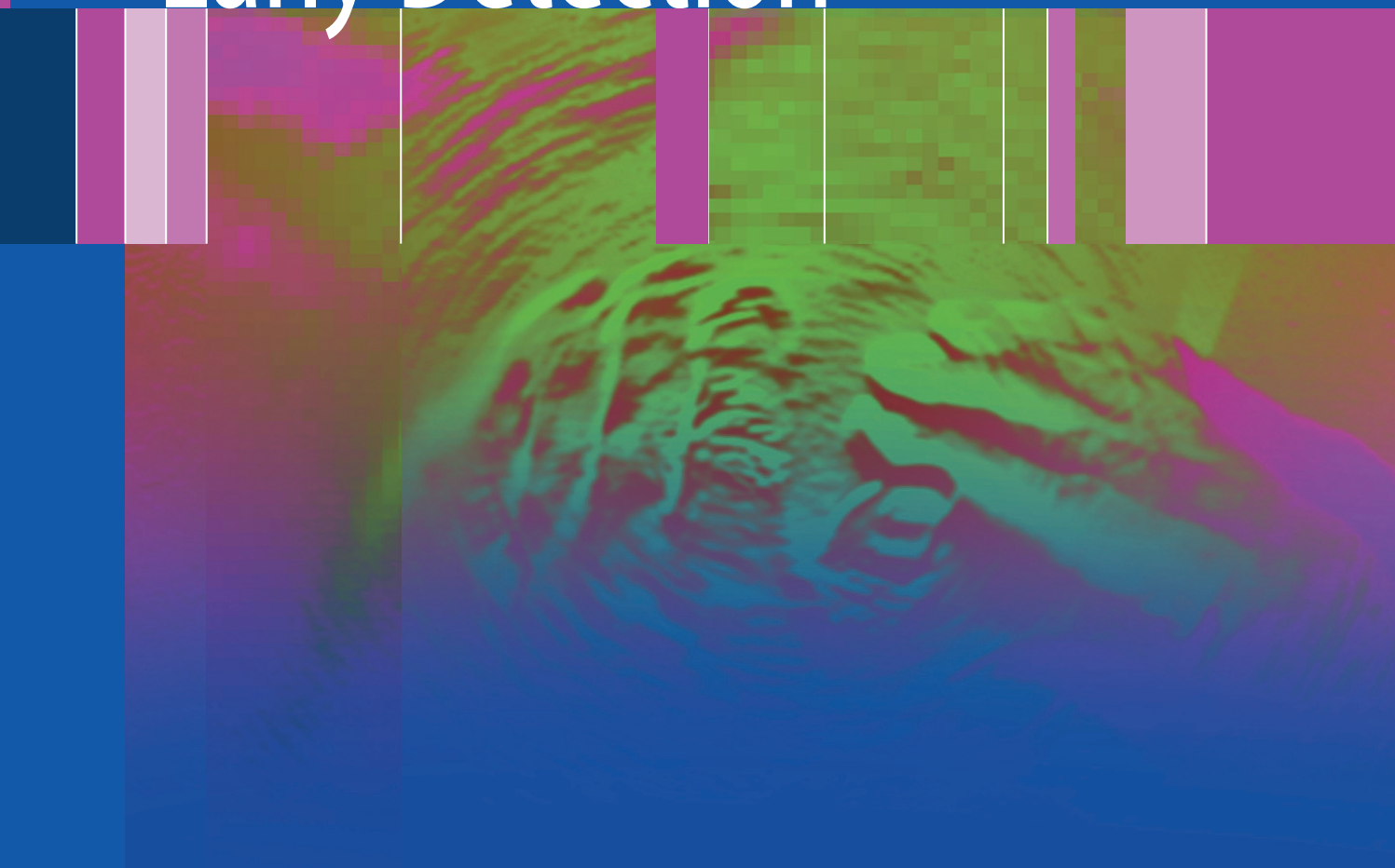
Edward Sauter • Mary B. Daly
Editors

Breast Cancer Risk Reduction and Early Detection



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Preface

While many comprehensive texts have been written on the treatment of breast cancer, the most common cancer among women, there are relatively few which cover in depth the prevention and early detection of the disease. The goal of this work is to present what experts in the field feel is the current knowledge and future direction of breast cancer prevention and early detection. We begin Part I of the book with a review of risk factors, both genetic and environmental. We next review progress in the use of chemoprevention. Notably, chemoprevention risk reduction studies have led to FDA approval of two medications which measurably reduce disease incidence among women at increased risk, although with some risk of treatment related side effects. Newer agents in the pipeline, which may also reduce risk among normal risk women, are also discussed. Surgical risk reducing strategies complete the section on prevention, including both the benefits and downsides to this more aggressive approach.

Even with aggressive prevention strategies, some women will develop breast cancer. For these women, early detection is critical to minimize disease spread and maximize long term survival. Part II of this book reviews current and upcoming approaches to early detection. Imaging strategies, including mammography, breast ultrasound, MRI, and PET imaging are reviewed. The potential for molecular tumor targeting to detect disease prior to the formation of a mass visible by anatomic imaging is presented. We complete our review with breast specific intraductal approaches and systemic evaluation of cells and cell components which may ultimately lead to breast cancer detection at its earliest stage, years prior to the formation of a tumor mass.

We hope that this book satisfactorily addresses the current and future issues related to breast cancer prevention and early detection, and stimulates new ideas which will contribute to reducing the burden of this disease.

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Part I
Prevention

Chapter 1
Risk Factors

Polly A. Newcomb and Karen J. Wernli

Abstract Important improvements have occurred in the past several years in our understanding of the causes and prevention of breast cancer. Age, family history of breast cancer, and experiences of reproductive life have long been known to be associated with breast cancer risk. More recently, new factors have emerged, including obesity, low physical activity, alcohol intake, and exogenous hormone use. Of these new factors, many appear to be related to perturbations in circulating estrogens, which are believed to be the major cause of breast cancer. Although there is a high level of interest in environmental causes of breast cancer, very few common exposures have proved to be associated with the disease. Although some of the factors that increase risk are not amenable to change, many are meaningfully modifiable, even when change is undertaken later in life.

Keywords Risk factors · Breast cancer epidemiology · Incidence

Key Issues

- Breast cancer incidence increased through 2000, when sharp declines began to occur, reflecting changes in risk factors.
- The strongest risk factors for breast cancer are being female and increasing age.
- A family history of breast cancer increases risk of this disease. While rare, a genetic mutation in the *BRCA1* or *BRCA2* genes is a strong risk factor.
- Reproductive experiences, such as nulliparity, late age at first birth, and late menopause are associated with risk. Lactation, especially of long duration, is associated with reduced breast cancer risk.
- Breast density is a powerful and readily assessed risk factor for breast cancer.
- Lifestyle factors associated with increased breast cancer risk include obesity, low physical activity, higher alcohol intake, and the use of exogenous hormones.
- Most environmental or occupational exposures have not been shown to increase breast cancer risk.
- A substantial proportion of postmenopausal breast cancers, the most common form of this disease, can be prevented by modifying known risk factors.

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Introduction

The rapidly increasing and high incidence of breast cancer over the past few decades supports the hypothesis that factors determining breast cancer risk have changed.[1,2] Some of this change can be directly attributable to a reduction of protective factors (e.g., increasing parity, early age at first birth) in a higher proportion of women.[3] Other factors which are known to increase breast cancer risk (i.e., obesity, low physical activity, and the use of exogenous hormones) have become more common. In addition to these changes in risk factors, breast cancer screening has impacted disease incidence. Mammography artifactually increased breast cancer incidence in the short-term by advancing the lead time for prevalent disease[4–6] and possibly in the long-term by identifying lesions with limited malignant potential (see Chapter 10 "screening").[7] In general, greater lifetime exposure to estrogen, influenced by endogenous and exogenous risk factors, increases risk of breast cancer. Although many exposures that increase risk are not readily modifiable, some behaviors can be adopted to decrease risk.

Demographic Factors

Age

Age is the strongest risk factor for breast cancer in women. The incidence of breast cancer increases steeply with age with the greatest rate increase in postmenopausal women, where the risk doubles with each decade of life up to age 80 (Fig. 1.1).[1] The decline in incidence rates after age 80 may reflect lower rates of screening leading to incomplete detection. Breast cancer in men is rare,[2] and presents a pattern of increasing incidence with age that is more consistent with most non-hormonal epithelial cancers, demonstrating that risk factors differ markedly from those in women.

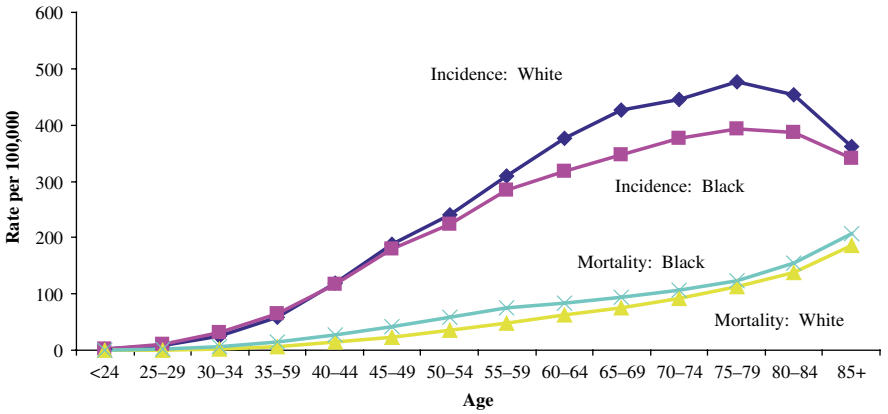

Fig. 1.1 Incidence and mortality rates of female breast cancer by age and race, USA, 2000–2004 (Data from Surveillance, Epidemiology, and End Results (SEER) Program, SEER 17 registries, 2000–2004, Division of Cancer Control and Population Science, National Cancer Institute, 2007.)

Race

There are differences in breast cancer incidence according to race and ethnicity.[1] The incidence of breast cancer is highest in white women, followed by black and Hispanic women, with the

lowest rates in Asian women.[1] These disparities might reflect multifactorial inherited factors, genetic differences in the biology of the tumors, or cultural differences (e.g., maternal age at first birth).[8,9]

Socioeconomic Status

Breast cancer occurs more often among women in higher socioeconomic groups as measured either by income or education,[10] as well as geographic locale.[11] This association may be attributable to the constellation of risk factors that are correlated with high socioeconomic status, including nulliparity and later age at first birth.[8]

Genetics

Family History

Breast cancer tends to cluster in families. Women with a family history of breast cancer, particularly in a first-degree relative, have approximately double the risk of developing breast cancer compared to women without such a history.[12] Risk of breast cancer is higher if the relative was diagnosed at a younger age (diagnosed at <40 years old, relative risk or RR = 6) or if more than one relative was affected (RR = 3–4) [13] (Fig. 1.2).

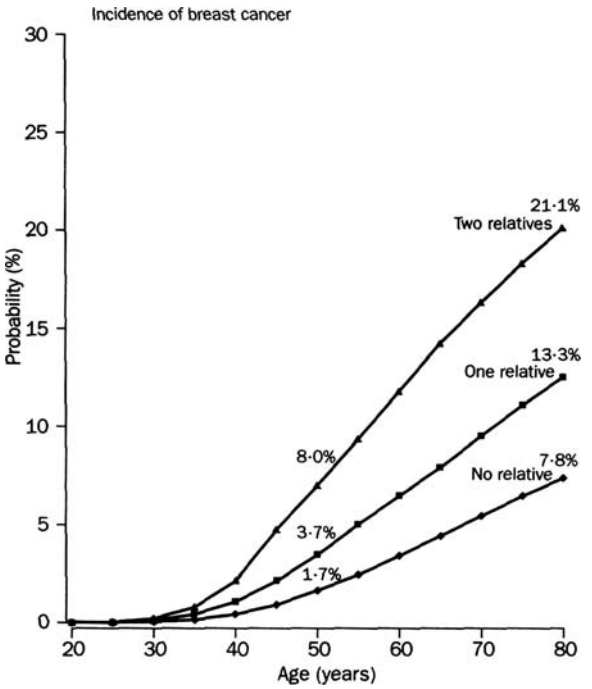

Fig. 1.2 Probability (%) that women in more developed countries who are free of breast cancer at age 20 will develop the disease (incidence) by various ages, according to the number of affects relatives (Reprinted with permission from Collaborative Group on Hormonal Factors in Breast Cancer[12])

BRCA1 and *BRCA2*

In the 1980s, studies of high risk families provided evidence of an autosomal dominant inheritance of breast cancer.[14,15] Gene linkage studies pointed to loci on chromosomes 13 and 17,[16,17] and cloning identified two genes, *BRCA1* (on chromosome 17) and *BRCA2* (on chromosome 13), that appear to be associated with the majority of inherited breast cancers, which account for 2–5% of all breast cancers.[18] Depending upon the populations considered, lifetime risk of disease ranges from 20 to 80%.[19] *BRCA1* and *BRCA2* are tumor suppressor genes with numerous important cell functions, including transcription, regulation of cell cycle checkpoints, genomic stability, and DNA repair.[20–22]

Other Genes

Other genes are also involved in breast cancer risk.[23,24] Women with the rare Li-Fraumeni syndrome have a very high risk of early onset breast cancer and other cancers,[25] which is caused by mutations in the *p53* tumor suppressor gene.[26] Due to the very low prevalence of this syndrome, it is responsible for a low number of breast cancers.[13] Women who are autosomal recessive for the very rare ataxia telangiectasia gene (*ATM*) are at nearly 100-fold greater risk for cancers, including breast cancer.[27] The number of *ATM* heterozygotes is much larger, about 1% of the population, and they have a fourfold increased risk of breast cancer.[28] Women with Cowden's disease have a mutation in the *PTEN* tumor suppressor gene.[29] Nearly 50% of women with this disease develop breast cancer by age 50.[30]

It is likely that most of the genetic susceptibility to breast cancer is due to alleles with a low to moderate penetrance. That is, they confer a small amount of risk, but are very common and as such the population attributable risk is potentially high.[19,31] While intensive investigations have identified gene polymorphisms statistically associated with increased breast cancer risk, studies have been inconsistent. Pooling data from these studies within a large consortium, such as the Breast Cancer Association Study (BCAS), will help clarify associations of polymorphisms with breast cancer as well as rare polymorphisms and gene-environment analyses that will require large sample sizes. Genome-wide association studies will identify new polymorphisms, and these will also need to be validated in larger consortium efforts.

