the Aegean, Depart. of Statistics and Actuarial – Financial Mathematics. He has participated in many Research Projects in Greece and in European Union. He is the writer of many papers in the areas of Informatics and Statistics.

Sofia Zyga has worked as a Renal Nurse since 1998. She has a Postgraduate Degree in Organization and of Health Services Administration and also a PhD in Clinical Nephrology. She is an Assistant Professor in Fundamentals of Nursing at the University of Peloponnese and a member of the Committee dealing with Health, Education, and Culture in the local prefecture government. Her professional and research interests focus on renal nursing and nursing education. She is the author and (co)editor of over 30 publications in International and Greek Scientific Journals. She is also Member of Scientific and Organizing Committee in Scientific Nursing Congresses.

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