The relationship of breast cancer risk to family history is due to both high penetrance gene mutations and low penetrance polymorphisms, together with shared environmental factors.

Reproductive and Hormonal Factors

Reproductive events, including menarche, pregnancies and live births, lactation, and menopause all mark important and sustained changes that can influence breast cancer risk. Incidence may be altered by the effects of reproduction's physical changes to breast tissue as well as long-term alterations in hormonal exposures.

Menarche and Menopause

Increasing age at menarche is associated with decreasing breast cancer incidence; with each one year increase in age at menarche, risk of breast cancer decreases by 5%.[32] Concomitantly, increasing the reproductive span with a late age at menopause increases the risk of breast cancer, presumably

through greater lifetime exposure to circulating hormones. Secular changes in age at menarche and menopause in women born in the first-half of the twentieth century have been notable,[33] and likely has had implications for breast cancer incidence.

Parity

Parity, specifically at an early age, is associated with a decreased risk of breast cancer. Compared to nulliparous women, parous women are at 17–41% decreased risk of breast cancer, depending upon their age and parity.[32] The reduction in risk is not immediate. Indeed, risk is actually increased in the first 10 or so years following a pregnancy,[34–36] likely due to an increase in the acute proliferating effects of pregnancy. Overall though, based upon a reanalysis of 53 observational studies, for each additional pregnancy, breast cancer risk is reduced by about 7%.[37] Repeated pregnancies very likely provide maximal breast epithelial cell differentiation prior to the accumulation of further DNA damage that occurs throughout adult life.[38]

Age at First Birth

The timing of first pregnancy is an important determinant of breast cancer risk, reflecting the benefits of final maturation of terminal ducts of the breast at an early age with hormonal exposures for the first pregnancy.[39] Compared to women under age 18 at the time of first pregnancy, women whose first birth occurred at age 35 years or older had a 60% increased risk of breast cancer.[32]

Lactation

Lactation further decreases risk of breast cancer in parous women, although the overall reduction in risk varies substantially within the population studied.[40] Based upon the pooled re-analysis of 51 observational studies, the relative risk for breast cancer decreases by 4.3% for every 12 months of breastfeeding (Fig. 1.3).[37] The risk reduction appears to be greatest among women with high parity, where the risk reduction due to breastfeeding may be as great as 50%,[41] and among premenopausal women with lactation durations $\geq$2 years, where the breast cancer risk reduction may be 30%.[42]

Intrauterine Environment

Exposures very early in life, including in utero, may be relevant to breast cancer risk.[43] Although results are not entirely consistent, factors that may reflect high levels of uterine estrogens, such as twins,[44] high birth weight,[45] left-handedness,[46] and older maternal age [47] are associated with modest increases in risk of breast cancer in offspring. In a recent review and meta-analysis of 57 studies, an increased risk of breast cancer was associated with increased birth weight with 8% increased risk per 1 kg excess weight.[48] Birth experiences that are associated with low levels of pregnancy estrogens, such as preeclampsia and higher birth order, have been associated with decreased breast cancer risk in daughters.[48] Exposures that occur shortly after birth, such as being breastfed, decrease the risk of breast cancer in daughters.[49]

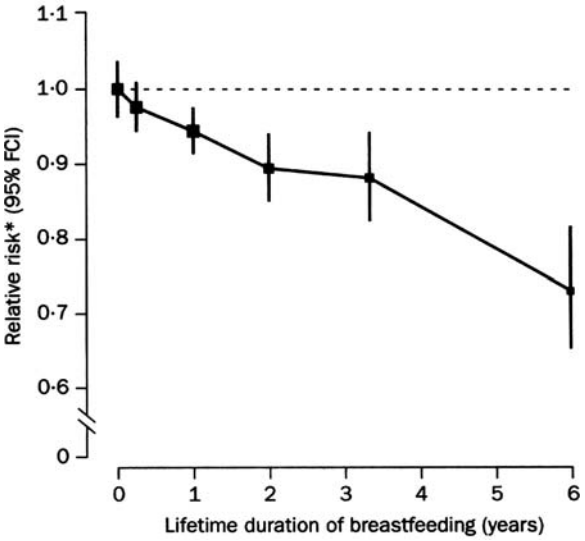

Fig. 1.3 Relative risk of breast cancer in parous women in relation to lifetime duration of breastfeeding (Reprinted with permission from Collaborative Group on Hormonal Factors in Breast Cancer [37])

Exogenous Hormones

Oral Contraceptive Pills

The proliferative effects of endogenous hormones support observations that exogenous exposure to hormones, primarily estrogen and progestin, is associated with breast cancer risk. Oral contraceptives provide a steady low level of hormones, which may be higher and more consistent than those occurring naturally.[50] Overall, oral contraceptives users have about the same risk of breast cancer as non-users, based upon the Oxford reanalysis.[51] There is some evidence that current or recent users of oral contraceptives might modestly increase their risk of breast cancer;[51] however, this increased risk disappears with cessation.[52] The underlying risk may be important; in one study, recent and current users of oral contraceptives aged 35–45 years had a statistically significant two-fold increased risk of breast cancer compared to never users.[53] Such an observation warrants consideration in older users of these common medications.

Postmenopausal Hormone Therapy

World-wide epidemiologic data now confirm that postmenopausal hormone therapy use is associated with increased breast cancer incidence. The Collaborative Group on Hormonal Factors in Breast Cancer pooled and reanalyzed the data from most observational studies.[54] They reported a modest increase in the risk of breast cancer associated with ever use of hormone therapy compared to never use (relative risk [RR] = 1.14; $p<0.001$), with evidence of an increasing risk with increasing duration of use ($p = 0.003$). The risk of breast cancer was increased among current users (RR = 1.21; $p<0.001$), but not among past users (RR = 1.07; $p = 0.10$). This association was only appreciable after long-term use, and returned to baseline after discontinuation. When stratified by hormone therapy type, current users of unopposed estrogen (E-alone) for 5 years or longer had a 1.34-fold increased risk of breast cancer, and current users of estrogen plus progestin (EP) for 5 years or longer had a 1.53-fold increased risk. Also noteworthy are results from eight recent observational studies from the US and Europe. Each study determined that use of EP was more strongly associated with breast cancer risk than is E-alone.[55–62]

Perhaps the most compelling data on the effects of types of hormone therapy and breast cancer risk comes from the Women's Health Initiative (WHI) randomized controlled-trials, which found that EP users had an elevated risk of breast cancer [hazard ratio (HR) =1.26, 95% CI 1.00–1.59].[65] This trial was stopped in 2002 after 5.2 years of follow-up when the risks of EP use were found to outweigh its benefits. In contrast, after 7 years of follow-up in the E-alone trial, E-alone users had a non-statistically significant reduced risk (HR = 0.77; 95% CI 0.59–1.01) of breast cancer.[63] Thus, based upon these results, there is little doubt that use of EP but not E-alone, as used in this older population, adversely affects the risk of breast cancer; therefore current recommendations for use are limited to the acute amelioration of menopausal symptoms.[64] It must be noted that, although there may indeed be biological reasons for the disparate risk estimates in the EP and E-alone groups, the study groups necessarily had different inclusion and exclusion criteria, which may have impacted the observed results.[65]

The results of the WHI and other epidemiologic studies have changed prescription practices of hormone therapy in the US and, indeed, the rest of the world. In a study conducted within health plans in the US, the proportion of women using hormone therapy had fallen approximately 40% for EP use and 20% for E-alone use.[66] Similarly, the study of prescription drug use by Hersh et al. observed drops in use by 66% for EP and 33% for E-alone.[67] While the prevalence of hormone therapy use has dropped considerably among women aged 40–80 years in the US, an estimated 7.9% are still current EP users and 9.1% are current E-alone users.[66] In a recent analysis of SEER data, the recent decline in breast cancer incidence is likely attributable to the decrease in the use of hormone therapy.[68]

Estrogen Antagonists

Because breast cancer is a hormonally-driven process, drugs that act as estrogen antagonists (selective estrogen-receptor modulators or SERMS) have been shown to reduce breast cancer incidence.[69] In the first randomized-controlled trial of tamoxifen to prevent breast cancer, high-risk women defined by a Gail model score were randomized to tamoxifen or placebo in the Breast Cancer Prevention Trial (National Surgical Adjuvant Breast and Bowel Project [NSABP P-1]).[70] After 5 years, women randomized to tamoxifen had a 49% reduced incidence of invasive breast cancer compared to placebo ($p < 0.0001$).[71] Risk reductions were limited to estrogen-receptor positive tumors, regardless of age. More recently, raloxifene, a second generation SERM, which is commonly used to prevent osteoporosis, has also been shown to reduce breast cancer risk.[72–74] In a large, randomized-controlled trial, the Study of Tamoxifen and Raloxifene (STAR, NSABP P-2), raloxifene was as effective as tamoxifen in reducing invasive breast cancer incidence after 5 years of treatment, with fewer side effects.[75] New chemopreventive drugs are under evaluation. Based largely on the results of clinical treatment trials using the aromatase inhibitor anastrozole,[76] two large clinical prevention trials testing aromatase inhibitors are now underway.[77] Several other agents show promise in breast cancer prevention, targeting pathways relevant to ER negative lesions (See Chapter 3, Chemoprevention).

Benign Breast Disease and Breast Density

Benign Breast Disease

It was initially reported in the 1950s that a history of benign breast disease might increase the risk of breast cancer.[78] These early reports had difficulties in identifying benign breast disease cases,

following-up study subjects, and utilizing a comparison population. Nonetheless, the overall consensus from years of research suggests that a history of benign breast disease does increase the risk of breast cancer.[79,80]

Overall, women with benign breast disease without hyperplasia have a 1.5-fold increased risk of breast cancer compared to women without benign breast disease (OR = 1.5, 95% CI 1.3–1.9). The risk of breast cancer among women with hyperplasia varies by whether or not atypia is present; women demonstrated to have hyperplasia with atypia have a 2.6-fold increased risk of breast cancer (OR = 2.6, 95% CI 1.6–4.1), but only a 1.8-fold increased risk in women without atypia (OR = 1.8, 95% CI 1.1–2.5). Women with a fibroadenoma have an independent increased risk for breast cancer (OR = 1.7, 95% CI 1.1–2.5).[81]

The risk of breast cancer associated with benign breast disease differs by menopausal status. Among premenopausal women, the relative risk of breast cancer associated with atypical hyperplasia is 5.9 (95% CI 2.9–13.2). By comparison, among postmenopausal women, the risk of breast cancer associated with atypical hyperplasia is 2.3 (95% CI 0.9–5.9), suggesting that atypia is more important in premenopausal women.[82]

The development of benign breast disease is influenced by some but not other breast cancer risk factors. The increased risk of breast cancer associated with a diagnosis of hyperplasia with or without atypia is not modified by ethnicity. In a recent multiethnic cohort, Worsham and colleagues detected no differences in breast cancer risk and benign breast disease among African American and non-African-American women.[83] In the WHI, the risk of development of benign breast disease 5.5 years post-randomization was 1.74 (95% CI 1.35–2.25) among women randomized to estrogen plus progestin hormone therapy compared to women in the placebo arm.[84]

Histological type of the benign lesion may influence risk. The risk of breast cancer associated with atypical hyperplasia appears to be stronger among women who had lobular compared to ductal lesions. In the Nurses Health Study, women with benign breast disease who had atypical lobular hyperplasia had a fivefold increased risk of a breast cancer while women with atypical ductal hyperplasia had a 2.4-fold increased risk of breast cancer compared to women with nonproliferative benign breast disease.[85] Other studies have detected similarly elevated risks between atypical lobular and atypical ductal hyperplasia.[83,86]

The latest body of research is evaluating genetic differences in cases of benign breast disease to identify women at increased risk for breast cancer. In a nested case-control study in the National Breast Screening Study (NBSS) in Canada, Rohan and colleagues determined that women with benign breast disease who had overexpression of p53 had a 2.55-fold increased risk (95% CI 1.01–6.40) of breast cancer compared to women with benign breast disease but without p53 overexpression.[87] The authors did not stratify by the presence or absence hyperplasia or atypia. Thus, benign breast disease maybe be sensitive to the same risk factors as for invasive cancer, and therefore should be considered in the causal pathway.

Breast Density

High mammographic breast density is considered one of the strongest risk factors for breast cancer.[88–90] Among women with more than 75% breast density, the risk of breast cancer is more than four times that of women with much less dense breasts.[90] Mammographic density is defined and measured by the amount of radiodense areas, which represent epithelial tissue and stroma.[91] There are several mechanisms by which to measure density, including Wolfe's scheme and the percentage of breast tissue that is dense measured by radiologists or a computer-assisted program.[91] All reported methods appear to be valid in assessing breast density. Breast density is associated with epithelial proliferation and with stromal fibrosis.[89]

There is a direct relationship between increasing breast density and breast cancer risk. A recent analysis of breast density and cancer risk utilizing three nested case-control studies demonstrated statistically significant odds ratios of 1.8 (10 to <25%), 2.1 (25 to <50%), 2.4 (50 to <75%), and 4.7 (≥75%) compared to women with <10% (p-trend<0.0001).[92]

Breast density and invasive breast cancer appear to be influenced by the same risk factors. In an ancillary study from the WHI randomized controlled-trial, women who were adherent to treatment of estrogen plus progestin hormone therapy at year 1 had a mean increase in density of 7.7% (95% CI 5.9–9.5%) compared to women in the placebo group who had a mean decrease in density of 1.1% (95% CI 0.3–1.9%).[93] Increased breast density is also associated with premenopausal status, younger age, nulliparity, older age at first birth, use of hormone therapy, and increasing body mass index.[90,94] To date, IGF-1 in premenopausal women and prolactin in postmenopausal women are associated with increased mammographic density.[90] Indeed, because of the parallels between risk factors for breast density and breast cancer, mammographic breast density has been called an "intermediate phenotype" for breast cancer.[90] Therefore, the addition of breast density to a modified Gail model significantly improved the predictive value of this risk model.[95]

Body Size and Physical Activity

Many aspects of body habitus influence the risk for breast cancer. Greater size as measured by height, weight, and the composite measurement of body mass index (BMI, kg/m^2) are related to increased breast cancer risk after menopause.[96]

Height

Increasing height is associated with increasing risk of breast cancer, particularly in postmenopausal women. In a pooled analysis, the relative risk of breast cancer per increment of 5 cm increase in height was 1.02 (95% CI 0.96–1.10) in premenopausal women and 1.07 (95% CI 1.03–1.12) in postmenopausal women.[97] These differences were confirmed by a large analysis from The European Investigation into Cancer and Nutrition (EPIC).[98]

Obesity

Increasing BMI is also associated with an increased risk of breast cancer. In a pooled analysis from seven large prospective studies, the authors suggest that increasing adult BMI is associated with an increased risk of breast cancer in postmenopausal women, but it shows evidence of no association or a possible decreased risk among premenopausal women.[97] There is strong evidence that weight gain in adult life is associated with a greater risk of breast cancer. In a large population-based study, for each 5 kg weight gain since the lowest adult weight, breast cancer risk increased by 8%,[99] while weight loss, particularly at younger ages is related to decreased risk.[100] The relationship between BMI and breast cancer risk appears to differ by hormone therapy use. Lahmann et al. demonstrated that women who were not hormone therapy users had 1.3-fold increased risk of breast cancer associated with a BMI of 25–29.9 or ≥30 compared to lean women.[98] Among women who were hormone therapy users, there appears to be no association with weight, and the suggestion of a decreased risk among women with a BMI over 30.[101]

Obesity in girls and adolescents appears to be related to a reduced risk of premenopausal breast cancer. The most recent analysis from the Nurses Health Study II suggests that women reporting being the most overweight during childhood (<10 years) and adolescence (10–20 years) had a 52% reduced risk of premenopausal breast cancer (95% CI 0.51–0.55).[102] The suggested pathways involve the relationship between obesity and many hormones, especially at the time near the onset of puberty. For example, overweight girls have a younger age at menarche.[103]

Physical Activity

Obesity and physical activity are closely related. Numerous epidemiologic studies have observed a reduction in breast cancer risk with physical activity.[104] Decreases are generally 20–40%, and observed in the most active compared to the least active women, both from occupational and recreational activities. Evidence indicates a greater consistency in a protective effect of physical activity on postmenopausal breast cancer; studies are limited regarding an effect in premenopausal women.[105] Other studies have examined timing, intensity, type, and modifiers of physical activity. For example, an early study showed a halving of breast cancer risk with vigorous physical activity during ages 14–22 years (OR = 0.6, 95% CI 0.4–0.7), but lifetime activity was not collected.[106] Other studies have shown that the benefits of physical activity are limited to women without a family history of breast cancer,[107] including carriers of BRCA gene mutations.[108] In 2002, there was "convincing" evidence for an inverse association between physical activity and breast cancer risk.[109] Though specific biological mechanisms have not been established, animal and human studies suggest physical activity influences a broad physiological spectrum, including sex hormone levels, insulin and insulin-like growth factors, immune function, and general energy balance.[104,110]

Behavioral Factors

Alcohol

Alcohol consumption at all ages is consistently associated with an increased risk of breast cancer.[111] A pooled-analysis of more than 50 studies showed that the risk of breast cancer was 1.32 (95% CI 1.19–1.45) for women consuming two to three drinks per day compared to non-drinkers. Risk is dose-dependent, with risk increasing by about 7.1% for each additional 10 g of alcohol consumed per day.[112] There does not appear to be a minimum threshold, so even one drink per day predicts modestly elevated risk.[113] Although the effect appears to be present for all types of alcoholic beverages, in some studies wine consumers appear to have an attenuated risk, perhaps due to residual confounding.[114] Subgroups of women may be at greater risk of disease because of other breast cancer risk factors. In one study, alcohol consumption was associated with a two-fold increased breast cancer risk in women with low BMI (<25).[115] Although the mechanisms are not completely clear, it may be that alcohol increased circulating levels of estrogen and androgens,[116] and increases the susceptibility to hormones, the effects of which may be mediated by folate metabolism.[117]

Diet and Nutrition

The tremendous international variation in cancer rates and potential anticarcinogenic constituents of food suggest that diet may be important in breast cancer epidemiology.[118] However, diet is a complex behavior, and studies of micronutrients, macronutrients, food items, and overall patterns of consumption in relation to risk have not been consistent or strong.

Neither observational nor experimental data appear to support an important relationship between diet and breast cancer risk. In a pooled reanalysis of cohort studies, the risk of breast cancer was 1.00 (95% CI 0.98–1.03) per 5 kg increase in dietary fat consumption.[119] In the WHI, women randomly assigned to a dietary modification to maintain only 20% of their calories from fat did not experience a significantly different breast cancer risk than women in the placebo group who maintained their usual diets (HRR, 0.91; 95% CI 0.83–1.01) after 8 years of follow up.[120]

Intake of fruits and vegetables was not associated with breast cancer for the highest to lowest category of consumption (RR 0.96, 95% CI 0.889–1.04).[121] Examination of specific nutrient groups, including milk products, soy intake, fiber, and other micro- and macronutrients, have also not indicated an association with breast cancer risk.[122]

Inconsistencies in previous research may be attributable to the collection of dietary intake information, particularly the validity of those items most relevant to breast cancer risk. For example, using a sub-study of the WHI Dietary Modification Trial, dietary fat identified from food records was associated with an elevated adjusted relative risk of breast cancer and a statistically significant trend, but this dietary fat association was not observed when evaluated from food frequency questionnaires.[123] Improved methodologies in dietary assessment may clarify the role of diet in breast cancer incidence.

Medications

The influence of regular use of several medications on breast cancer risk has been studied. Common non-steroidal anti-inflammatory agents (i.e., aspirin, ibuprofen) have been associated with decreased risk of breast cancer in some[124] but not all studies. Other medications, such as statins, have been associated with increased risk of disease.[125] Reports of other drugs, such as antidepressants, have not shown an association.[126]

Smoking

Despite extensive study, data regarding the relationship between smoking and breast cancer remains inconclusive. The most recent review of the literature suggests that there is no evidence of a reduction in risk, and there may in fact be a modest increased risk associated with increasing duration and frequency of smoking.[127] Researchers have demonstrated an increased risk of breast cancer associated with duration, intensity, cumulative exposure, and latency among long-term smokers.[128] Exposure to passive smoking either from parents as children or a partner/spouse has not been associated with breast cancer.[129]

Increasingly, researchers are investigating the association between smoking and breast cancer by genotype which detoxify or activate the chemicals in tobacco smoke. In a recent meta-analysis, Terry

and Goodman demonstrated that the relative risk for smoking associated with breast cancer was 1.2 among NAT2 rapid acetylators (95% CI 1.0–1.5) and 1.5 among NAT2 slow acetylators (95% CI 1.2–1.8).[130]

Environmental and Occupational Factors

The increases in breast cancer incidence with industrialization and urbanization suggest that there are environmental components to breast cancer risk. However, despite the investigation of numerous environmental or occupational exposures in association with breast cancer risk, few have been demonstrated to be important etiologically.

Pesticides

Much attention to environmental risk factors for breast cancer has focused on exposure to dichlorodiphenyl-trichloroethane (DDT) and its metabolite, dichlorodiphenyldichloroethylene (DDE). DDT was originally used as a pesticide agent but it is no longer used in the US.[131] Some have suggested that it acts as an estrogen within the environment and the human body, and have shown estrogenic effects in animal models.[132] The unknown consequences of these agents have led investigators to research its association with breast cancer. Despite a plausible biologic rationale and laboratory support, most research suggests that there is no association between DDT and breast cancer risk.[133] Recent studies have investigated women who develop breast cancer at a younger age [134] or in populations where DDT has been used more recently (i.e., Mexico),[135] and have shown some increasing breast cancer risk with increasing exposure based on blood levels.

The association between polychlorinated biphenyls (PCBs) and breast cancer has also been examined in a multitude of studies.[131] The majority of investigations have shown no overall association between PCBs exposure and breast cancer, and there was no evidence of a dose-response relationship by duration of exposure or body burden. More recent studies are investigating genetic variation, which suggests women are at increased risk of breast cancer due to exposure to PCBs based on the metabolizing genes in the cytochrome *p*450, specifically CYP1A1.[136–138]

Other Agents

Other environmental risk factors have been studied in relation to breast cancer risk. A recent analysis of the heavy metal cadmium, a by-product of industrialization, suggests that higher body burden of cadmium is associated with an increased risk of breast cancer. Women with a creatinine-adjusted cadmium level in the highest quartile had more than twice the breast cancer risk of those in the lowest quartile (OR = 2.29, 95% CI 1.3–4.2) (p-trend = 0.01).[139] A recent report suggested that greater exposure to traffic emissions at the time of menarche is associated with an increased risk of breast cancer in pre- (OR = 2.05, 95% CI 0.92–4.54) but not in postmenopausal women.[140]

Occupational Exposures

Occupational health studies in women are difficult to accomplish given that few women are exposed to the agent of interest through their work and few women are working in a relevant occupation,

often leading to low statistical power to assess an outcome such as breast cancer. Exposure to electromagnetic fields (EMF) is fairly common in occupational settings. Several studies have shown a slight but statistically significant elevation in risk of breast cancer in women with the highest levels of EMF exposure.[141,142] Increased risks of breast cancer have been associated with employment as nurses,[143] hairdressers,[144] and flight attendants.[145,146] Occupational exposures of textile workers has been speculated to increase breast cancer risk, but the largest cohort study on this topic did not detect a statistical association with textile hazards and breast cancer risk.[147] Some occupations are associated with increased physical activity. Women with heavy activity occupations in a large case control study had a decreased risk of breast cancer compared to women with sedentary work ($p = 0.007$).[148]

Working as a nurse has been associated with an increased risk of breast cancer.[143] One hypothesis for this association is related to employment at night or shift work. Exposure to light at night suppresses the night surge of melatonin, and the reduction of melatonin is thought to result in increased circulating estrogen. Initial reports have suggested that women who have worked at night for longer durations have increased risks of breast cancer.[149,150] In 2007, the International Agency for Research in Cancer classified exposures that involve circadian rhythm disruption as a probable human carcinogen (Group 2a).[151]

Radiation

The breast is very susceptible to the damaging effects of radiation.[152,153] In general, risk depends upon dose, age, and time since exposure.[154] Radiation exposures in women are most common in the medical care setting, such as chest radiation for benign breast disease,[155] scoliosis,[156] and radiation for cancer treatment (i.e., Hodgkin's disease).[157] The breast is particularly sensitive to the effects of ionizing radiation during puberty, even at low doses.[158] Women living in Hiroshima who were under 20 years of age when the atom bomb was dropped had a nearly 15-fold increased risk compared to unexposed women. This increased risk was far greater than for older women in the same area.[159]

Summary

Many risk factors for breast cancer are inextricably tied to our modern lifestyle, and clearly there are causes for breast cancer that remain unknown. The frustration of breast cancer epidemiology has been that the strongest risk factors (i.e., known genetic or heritability syndromes) are rare, and some of the most common risk factors (i.e., age) are not amenable to change. The *in toto* proportion of explained population attributable risk from known factors ranges from 15 to 55%.[160–162] These studies, however, include all established risk factors,[163] yet the modifications of most reproductive and medical history factors are not consistent with the current goals of society. However, women who wish to reduce their risk, particularly since the incidence is highest in the later adult years, can substantially decrease their risk of disease by changing some behaviors,[164] including reducing alcohol intake, maintaining a healthy weight, and pursuing regular physical activity. Such a purposeful change would result in a 41% reduction in breast cancer incidence in postmenopausal women. The search for modifiable risk factors must continue, and should creatively examine the interplay of known factors to target women at greater risk to tailor risk reduction interventions.

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Chapter 2
Lifestyle Factors and Risk of Breast Cancer: A Review of Randomized Trial Findings

Garnet L. Anderson

Abstract Observational and pre-clinical studies suggest a role for multiple lifestyle factors in the etiology of breast cancer, but few of these have been tested in full-scale randomized trials and fewer still have been shown to affect breast cancer risk. The clearest evidence for a modifiable risk factor is use of menopausal estrogen plus progestin therapy, where randomized trial evidence confirms an increased risk with longer durations of exposure and studies in multiple populations document that a decrease in use results in reduced rates. The effects of estrogen alone on breast cancer risk are less clear as the only large trial on this topic suggests a possible reduction and that timeframe of exposure may be an important effect modifier. The role of diet has not been adequately resolved, in part because of the methodological difficulties. Randomized trial data are strongly suggestive of the benefits of a low-fat diet on breast cancer risk but no other nutrients tested in trials of dietary supplements have yielded benefits for breast cancer or total cancer in women. Similarly, one large randomized trial of low-dose aspirin has not shown an effect on breast cancer. The contrasts between the observational studies that motivated the randomized trials and the results of the trials emphasize the need for more efforts to test other lifestyle factors in full-scale randomized trials.

Keywords Diet · Nutritional supplements · Menopausal hormone therapy · Aspirin

Key Issues

- Lifestyle factors are behaviors or exposures that are modifiable at the individual level.
- Observational studies, and particularly international observational studies, point to lifestyle as likely having a role in breast cancer risk.
- Randomized trials are often required to assess the effects of lifestyle factors because of the methodologic challenges of the observational studies: measurement problems, modest effect sizes, confounding and differential screening/detection.
- Estrogen plus progestin therapy in postmenopausal women increases the risk of breast cancer and reductions in its use are associated with a rapid decline in incidence rates.
- The effect of unopposed estrogen therapy on breast cancer rates in postmenopausal women is less clear and the effects may vary by age at first use.

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- Randomized trial data strongly suggest that a low-fat diet provides a modest reduction in risk of breast cancer after menopause.
- None of the micronutrients tested, including β-carotene, vitamin C, vitamin E, folic acid, vitamin B6 and B12, calcium and vitamin D and selected multi-nutrient supplements have been shown to have an effect on either breast cancer or total cancer incidence in women.
- Low-dose aspirin offers women no protection for breast cancer or total cancer risk.

Introduction

The large international variation in breast cancer rates[1,2] and more specifically studies that demonstrate a change in breast cancer rates with migration from low incidence countries to high incidence countries[3,4] provide evidence that environmental and lifestyle factors modify breast cancer risk. Many lifestyle factors, here defined as risk factors that are amenable to change at the individual level, have been examined and found to be associated with breast cancer risk. The most commonly considered are diet, physical activity, smoking, and alcohol consumption, but choices such as regular use of dietary supplements or medications, reproductive choices and lactation represent aspects of women's lifestyle that are associated with breast cancer.

Definitive evidence to support a causal influence of most lifestyle factors on breast cancer or other health conditions is scarce. Most of our current understanding arises from observational studies. In addition to the usual limitations of non-experimental data, many observational studies of lifestyle are faced with additional methodological challenges: difficulties in measuring lifestyle choices, limited variability within populations for selected behaviors, modest effect sizes, and correlation among lifestyle factors and between lifestyle and other exposures, including cancer screening, in free-living populations. At best, these complexities constrain the inference that can be made; often they contribute to conflicting results. Thus, it is increasingly clear that unless a lifestyle factor has a very strong effect on health, definitive evidence requires a randomized trial.

The first trial to document a reduction in breast cancer risk with any intervention was a randomized, double-blind, placebo-controlled trial of tamoxifen.[5] Since that time a few other large scale studies have tested effects of diet, specific nutrient supplements, aspirin and hormone therapy on breast cancer incidence. Although some of these were motivated by other chronic disease hypotheses, these trials provide the clearest information regarding the role of these factors on breast cancer risk. The most noteworthy of these efforts include the Women's Health Initiative (WHI), a large randomized trial testing three disease prevention strategies, hormone therapy, a low-fat diet, and calcium and vitamin D supplementation in a partial factorial design,[6,7] the Women's Health Study (WHS), which tested low-dose aspirin, vitamin E and β-carotene[8] in a factorial design, and the Women's Antioxidant Cardiovascular Study (WACS), another randomized trial testing the effects of vitamin C, vitamin E and β-carotene in a $2\times2\times2$ factorial design.[9]

Nutrition and Breast Cancer

When one considers lifestyle and health, nutritional factors are often at the forefront. The diet and disease question can be posed in different terms: specific foods, macro or micro-nutrients, eating patterns (e.g., vegetarian, Mediterranean, religious), and food preparation methods. Variation in specific nutrient consumption or in eating patterns may be limited within a population and may be correlated with other lifestyle factors. Further, the relative risks associated with different dietary factors are

generally thought to be modest and the lifestyles must be sustained for some interval to reach their full effect. While various observational study designs have been used to examine these questions, all face noteworthy hurdles. Prospective studies require very large sample sizes and long-term follow-up to have adequate statistical power. Retrospective studies, though more efficient, generally rely on dietary recall over a long period of time, a particular concern since dietary behaviors and memory may be altered by the presence of the disease. Not surprisingly, inconsistencies in results are common.

Macronutrients

One of the strongest and longest posed hypotheses has been that a diet high in total fat is associated with increased breast cancer incidence. Analyses of international data found a strong association between breast cancer incidence and fat disappearance, which was well-summarized as a linear trend with a 50% lower fat consumption associated with a 50% lower breast cancer incidence.[10] A summary of case-control data showed a more modest but statistically significant increasing trend in breast cancer risk across quartiles of dietary fat intake.[11] Prospective cohort studies did not support this hypothesis.[12,13] A more recent review of the international data reported 11 case-control studies supporting increased breast cancer risk with greater fat intake, 5 showing decreased risk.[14] In the same review, nine cohort studies addressed this question; of these, six reported increased risk and three reported reduced risk with increased fat consumption.[14]

Between 1993 and 2005, the WHI tested the low-fat diet and breast cancer hypothesis in a large scale randomized primary prevention trial. The trial randomized 48,835 postmenopausal women aged 50–79 to their usual diet (60%) or to a dietary behavioral intervention (40%). Women in the intervention group were taught strategies to reduce their dietary fat intake to 20% of energy and to increase their consumption of fruits, vegetables, and grains. The design assumed that the difference in percent energy from fat intake between intervention women and women in the usual diet group would be 13% at 1 year and that most of this difference would be maintained throughout the planned 9-year follow-up period. In addition, a 10-year lag time to full effect was incorporated, as suggested by the international data.[10] Based on these assumptions, the trial was designed to detect a 14% reduction in breast cancer incidence.[7]

Statistically significant reductions in reported percent energy from fat were found (mean 10.7% at year 1, diminishing to 8.1% at year 6) but these did not reach trial goals. Intervention women also reported small but statistically significant increases in fruit and vegetable consumption (mean of one serving per day) and grain servings (mean < one serving per day).[15]

After a mean 8.1 years of follow-up, the hazard ratio (HR) for invasive breast cancer was 0.91 (95% confidence interval [CI] 0.83–1.01) in 1,727 cases (Fig. 2.1).[15] Total cancer incidence was not affected (HR 0.96; 95% CI 0.91–1.02 in 4,986 cases). No effect was seen on breast cancer mortality (HR 0.77; 95% CI 0.48–1.22 in 80 breast cancer deaths) or on total cancer mortality or total mortality. The 9% breast cancer risk reduction did not reach statistical significance ($p = 0.09$), but was consistent with the level of risk reduction projected from the trial design after accounting for the level of adherence, i.e., the trial achieved roughly 70% of intervention goals and observed approximately 70% of the expected 14% reduction in incidence[15] (Fig. 2.1).

In further analyses, the observed reduction appeared to be concentrated in progesterone receptor (PR) negative disease (HR 0.76; 95% CI 0.63–0.92) with no reduction observed in PR positive disease, and a suggestion of the strongest risk reduction in ER positive/PR negative disease. In exploratory analyses, an interaction ($p = 0.04$) with baseline fat intake was observed – among the women consuming the highest percent energy from fat at baseline (>36.8% calories from fat), the

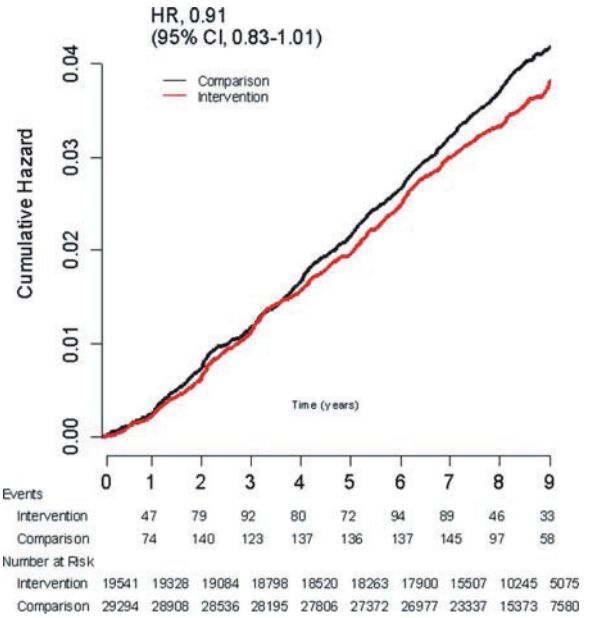

Fig. 2.1 Kaplan-Meier estimates of the cumulative hazard for invasive breast cancer. CI indicates confidence interval; HR, hazard ratio (Reprinted with permission from Prentice et al.[15] Copyright © 2006 American Medical Association. All rights reserved)

intervention group achieved the greatest absolute change in dietary fat intake (12.2% ± 7.0%) and experienced the largest risk reduction (HR 0.78; 95% CI 0.64–0.95). Evidence for a trend with baseline fruit and vegetable intake was not as significant and there was no evidence of interaction with baseline grain consumption.[15] Thus, while the trial does not provide definitive results, the data are strongly consistent with the underlying hypothesis.

Limitations of a behavioral intervention study such as the WHI dietary modification trial include lack of ability to control adherence to the intervention goals, and the inability to identify the specific component of this dietary intervention that may lead to risk reduction. In addition, this study included only postmenopausal women; it is unknown whether these dietary changes if made earlier in life, and in that sense more reflective of the international studies, would have a stronger effect on breast cancer risk.

Micronutrients

The most prominent micronutrient hypothesis over the last two decades has been in the role of antioxidants, with regard to both cancer risk and chronic disease prevention more generally. This hypothesis has been tested in several large scale prevention trials using dietary supplements, often with doses at or above the recommended daily allowance at the time for dietary consumption in the general population. The design of these trials was usually based on cardiovascular disease, total cancer, or other site-specific cancers but they provide some of the most reliable information to date on the effects of these agents on breast cancer risk.

Vitamin A/β-carotene

Several full-scale prevention trials of vitamin A or β-carotene (pro-vitamin A) have been conducted: the α-Tocopherol and β-Carotene Cancer Prevention Study (ATBC),[16] the Carotene and Retinol Efficacy Trial (CARET),[17] the Physician's Health Study (PHS),[18] and the Women's Health Study.[19]

Most of these trials were focused on men, however. The ATBC trial reported an increased risk of lung cancer (hazard ratio [HR] 1.18; 95% CI 1.03–1.36) and no effect on other cancer incidence with 20 mg β-carotene daily in this trial of 29,133 male smokers in Finland after 5–8 years of follow-up.[16] Shortly thereafter the US based CARET trial was stopped early, after a mean follow-up of approximately 4 years, based on the observation of an adverse effect of supplement use on lung cancer incidence (HR 1.28; 95% CI 1.04–1.57) in 18,314 male and female heavy smokers and asbestos-exposed men randomized to either 30 mg β-carotene plus 25,000 IU of retinol per day or placebo.[17] The PHS, which randomized 22,071 male physicians in the USA to 50 mg β-carotene every other day, reported no effect on lung cancer or other cancer incidence after an average 12 years of follow-up.[18] These results undermine the general antioxidant and cancer association but do not provide direct information on the effects of β-carotene on breast cancer.

The WHS randomized 39,876 women aged 45 and older to 50 mg β-carotene every other day or placebo in a factorial design that also tested aspirin and vitamin E.[8] The β-carotene arm was terminated early, after a median duration of exposure of 2.1 years, prompted mainly by the results of the preceding trials. Follow-up continued with the other trial interventions. With a median 4.1 years of follow-up, no effect of β-carotene was observed on total cancer incidence and specifically there was no difference in the number of reported breast cancer cases (Table 2.1).[19]

The WACS was a somewhat smaller trial that tested β-carotene as well as vitamins C and E in a 2×2×2 factorial design among women health professionals 40 years of age or older who were at increased risk of cardiovascular disease. The trial randomized 8,171 women to either 50 mg β-carotene every other day or placebo. In the subset of 7,627 who were cancer-free at baseline, no effect was seen for total cancer or breast cancer incidence after an average 9.4 years of follow-up (Table 2.1).[20]

Vitamin C

Two large scale prevention trials provide information on the role of vitamin C in cancer risk. In WACS, women were randomized to receive either 500 mg of ascorbic acid daily or a placebo. Vitamin C did not reduce the risk of breast cancer or total cancer (Table 2.1).[20] The only other large scale trial (PHS) tested this same dose in men and found a similar overall null finding for total cancer incidence (relative risk [RR] 1.01; 95% CI 0.92–1.10) after a mean 8.0 years of follow-up.[21]

Vitamin E

Vitamin E (or α-tocopherol), a potent antioxidant, has been tested in several prevention trials, primarily in men for its impact on incidence of lung cancer,[16] prostate cancer,[21,22] and cardiovascular disease.[23] None of these studies reported a significantly reduced risk of cancer. Both the WHS and the WACS randomized women to vitamin E (600 IU on alternate days) or placebo. In the WHS, there was no effect of vitamin E on breast cancer incidence or on total cancer incidence (Table 2.1) over an average 10.1 years of follow-up.[24] Similar results were found after the average 9.4 years of follow-up in the smaller WACS (Table 2.1).[19]

The HOPE trial and its extension, the HOPE-TOO trial, randomized 9,541 subjects with vascular disease or diabetes to 400 IU vitamin E per day or placebo. After a median follow-up of 7.0 years, no significant effect of supplements was observed on breast cancer or total cancer incidence (Table 2.1).[25]

Table 2.1 Findings from randomized, double-blind, placebo-controlled trials of micronutrients on breast and total cancer incidence in women

Nutrient(s)		Women randomized	Mean or median follow-up (yrs)	Cancer cases				
Study	Dose(s)			Site	Active	Placebo	RR	95% CI
β-Carotene								
WHS[19]	50 mg/2 days	39,816	4.1	Breast	169	168	NA	NA
				Total	378	369	1.03	0.89–1.18
WACS[20]	50 mg/2 days	8,171	9.4	Breast	129	128	1.01	0.79–1.30
				Total	311	313	1.00	0.85–1.17
Vitamin C								
WACS[20]	500 mg/day	8,171	9.4	Breast	135	122	1.11	0.87–1.41
				Total	329	295	1.11	0.95–1.30
Vitamin E								
WHS[24]	600 IU/2 days	39,876	10.1	Breast	616	614	1.00	0.90–1.12
				Total	1,437	1,428	1.01	0.94–1.08
HOPE[25]	400 IU/day	9,541	7	Breast	29	25	0.86	0.50–1.47
				Total	552	586	0.94	0.85–1.06
WACS[20]	600 IU/2 days	8,171	9.4	Breast	127	130	0.98	0.77–1.25
				Total	300	324	0.93	0.79–1.09
Antioxidant multivitamins								
SU.VI.MAX[29]	120 mg/day vit C 30 mg/day vit E 6 mg/day β-carotene 100 μg/day selenium 20 mg/day zinc	7,715	7.5	Breast	95	199	0.95	NA
				Total	179	171	1.04	0.85–1.29

Table 2.1 (continued)

Nutrient(s)				Cancer cases				
Study	Dose(s)	Women randomized	Mean or median follow-up (yrs)	Site	Active	Placebo	RR	95% CI
Folate and vitamin B								
WACS[34]	2.5 mg/day folic acid 50 mg/day vit B6 1 mg/day vit B12	5,442	7.3	Breast Total	70 187	84 192	0.83 0.97	0.60–1.14 0.79–1.18
Calcium and vitamin D								
WHI[39,41]	1,000 mg/day Ca 400 IU/day vit D	36,282	7.0	Breast Total	528 1,634	542 1,655	0.96 0.98	0.85–1.09 0.91–1.05
Lappe[42]	1,400 mg/day Ca 1,400 mg/day Ca^{+} 1,100 IU/day vit D	288 placebo 445 Ca 446 $Ca^{+}D$	4.0	Total	17 13	20	0.40 0.53	0.20–0.82 0.27–1.03

NA – Not available

Multiple Nutrient Supplements

In addition to these single nutrient trials, a few such efforts have tested the antioxidant hypothesis using micronutrient combinations. The first two trials were conducted in Linxian China, testing the effects of selected vitamins and minerals in the general population, where nutrient intake was generally low, and in a high risk population. Participants in the general population trial were randomized in a fractional factorial design to either placebo or one of seven arms, each with a different combination of vitamins and minerals including β-carotene, retinol, vitamin C, vitamin E, niacin, riboflavin, molybdenum, selenium and zinc daily for over 5 years.[26] No effect on cancer incidence or mortality was observed for any of these supplement combinations except those containing β-carotene, vitamin E and selenium, where a reduction in stomach cancer and total cancer mortality was observed, a difference that has persisted through 5 additional years of follow-up.[27,28] In the parallel trial in 3,318 participants with esophageal dysplasia, participants were randomized to placebo or a daily supplement containing 14 vitamins and 12 minerals. A reduction in rates of esophageal dysplasia for up to 6 years of follow-up was reported.[27]

The Supplementation en Vitamines et Mineraux Antioxydants trial (SU.VI.MAX) randomized 13,017 French adults, ages 45–60 years to placebo or a single daily capsule containing 120 mg vitamin C, 30 mg vitamin E, 6 mg of β-carotene, and 20 mg of zinc. After a median 7.5 years of follow-up, no effect of these supplements was found on total cancer incidence in the entire population (RR 0.90; 95% CI 0.76–1.06 in 562 cases). There was evidence of an interaction with sex ($p = 0.02$) however, suggesting that men experienced some benefit from the supplements but women did not (Table 2.1). The numbers of breast cancers observed among women in the two groups were similar (Table 2.1).[29]

The Heart Protection Study tested the antioxidant and cardiovascular disease hypothesis in 20,536 UK adults aged 40–80 with coronary disease or occlusive arterial disease or diabetes. Participants were randomized to daily supplementation with 250 mg vitamin C, 600 mg vitamin E and 20 mg β-carotene or a matching placebo. After 5 years of supplementation, there was no effect on total cancer incidence (RR 0.98; 95% CI 0.89–1.08 in 1,617 cases).[30] Neither breast cancer rates nor total cancer rates in women were reported.

Together these trials provide strong evidence that antioxidants, supplied through either single or multi-nutrient supplements, do not provide protection against breast cancer or cancer risk in general. Whether the discrepancy between the observational studies and these trials arises from the manner in which these vitamins were consumed, differences in duration, timing, dose, or lack of power to detect more modest effects in the trials or in residual confounding or systematic measurement error in the observational studies remains to be determined.

Vitamin B and Folate

Recent interest has turned to other micronutrients. Folate, methionine, riboflavin, and vitamins B-6 and B-12 are nutrients involved in one-carbon metabolism. Because grain products are routinely fortified with folic acid to help prevent neural tube defects, it is important to know the long-term effects of these supplements on other health conditions. Folate has been shown in case-control studies, but not prospective studies, to be associated with lower risk of breast cancer, with some evidence for a stronger benefit for women consuming moderate or high amounts of alcohol.[31] Further inconsistencies regarding the relationship with pre- and postmenopausal disease and with ER and PR status have been raised.[32,33]

The WACS was expanded to include a randomization to folic acid (2.5 mg/day), vitamin B6 (50 mg/day) and vitamin B12 (1 mg/day) vs placebo. In the 5,442 women aged 42 years and older

who were randomized, neither breast cancer incidence nor total cancer incidence rates were affected by supplements (Table 2.1).[34]

One smaller placebo-controlled trial of two doses of folate supplements in 2,928 pregnant women was conducted in the UK in 1966–1967. Using linkage to the National Health Service Central Registry to ascertain cause of death through 2002, a non-significant increased risk of breast cancer mortality was found with 0.2 mg/day (RR 1.56; 95% CI 0.38–3.41) and with 0.5 mg/day (RR 2.02; 95% CI 0.88–4.72) in 31 cases.[35] Limitations of this trial include the small sample size, limited duration of intervention, lack of data on breast cancer incidence and an unknown randomization scheme.

Calcium and Vitamin D

Another recent nutrient and cancer hypothesis is related to vitamin D and calcium levels. Serum vitamin D levels are influenced by exposure to sunlight, dietary supplements and dietary intake, primarily through fortified dairy products. Observational studies examining the relationship between breast cancer risk and dairy product consumption, use of supplements, and estimates of sunlight exposure through geographic residence and outdoor activity have found supportive evidence of a protective effect of vitamin D with some inconsistency in the effects before and after menopause.[36–38] Whether vitamin D or calcium or both are involved cannot be adequately determined from these studies.

The WHI randomized 36,202 postmenopausal women to either 1,000 mg of calcium combined with 400 IU of vitamin D daily or matching placebo in divided doses. The primary outcome for this trial was hip fracture incidence. With an average of 7 years of follow-up, no effect on breast cancer incidence was observed (HR 0.96; 95% CI 0.88–1.06 in 1,074 cases, Table 2.1),[39] nor was there an effect on benign proliferative breast disease[40] or total cancer incidence (Table 2.1).[41] Further, in a nested case-control study, baseline serum vitamin D levels were found to be correlated with total vitamin D intake, BMI and physical activity but were not associated with breast cancer risk after adjustment for BMI and physical activity, suggesting these other factors may be stronger predictors of risk than serum vitamin D levels.[39]

In a small single-center study conducted by Lappe and colleagues, 1,179 postmenopausal women were randomized to 1,400–1,500 mg supplemental calcium, supplemental calcium plus 1,100 IU vitamin D or placebo to test for effects on fracture rates. After 4 years of treatment, a significant difference was observed in total cancer incidence rates between groups, with a large but non-significant reduction found with calcium alone (RR 0.53, 95% CI 0.27–1.08) and a slightly larger reduction with the addition of vitamin D (RR 0.40; 95% CI 0.20–0.82).[42]

The WHI and Lappe trials differ in dose, duration, and study population. The Lappe study[42] supports the hypothesis that among women with relatively high baseline serum 25(OH)D levels (71.8 ± 20.3 nmol/L), a large daily dose of calcium and vitamin D may reduce cancer incidence but the estimates are imprecise. The WHI, with the large, national sample, and longer-term intervention provides a more complete assessment of effects of these supplements but at a dose of vitamin D considered by some to be ineffective. The adverse effect on kidney stones reported by the WHI[43] will need to be factored into any future trials looking at larger doses.

Medication Use

Hormone Therapy

The hypothesis that female hormones are associated with breast cancer risk developed out of the literature on reproductive factors (age at menarche, age at first birth, parity). The findings of higher

risk for women with earlier age at menarche, later age at first birth, and lower parity pointed to an adverse role of increasing levels of endogenous estrogens.

Menopausal hormone therapy (HT) was first approved by the US FDA to relieve menopausal symptoms in the early 1940s. Initially, the vast majority of hormone use was of a single preparation – conjugated equine estrogens (CEE). Its use grew over the next three decades with CEE being the main preparation used. In the mid-1970s, however, unopposed estrogens were shown to have a carcinogenic effect on the endometrium and its use rapidly declined. When the protective effect of progestin on the uterus was established in the mid-1980s, interest in HT was rekindled and guidelines for use were developed based on a stratified approach to care for women with menopausal symptoms: women who had had a hysterectomy were given estrogen alone and women with an intact uterus were either given a combination of estrogen and progestin or were given estrogen alone with frequent endometrial monitoring.[44–46] By the mid-1990s, approximately 90 million prescriptions were filled annually for 15 million women.[47]

Medroxyprogesterone acetate (MPA) has been the most commonly used progestin product though, again, many different forms of progestin are available.[47] Progestins have been prescribed in daily, cyclic, and sequential regimens at different doses in an attempt to balance endometrial safety, convenience and side effects.

The prevalence of HT use motivated a large number of observational studies examining its effects on chronic diseases. Early studies did not always distinguish between types of HT but generally captured women exposed to CEE. As the prevalence of progestin use increased, analyses began to examine unopposed estrogen and combined estrogen/progestin separately. Together these studies suggested an association between prolonged exposure to HT and an increased risk of breast cancer.[48] The analyses of progestin effects, somewhat limited in their interpretation because of the variability in progestin regimens and the shorter history of exposure, suggested a generally similar pattern of association.[48]

Between 1993 and 1998, the WHI enrolled 27,341 postmenopausal women aged 50–79 years of age from 40 US clinical centers into one of two hormone trials testing whether HT would reduce risk of coronary heart disease: 16,608 women with an intact uterus were randomized to combined estrogen plus progestin (CEE.625 mg/day + MPA 2.5 mg/day) vs placebo and 10,733 women with prior hysterectomy were randomized to estrogen alone (CEE.625 mg/day) vs placebo.[7]

Combination Estrogen/Progestin Therapy

The WHI estrogen plus progestin trial was stopped early, in 2002, based on the finding of an increased risk of breast cancer and an overall assessment of risks exceeding benefits.[49] The invasive breast cancer hazard ratio over the 5.6 year average follow-up was 1.24 (95% CI 1.01–1.54, weighted $p = 0.003$) (Table 2.2)[50] and was nearly identical to the breast cancer hazard ratio (HR:1.27; 95% CI 0.84–1.94) reported from the smaller Heart Estrogen/Progestin Replacement Study, a randomized secondary cardiovascular prevention trial in 2763 women that used the same combined hormone regimen.[51] Secondary analyses of the WHI trial suggested increasing risk with prior exposure, longer follow-up, and greater adherence to therapy.[49,50,52] There was no evidence of interactions with other breast cancer risk factors. Estrogen plus progestin increased the incidence rates for both ductal and lobular cancer as well as receptor positive and negative tumors. The risk of in situ disease was not significantly elevated (HR 1.18; 95% CI 0.77–1.82).[50] Estrogen plus progestin was also found to increase mammographic density[53] and the risk of benign proliferative breast disease (HR 1.74; 95% CI 1.35–2.25).[54]

Table 2.2 Results of the WHI randomized trials and observational study on estrogen plus progestin and estrogen alone on breast cancer incidence [60,61]

		Estrogen + Progestin		Estrogen-alone	
Study group		HR	95% CI	HR	95% CI
Clinical Trial (CT)		1.24	1.01–1.54	0.80	0.62–1.04
No prior HT use		1.13[a]		0.61[a]	
Prior HT use		1.86[a]		0.95[a]	
Observational Study (OS)					
No prior HT use		2.20[a]		1.09[a]	
Prior HT use		2.07[a]		1.11[a]	
Combined CT/OS[b]	Years since initiation of current HT				
No prior HT use	0–2	0.98	0.56–1.72	1.44	0.54–3.84
	2–5	2.01	1.41–2.86	1.15	0.57–2.32
	5+	2.85	2.29–3.54	1.00	0.54–1.84
Prior HT use	0–2	1.28	0.66–2.51	1.63	0.68–3.91
	2–5	2.56	1.54–4.24	0.82	0.42–1.57
	5+	3.30	1.90–5.73	0.91	0.49–1.69
5-year increase in gap time		0.81	0.71–0.91	0.85	0.73–0.98

[a]Age-adjusted incidence ratio.
[b]Based on multivariate models that controlled for age, body mass index, education, smoking, alcohol intake, general health, physical activity, family history of breast cancer, Gail model estimated risk of breast cancer, bilateral oophorectomy and for women with prior HT, duration of prior HT use (not including the current episode). These models restricted the CT and OS to have the same HT effects up to a factor estimated as 1.30 (95% CI 0.69–1.53) for estrogen + progestin and 1.07 (95% CI 0.60–1.93) for Estrogen alone.

Results of the WHI trial suggested that estrogen plus progestin treatment delays diagnosis. Tumors in women assigned to combined hormones were larger and at somewhat more advanced stage than those in the placebo group. Even though there was increased risk after 5 years, breast cancer incidence rates were non-significantly lower in the estrogen plus progestin group for the first 2 years of follow-up.[50] The active and placebo groups had comparable mammography rates throughout since these were required by protocol, but the frequency of abnormal mammograms and of biopsies was greater in the active hormone group.[55] Together these results suggest that estrogen plus progestin serves as an active agent in postmenopausal breast tissue to promote carcinogenesis and reduces the sensitivity and specificity of mammography, leading to delayed diagnoses as well as a higher rate of unnecessary biopsies.[55]

Estrogen Alone

The effect of unopposed estrogen on breast cancer risk is less clear. The WHI estrogen alone trial was also stopped early, after an average of 6.8 years of follow-up, based on an increased risk of stroke.[56] The hazard ratio for breast cancer over this interval was 0.80 (95% CI 0.62–1.04) (Table 2.2), narrowly missing statistical significance for a protective effect but ruling out an increased risk in this population.[57] Secondary analyses found statistically significant interactions with family history of breast cancer, history of benign breast disease, as well as a summary risk estimate based on the

modified Gail model, each suggesting some degree of protection among women at lower risk and an elevated hazard ratio among women with the established risk factor. The apparent protective effect was observed only in localized disease and ductal carcinoma; there was no evidence of a reduced risk in more advanced disease or lobular tumors. Hazard ratios for both receptor positive and negative disease were less than one. Women in the estrogen alone arm had slightly larger tumors than those in the placebo arm and were more likely to have positive lymph nodes.[57] Additional analyses showed that estrogen alone increased the risk of mammograms requiring early recall[57] and of benign proliferative disease.[58]

Further Analyses of Hormone Therapy Effects

The WHI trial results apply to the two HT regimens tested, yet many others are available. Since it is unlikely this effort will be repeated for other forms of HT, observational studies are needed to expand the inference beyond the specific interventions tested. The Million Women Study (MWS), the largest study of breast cancer conducted to date, examined the relationship between current and past use of HT and found that among 1,084,110 UK women aged 50–64 years, current users of combination hormones were twice as likely as non-users to develop breast cancer (relative risk [RR] 2.0; 95% CI 1.88–2.12). Current users of estrogen alone also had elevated risks relative to non-users but lower than estrogen plus progestin (RR 1.30, 95% CI 1.22–1.38). There was no evidence of variation in these estimated risks with type of estrogen (equine estrogens or estradiol), type of progestin (MPA, Norethisterone, or Norgestrel/levonorgesterel), regimen (sequential vs continuous) or formulation (oral, transdermal, or implanted estrogens). Both types of hormones showed increasing risks with duration of exposure but past users exhibited no elevation in risk relative to never users. In addition to the large sample size which afforded very precise estimates of risk, and the usual control for confounding, this study was able to address concerns of differential breast screening by enrolling women only through the United Kingdom's National Health Service Breast Screening Programme, assuring comparable breast screening among both HT users and non-users.[59]

The difference between the randomized trials and observational data with regard to the magnitude of effect associated with combined hormones and the direction of effect for estrogen alone is noteworthy. Most of the hormone use captured in the observational studies was of the same agents and doses tested in the WHI so differences in hormone preparations cannot explain these discrepancies. Changes in screening practices over time might explain the variation between the motivating studies and WHI, but the Million Women Study effectively controlled for screening practices. Other possible explanations are residual confounding or selection bias and differences in other aspects of exposure such as duration and timing.

To understand these differences, Prentice and colleagues conducted a series of analyses of pooled data from the WHI trials and the parallel WHI observational study (OS) of nearly 94,000 women.[60,61] For each trial, a corresponding sample was defined consisting of women in the observational study that were in general eligible for that trial but refused randomization. Specifically, for the cohort looking at estrogen plus progestin, women in the observational study were included if they had a uterus at entry, had a mammogram in the last 2 years and were either taking combined estrogen plus progestin therapy or were not taking any HT. For estrogen alone analyses, the observational study participants selected reported a prior hysterectomy and a mammogram in the past 2 years and used either unopposed estrogen or no HT. The trials and observational study were conducted in parallel in the same clinical centers with almost identical methods thereby facilitating data pooling. The observational study cohorts were analyzed as if they were trials – women were placed in the HT user or non-user groups depending on their reported use at baseline.

In simple age-adjusted analyses, the WHI observational study estimates of effects of hormones better aligned with MWS and other observational studies than with the WHI trials (Table 2.2). Adjusting for other confounders, and for time dependent effects reduced the discrepancies with the trials but important differences remained.[60,61] The identification of prior hormone use as an effect modifier[50,52] led to modeling of the so-called "gap-time," the interval between cessation of menses and first use of hormone therapy. By modeling gap time as well as prior hormone therapy and time since initiation of therapy (i.e., randomization for trial participants), the estimates from each trial and the corresponding observational study cohort were brought into excellent alignment.[60,61]

The resultant pooled analyses indicate that, among women who started HT at the time of menopause (gap time = 0) the risk of breast cancer associated with estrogen plus progestin therapy doubled after 2 years of exposure with some variation in level of risk by prior hormone use (Table 2.2). A delay in hormone initiation, i.e., an increase in gap time, would reduce these risks by an estimated 19% per 5-year increment.[60]

In analyses of estrogen alone the three time variables were again important. For women who started therapy at the time of menopause (gap time = 0), there is no clear evidence of a reduced risk of breast cancer; any reduction seems limited to those women with large gap times as each 5-year delay is associated with an estimated 15% reduction in risk (Table 2.2).[61]

The effect of these findings on breast cancer rates has been documented in multiple populations. In the first report from the US using SEER data, an 8.6% (95% CI 6.8–10.4) reduction in annual breast cancer incidence was reported for 2004 relative to 2001[62] that paralleled the substantial drop in hormone therapy prescriptions which occurred when the first WHI results were released.[47] Limitations of this study included the inability to link hormone use to cancer incidence on an individual basis or to control for screening mammography use. Subsequent reports from Australia,[63] Scandinavia,[64] and France[65] reported similar declines in incidence rates with changing HT use on the population level but not all analyses agreed,[66,67] raising the possibility that the decline in rates may be attributable to other factors, including change in use of screening mammography. Similar declines in cancer rates reported in a screening population lent support to the HT hypothesis.[68]

Further information from the WHI trials and observational study provides clear support for this finding. In the 3 years after WHI trial participants were asked to stop their study hormones, the breast cancer risk in women originally randomized to estrogen/progestin therapy remained elevated (HR 1.26; 95% CI 1.02–1.55),[69] suggesting some carry-over effect. More detailed analyses of the rates over time indicated that within 5 years of randomization to estrogen plus progestin the breast cancer rates nearly doubled, but within the 3 years after cessation of therapy, the adverse effect became non-significant (Fig. 2.2).[70] The parallel cohort of WHI observational study participants who used combined hormones at baseline also discontinued their use of hormones, similar to the general population; 75% of baseline users were still users in 2001 but only 41% reported HT use in 2003. Modeling the effect of hormone use over calendar time in this population with multivariate adjustment for potential confounders produced a hazard ratio function that was relatively constant over time at approximately 2.0 prior to 2001 and then dropped rapidly between 2001 and 2003 to a level not significantly greater than 1.0 (Fig. 2.3).[70]

The totality of the WHI data indicate that estrogen plus progestin increases breast cancer risk, with greater risk with longer exposure duration reaching a twofold increase after 5 years, with higher risk in women who initiate use soon after menopause. The increased risk appears to dissipate within approximately 3 years of cessation of therapy. Estrogen alone, however, does not have a clear effect on breast cancer risk in women initiating HT soon after menopause but may reduce risk among women starting HT at later ages. The contrast between these two trials suggests that MPA, the form of progestin used in WHI trials, may be the potent agent.

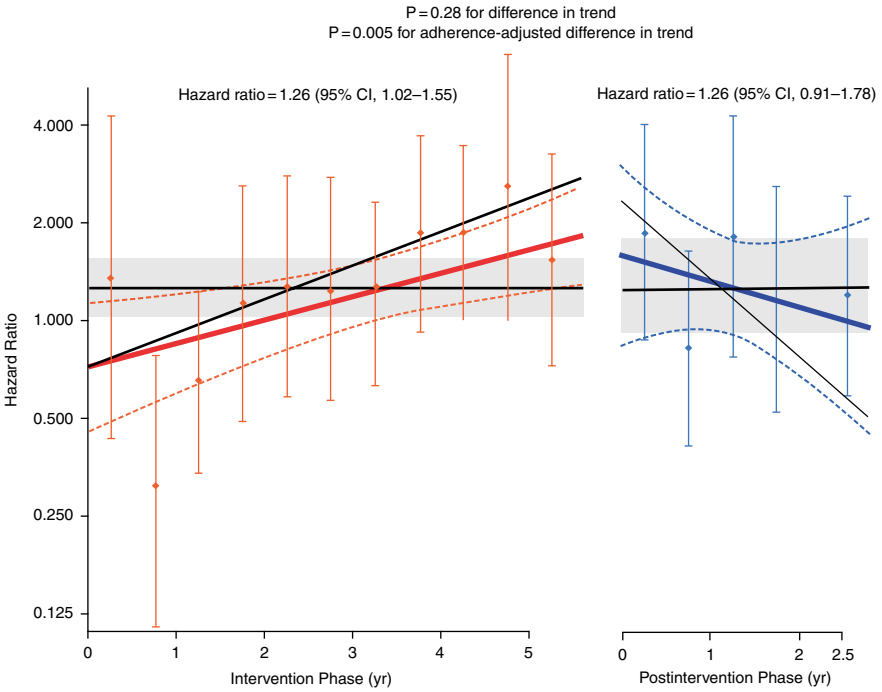

Fig. 2.2 Effects over time of estrogen plus progestin on the incidence of breast cancer in the WHI clinical trial (Reprinted with permission from Chlebowski et al.[70] Copyright © 2009 Massachusetts Medical Society. All rights reserved). Time-varying linear hazard ratios and 95% Cls (thick solid and dashed lines, respectively) are shown for the effect of conjugated equine estrogens plus medroxyprogesterone acetate on the risk of breast cancer as compared with placebo during the intervention and postintervention phases of the study. The shaded areas indicate the 95% Cls for the hazard ratios in the intervention and postintervention phases. The I bars show hazard ratios and 95% Cls according to an analysis based on events accumulated at 6-month intervals. The P value of 0.28 for a difference in trend is for the comparison of the hazard-ratio slopes in the two study phases in the primary, unadjusted analysis, and the P value of 0.005 is for a difference in trend from an analysis adjusted for adherence status, with censoring of events that occurred 6 months after a woman became nonadherent (defined as consuming < 80% of study pills or starting hormone therapy). The thin solid lines show the adherence-adjusted, time-varying linear hazard ratios

Aspirin and NSAIDS and Other Anti-inflammatory Medicine

The potential anti-carcinogenic effect of aspirin and non-steroidal anti-inflammatory medicines is based on their inhibition of the Cox-1 and Cox-2 pathways.[71] Observational studies have reported generally consistent modest risk reductions with aspirin use and to a lesser extent other nonsteroidal anti-inflammatory medications.[72]

To date, only one large scale trial has tested this hypothesis. The WHS randomized 39,876 women to low-dose aspirin (100 mg every other day) or a placebo between 1992 and 1996 and followed them until the planned termination in 2004. With an average of 10 years of follow-up, the overall invasive breast cancer HR was 0.98 (95% CI 0.87–1.09). No evidence was found for a differential effect by histology, grade, receptor status, tumor size or stage.[73]

Discussion

Large scale randomized trials examining lifestyle factors aimed at reducing the incidence of breast cancer or other chronic diseases have provided important answers to critical public health

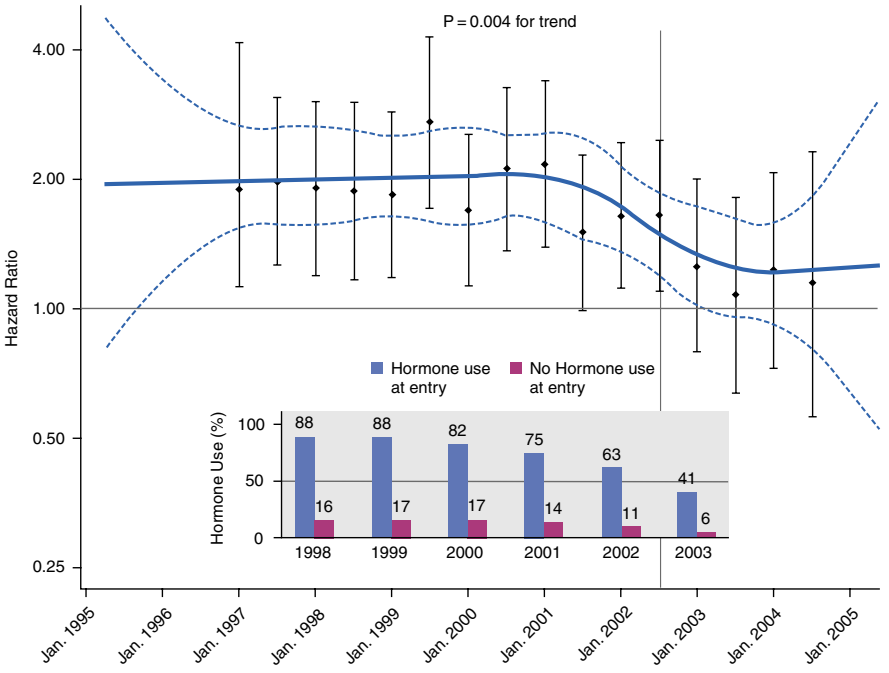

Fig. 2.3 Effects over time of estrogen plus progestin on the risk of breast cancer in the WHI observational study (Reprinted with permission from Chlebowski et al.[70] Copyright © 2009 Massachusetts Medical Society. All rights reserved). Smoothed time-varying, multivariable-adjusted hazard ratios and 95% confidence intervals (solid and dashed blue lines, respectively) for the comparison of participants who were taking estrogen plus progestin at study entry with those who were not are shown with the corresponding multivariable-adjusted hazard ratios and 95% confidence intervals from an analysis based on accumulated events at 6-month intervals (I bars). The vertical line indicates the announcement of the results of the clinical trial in July 2002. The bar graph shows the year-to-year percentages of participants who were taking hormones and those who were not

questions but have provided few clear choices women can make to reduce their risk of breast cancer.

The most definitive finding of these trials is the increase in breast cancer risk found with use of estrogen plus progestin therapy.[50] As demonstrated by both the follow-up after cessation of the intervention in the WHI trial[70] and in multiple other population-based studies,[62–65,68] reduction in estrogen plus progestin use resulted in a rapid and noteworthy decline in breast cancer rates.

The effects of estrogen alone on breast cancer risk are less clear. The suggestion of an overall protective effect of estrogen alone in the trial[57] may not apply to the average woman considering hormone use near the time of menopause.[61] Given the serious adverse health effects of estrogen on risk of stroke, blood clots,[56] and cognitive impairment in older women,[74] estrogen is an unacceptable breast cancer prevention approach for most women.

The preventive role of diet in breast cancer remains one of the more interesting and important areas for further study. Although the WHI low-fat diet trial did not provide definitive evidence on the fat and breast cancer incidence hypotheses, the totality of the evidence supports a modest risk reduction.[15] As a lifestyle change that is low in cost, without any serious adverse effects and therefore suitable for the majority of the population without close medical monitoring, it remains one of the more viable methods to address the public health question. Additional efforts are warranted to clarify this issue.

The multiple, large, high-quality randomized trials of dietary supplements[19,20,24,25,29,30,34,39] provide strong evidence that these agents, as delivered, have no appreciable effect either positive or negative, on breast cancer risk. While the lack of an adverse effect is reassuring given the high prevalence of use, the lack of benefit means there are few easy choices to effectively modify breast cancer risk.

Other aspects of diet deserve further consideration. To date, fruit and vegetable consumption, associated with cancer risk in observational studies, has been simplified into a limited set of micronutrients for testing in randomized trials. These trials provided rigorous tests of the specific supplement preparations in an easily implemented and monitored intervention but they differ from the motivating studies in the manner, form and timeframe in which the nutrients were consumed. Whether the reductionism employed in designing these trials resulted in loss of the critical risk modifying factor or other methodological factors confounded these results has not yet been determined.

The lack of an effect of low-dose aspirin on cancer risk reported by one trial, while disappointing, provides the important information that this very commonly used medication has no adverse effects on cancer risk but casts some doubt on the underlying hypothesis regarding the role of Cox-1/2 inhibitors. Further testing of other targeted agents may be warranted if safety concerns, such as those that have plagued other Cox-2 inhibitors[75] can be alleviated.

In addition to these important findings, these prevention trials provide an important cautionary reminder of the inference that can be derived from observational studies of lifestyle factors and cancer risk. The discrepancies observed between these two study designs are not easily resolved. Methodological limitations of each are generally recognized but in the context of lifestyle, the understanding of the issues may be critical in giving each their appropriate weight. Measurement problems and confounding of observational studies can be substantial. Limitations in clinical trials include timing and duration of the intervention. Additional efforts such as those pursued by Prentice and colleagues[60,61] and others[76] to analyze observational study data using the approach employed for clinical trial analyses provide some perspective on methodologic factors that may explain these differences. In addition, conducting more parallel observational studies and randomized trials may be useful to expand the inference that can be gained regarding a class of interventions when only one can be tested in a trial.

What do these trials imply for the role of other lifestyle factors on cancer risk? Current evidence regarding the relationship between breast cancer and lifestyle choices, such as alcohol use, smoking, physical activity, weight control/reduction, use of oral contraceptives, pregnancy and lactation is derived primarily from association studies with some supported by pre-clinical experiments. Some of these factors are not amenable to testing in randomized trials and, for these, the inference must be based on evidence accumulated from multiple sources and study designs. For others, such as physical activity, the lessons drawn from these randomized trials suggest that a full-scale randomized trial testing the effects of an exercise intervention on cancer incidence is needed to determine accurately and adequately the full range of potential health effects.

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Chapter 3
Breast Cancer Chemoprevention

Mary B. Daly

Abstract A significant amount of evidence has accumulated from randomized clinical trials supporting the use of pharmacologic agents for breast cancer risk reduction. All of these trials have capitalized on the known expression of estrogen receptors on many breast cancer cells, and the demonstrated efficacy of selective estrogen receptor modulators (SERMs) to treat breast cancer. Two SERMs, tamoxifen and raloxifene, have been studied extensively in randomized, controlled trials, and both have been shown to reduce the risk of invasive ER-positive breast cancer by approximately 50% in women at increased risk. Other benefits that have been documented are a decrease in fractures for both drugs, and a decrease in noninvasive breast cancer and benign breast disease for tamoxifen. The risk of venous thromboembolic events and vasomotor symptoms is increased for both drugs. In addition, tamoxifen is associated with a twofold increase in endometrial cancer, and a modest increase in cataracts and cataract surgery. Studies are underway to explore the role of aromatase inhibitors for breast cancer risk reduction. In addition, several novel pharmacologic and natural compounds are being considered for future trials.

Keywords Chemoprevention · Tamoxifen · Raloxifene · Biomarkers

Key Issues

- Extensive studies of both tamoxifen and raloxifene in the preventive setting have shown an approximate 50% reduction in the risk of invasive breast cancer. The risk reduction is confined to ER-positive tumors and is highest in women with a pre-existing history of atypical ductal hyperplasia or lobular carcinoma in situ.
- Although most of the trials conducted to date have used a 4- to 5-year treatment schedule, there is considerable data that the protective effect may persist for several years after the completion of active treatment.
- There is a two- to threefold increase in the risk of endometrial cancer and venous thrombotic events (VTEs) associated with 5 years of tamoxifen treatment. The incidence of cataracts and cataract surgery were both increased in women taking tamoxifen. Raloxifene is also associated

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with an increase in VTEs, but appears to spare the endometrium, and is not associated with cataract risk. Both drugs significantly increase the risk of vasomotor symptoms.
- Both tamoxifen and raloxifene have a beneficial effect on bone mineral density.
- Unresolved issues include the optimal duration of active treatment, the optimal age to initiate treatment, the role of genetic polymorphisms which may alter response to treatment, the role of tamoxifen or raloxifene in women with a hereditary risk of breast cancer, and the under-utilization of these agents by eligible women, particularly minority women.
- Other classes of agents, which target both ER-positive and ER-negative breast cancer, are being investigated. These include both pharmacologic and natural products. Efforts to identify accurate biomarkers of both risk and response to therapy are underway. The concept of using combination chemoprevention agents is also gaining support.

Introduction

A significant amount of evidence has accumulated from randomized clinical trials supporting the use of pharmacologic agents for breast cancer risk reduction. All of these trials have capitalized on the known expression of estrogen receptors on many breast cancer cells and the demonstrated efficacy of selective estrogen receptor modulators (SERMs) to treat breast cancer. The first of these agents, tamoxifen, a nonsteroidal antiestrogen, was introduced as a treatment for advanced breast cancer in the early 1970s. The knowledge gained with tamoxifen over the years in the treatment setting ultimately led to its testing as a preventive agent, and set the stage for the evolving science of cancer chemoprevention. This chapter will describe the findings of the major breast cancer chemoprevention trials and will suggest opportunities for future research.

Tamoxifen Trials

First introduced as a reproductive contraceptive, tamoxifen was found in the laboratory to block the binding of estradiol to human and rat mammary tumor estrogen receptors (ERs) and to prevent the growth of ER-positive mammary carcinomas. Subsequent clinical studies demonstrated the efficacy of tamoxifen in both the metastatic and adjuvant setting in improving disease-free and overall survival among women with ER positive breast cancer.[1] Experience with the drug over the past 30 years has clarified its risk-benefit ratio. In addition to its effect on ER positive breast cancer, tamoxifen reduces both the risk of subsequent contralateral breast cancers and the loss of bone mineral density. However, long-term tamoxifen treatment was also associated with an increased risk of endometrial cancer and of venous thromboembolism (VTE).[2] These observations of both estrogenic and antiestrogenic actions are now understood to result from the action of tissue-specific modulating effects which can determine the action of tamoxifen at a specific site.[3] Overall, the favorable risk/benefit ratio of tamoxifen led to several randomized trials which have established its role in significantly reducing breast cancer across a variety of risk groups.

Royal Marsden

The Royal Marsden Tamoxifen Breast Cancer Prevention Trial was a feasibility trial to demonstrate the ability to recruit and retain healthy pre- and postmenopausal women in a breast cancer prevention trial and to determine the safety profile of the drug in this setting. Healthy women aged

30–70 years were eligible on the basis of a family history of breast cancer. A total of 2,494 women were randomized to tamoxifen, 20 mg/day, or placebo for a total of 8 years. After demonstration of satisfactory accrual, compliance, and toxicity, the women continued to be followed in a blinded fashion for breast cancer incidence outcomes. After a median follow-up of 13 years, there was a statistically significant 39% reduction in ER positive invasive breast cancer in the tamoxifen treated arm. Of note, the risk reduction was greater in the post-treatment time period than in the 8-year treatment period, suggesting a long-term, cumulative effect of tamoxifen. The risk reduction was similar in pre- and postmenopausal women, in those who used hormone replacement therapy (HRT) and those who did not, and across strength of the family history. Gynecologic toxicity (vasomotor symptoms and vaginal discharge) was the most prominent adverse event.[4]

The National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention Trial (BCPT)

The largest randomized, double blind chemoprevention trial of tamoxifen was the NSABP BCPT begun in 1992. Eligibility criteria were based on level of risk and included: (1) age 60 years or older; (2) a history of lobular carcinoma in situ (LCIS); or (3) a 5-year predicted risk of breast cancer of 1.67% or greater, as determined by the Gail Model. The Gail Model is a multivariable logistic regression model which includes age, number of first-degree relatives with breast cancer, nulliparity or age at first birth, age at menarche, and number of breast biopsies.[5] The model was later modified to include race as a variable.[6] Altogether, 13,388 women aged 35–79 years were randomized to either tamoxifen at 20 mg/day (6,681 participants) or placebo (6,707 participants) for a total of 5 years.

The study was unblinded in March, 1998 when an independent data monitoring committee concluded that the primary end point of the trial had been attained and that tamoxifen significantly reduced the incidence of invasive breast cancer by 49% ($P > 0.00001$). With a median follow-up of 5.9 years, the risk reduction for noninvasive breast cancer was 50% ($P > 0.002$). Tamoxifen was effective in reducing the risk of breast cancer for all categories of age, predicted levels of risk, and strength of family history, but was confined to women with ER-positive breast cancer in whom the risk reduction was 69%.[7] The degree of risk reduction was highest for women with atypical hyperplasia and LCIS. The only significantly different quality of life outcomes between the two arms were more frequent hot flashes and vaginal discharge seen in the tamoxifen arm. There were no differences in depression, physical functioning, or sexual functioning.[8]

An updated report with 7 years of follow-up found a persistent 43% reduction in the risk of invasive breast cancer, and a 37% risk reduction in non-invasive breast cancer in the tamoxifen arm (See Fig. 3.1).[2] The risk for benign breast disease, including adenosis, fibrocystic disease, fibroadenoma, fibrosis, hyperplasia, and metaplasia, was also reduced by 28%, resulting in a 29% reduction in the number of breast biopsies in the tamoxifen arm. The reduction in benign breast disease was primarily observed among women aged 50 years or less.[9] An additional benefit seen in the tamoxifen arm was a 32% reduction in hip, spine and radius fractures.

Adverse events included a threefold increase in the risk of invasive endometrial cancer, a twofold increase in the risk of VTEs, and a modest but marginally significant increase in cataracts and cataract surgery in the tamoxifen arm (RR. 1.14). The increased risk of endometrial cancer was confined to women aged 50 years and greater, and 98% of the cancers were FIGO Stage I. No significant differences were seen in other cancers or in ischemic heart disease.[2] In addition to demonstrating a clinically significant reduction in the risk of invasive and non-invasive breast cancer in the tamoxifen

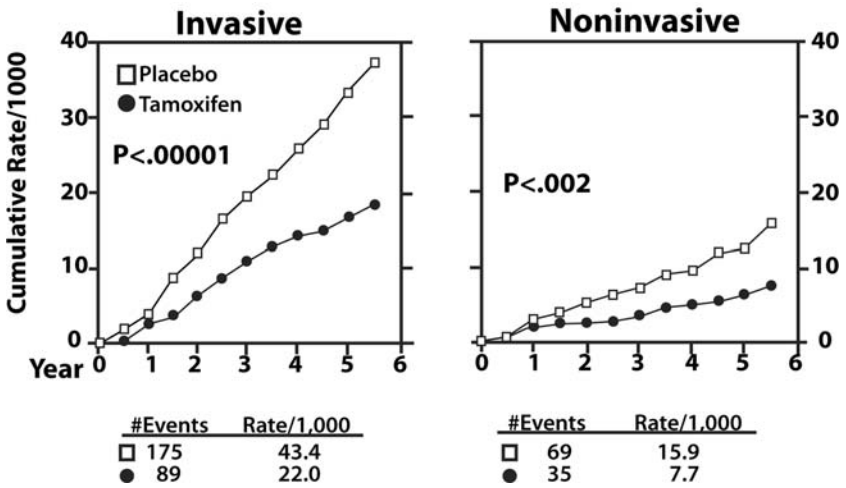

Fig. 3.1 Cumulative rates of invasive and noninvasive breast cancer occurring in participants receiving placebo or Tamoxifen. The *P* values are two-sided (From Vogel et al.[24] Reprinted with permission from the Oxford University Press)

arm, the BCPT provided evidence that tamoxifen could alter the course of pre-clinical pathologic changes in breast epithelium.

To synthesize the risks and benefits of tamoxifen for clinical decision making, Gail et al.[6] conducted a quantitative analysis of the major risks (endometrial cancer, stroke, pulmonary embolism, and deep vein thrombosis) and the major benefits (reduction of breast cancer and fractures). Relative risk estimates identified younger women and women over age 50 who have had a hysterectomy as deriving the most benefit from tamoxifen. The risk/benefit ratio was less clear among postmenopausal women, women who have not had a hysterectomy, and women without a history of LCIS.

The First International Breast Intervention Study – I (IBIS-I)

IBIS-I, a double-blind randomized trial of tamoxifen vs placebo, similar in design to the BCPT, was conducted in the United Kingdom between 1992 and 2001.[10] Eligible women were aged 35–70 years with risk factors indicating at least a twofold relative risk for women aged 45–70, a fourfold relative risk for women aged 40–44, and a tenfold relative risk for women aged 35–39. Factors determining risk were family history of breast cancer, nulliparity, and a history of atypical hyperplasia or LCIS. The use of HRT was permitted during the trial for the control of menopausal symptoms. A total of 7,145 women were randomized (3,570 in the tamoxifen arm and 3,575 in the placebo arm).

At a median follow-up of 95.6 months, the incidence of invasive and noninvasive breast cancers combined was 27% lower in the tamoxifen arm compared to the placebo arm. The risk of ER-positive invasive breast cancer was 34% lower in the tamoxifen arm. Consistent with findings from the BCPT, there was no reduction in ER-negative breast cancer. The benefit of tamoxifen was constant over time and persisted for at least 10 years suggesting a long-term alteration of cellular events. In this trial, no clear effect of tamoxifen was seen among women who used HRT during the trial. There was a 1.5-fold increase in endometrial cancer in the tamoxifen arm, and a twofold excess of VTEs. The

majority of endometrial cancers were FIGO Stage I and were observed in women aged 50 years and older. Both the endometrial cancers and the VTEs were seen only during the active treatment phase. A nested case-control analysis found that recent surgery, immobilization or fracture also contributed to the risk of VTE, suggesting that tamoxifen be withheld from women in those circumstances. Factor V Leiden and other prothrombin mutations were not associated with VTE.[11] Gynecologic and vasomotor symptoms were significantly more common in the tamoxifen arm, while breast tenderness was seen more frequently in the placebo arm. In this trial, HRT was not effective in the relief of hot flashes among women in the tamoxifen arm.[12]

The Italian Randomized Trial of Tamoxifen

A double-blinded, placebo controlled trial of tamoxifen was undertaken in Italy between 1992 and 1997. A total of 5,408 women, unselected for risk of breast cancer, all of whom had undergone hysterectomy, were randomized to 20 mg of tamoxifen (2,700 women) or placebo (2,708 women) for a total of 5 years. The estimated risk for breast cancer was considered to be somewhat lower in this group than that of the general population, because close to half had undergone bilateral oophorectomy. Initial findings failed to show a benefit of tamoxifen in reducing breast cancer risk.[13] Using baseline characteristics of the study participants, a group of women at high risk of ER-positive tumors was defined. The factors chosen which contribute to risk of ER-positive tumors were height greater than 160 cm, early age at menarche, nulliparity up to age 24 years, and the presence of at least one functioning ovary. Analyses restricted to women defined by their baseline risk estimation showed a significant risk reduction of 76% among those women in the high-risk group who were assigned to the tamoxifen arm. Similar to the other studies, there was an excess of hot flashes, vaginal discharge, and VTEs in the tamoxifen arm during the active intervention period.[14] In this study, conventional risk factors for atherosclerosis, namely older age, increased body mass index, hypertension, hypercholesterolemia, smoking, and a family history for coronary heart disease, contributed to the increased risk of VTE among tamoxifen users.[15]

A subset of women in the Royal Marsden and the IBIS-1 trials were recruited into an ancillary study to evaluate the psychosocial impact of taking tamoxifen. Standardized psychological measures were used to assess anxiety, emotional distress, sexual functioning, and symptom distress. Although scores varied considerably over time, changes in anxiety, mood, and sexual functioning were not related to treatment group.[16]

Raloxifene Trials

Raloxifene hydrochloride is a SERM which binds to estrogen receptors and blocks estrogen induced DNA transcription in the breast and endometrium. First evaluated for the treatment and prevention of osteoporotic fractures, raloxifene was also observed to reduce the rate of primary breast cancers among postmenopausal women. Four studies have evaluated the effect of this drug on multiple outcomes.

The Multiple Outcomes of Raloxifene Evaluation (MORE) Study

The MORE study was initiated in 1994 to study the effect of raloxifene on bone mineral density and osteoporotic fractures.[17] A total of 7,705 postmenopausal women with a diagnosis of osteoporosis,

as defined by radiographically evident vertebral fractures or a bone mineral density T score less than –2.5, were randomized to one of two doses of raloxifene (60 or 120 mg) or placebo for 3 years. All women in the study also received daily supplements of calcium (500 mg) and cholecalciferol (400–600 IU).

At 36 months of follow-up, there were significantly fewer new vertebral fractures among women in both raloxifene arms, 2.3% for those assigned to 60 mg/day, and 2.8% for those assigned to 120 mg/day, compared to 4.5% for women in the placebo arm. The risk for non-vertebral fractures did not differ significantly. Bone mineral density increased by 2.1 and 2.6% in the femoral neck and spine in the 60 mg/day group, and by 2.45 and 2.7% in the 120 mg/day group, while no improvement was seen in the placebo group. There was a threefold increase in VTEs in the raloxifene arms.

Although not powered to detect a significant difference in cancer outcomes, the MORE trial reported a 76% reduction in breast cancer risk in the raloxifene arms. This benefit was specific to ER-positive breast tumors. No effect of raloxifene was seen on the risk of endometrial cancer.[18] Raloxifene therapy did not significantly affect the risk of cardiovascular events in the MORE trial. However, among the subset of women with cardiovascular risk factors (including prior MI, prior percutaneous coronary intervention or CABG, the presence of diabetes, hypertension or hyperlipidemia, current smoking, and/or age 65 years or greater) at baseline, raloxifene treatment was associated with a 40% reduction in the risk of cardiovascular events.[19]

The Continuing Outcomes Relevant to Evista (CORE) Trial

Based on the reduction in risk of breast cancer observed in the MORE trial, the CORE trial was designed to evaluate the benefit of an additional 4 years of raloxifene therapy (at 60 mg/day) in women enrolled in the original trial.[20] All MORE trial participants randomized to raloxifene or placebo who had not developed a hormone-related malignancy were eligible for the CORE trial. A total of 4,011 women agreed to participate in the CORE trial. They were not re-randomized but remained in their MORE trial treatment assignment and remained blinded to treatment arm. Because the 60 mg/day and the 120 mg/day doses were similar in breast cancer risk reduction in the MORE trial, the investigators chose to use only the 60 mg/day dose for the CORE trial.

During the 4 years of the CORE trial, there was a 59% reduction in the incidence of invasive breast cancer, and a 66% reduction in the incidence of ER-positive invasive breast cancer. The incidence of invasive ER-negative breast cancer was similar in both arms. When data from the MORE and CORE trials were combined, there was an overall 66% reduction in the incidence of invasive breast cancer, and a 76% reduction in the incidence of invasive ER-positive breast cancer. Unlike the data for tamoxifen, there was no significant difference in the incidence of noninvasive breast cancer in the two treatment groups.[20] The increased risk for hot flashes and leg cramps seen in the MORE trial did not persist during the additional 4 years of the CORE trial. A twofold increase in VTEs, however, did persist. The risk reduction observed in the raloxifene-treated arm was present across all subsets of risk as defined by age, age at menopause, body mass index (BMI), serum estradiol level, prior estrogen therapy, family history of breast cancer and bone mineral density at baseline.[21]

The Raloxifene Use for the Heart (RUTH) Trial

Coincident with the MORE/CORE trials, an international multicenter randomized, double-blind placebo-controlled trial, the RUTH trial, was initiated to evaluate the effect of 60 mg/day of

raloxifene on cardiovascular outcomes and invasive breast cancer. A total of 10,101 postmenopausal women from 26 countries, who had either coronary heart disease (CHD) or CHD risk factors, were randomized to 60 mg/day of raloxifene or placebo and followed for a median of 5.6 years. Of the women in the RUTH trial, 35% had a 5-year Gail model score of $\geq$1.66%.[22]

Compared with placebo, raloxifene had no significant effect on the incidence or death from coronary causes, nonfatal myocardial infarction, or hospitalization for any acute coronary syndrome. The risks for fatal stroke and VTEs were significantly increased in the raloxifene arm. Consistent with other trials, raloxifene reduced the incidence of invasive breast cancer by 44%, with the benefit confined to the ER-positive invasive breast cancers.[23]

The Study of Tamoxifen and Raloxifene (STAR)

Given the encouraging results of the MORE and CORE trials, the STAR trial was launched by the NSABP to directly compare tamoxifen with raloxifene among women at increased risk for breast cancer.[24] Eligible women were those with a 5-year Gail model score of $\geq$1.66%, age 35 or greater, postmenopausal, and not taking any hormonal agents for at least the previous 3 months. A total of 19,747 were randomized to tamoxifen, 20 mg/day, or raloxifene, 60 mg/day, in a double-blind design, for a maximum of 5 years.

At a median follow-up of 3.9 years, there was no significant difference in the incidence of invasive breast cancer between the two treatment groups. In contrast, there were fewer cases of noninvasive breast cancer in the tamoxifen group (See Fig. 3.2).[24] The majority (54%) were ductal carcinoma in situ (DCIS), 36% were LCIS, and the remainder were mixed type. Although the difference did not reach statistical significance ($P = 0.052$), this finding was consistent with findings from the MORE, CORE, and BCPT trials.

The incidence of endometrial cancer was lower in the raloxifene arm, although the difference did not achieve statistical significance (See Fig. 3.3).[24] There was, however, a statistically significant reduction in cases of endometrial hyperplasia in the raloxifene arm, and significantly fewer women in the raloxifene arm underwent hysterectomy for any reason. Consistent with the previous trials, the majority of endometrial cancers diagnosed during the trial were found in women over the age of 50 and were primarily Stage I.

The risk for VTEs was 30% lower in the raloxifene arm (See Fig. 3.3),[24] although the risk for stroke was identical to that of the tamoxifen arm.[25] Hip, spine and Colles fractures of the wrist

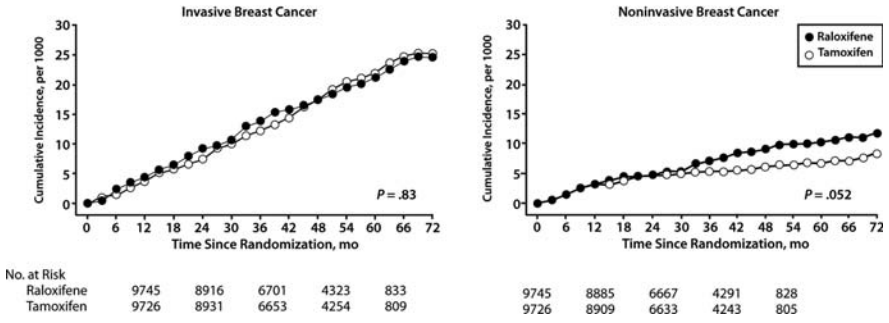

Fig. 3.2 Cumulative incidence of invasive and noninvasive breast cancer (From Vogel et al.[24] Copyright © 2006. American Medical Association, All rights reserved)

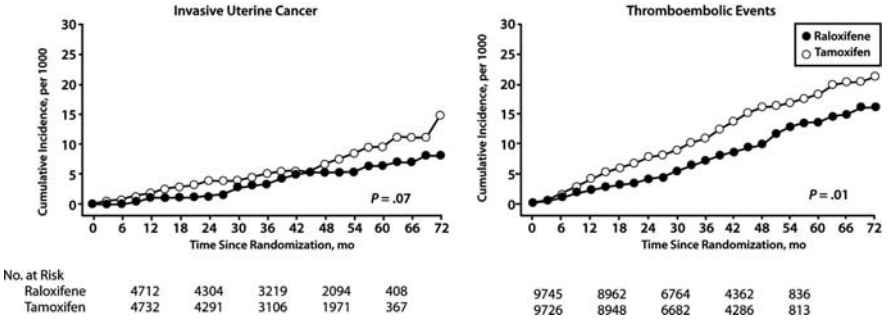

Fig. 3.3 Cumulative incidence of invasive uterine cancer and thromboembolic events (From Vogel et al.[24] Copyright © 2006. American Medical Association, All rights reserved)

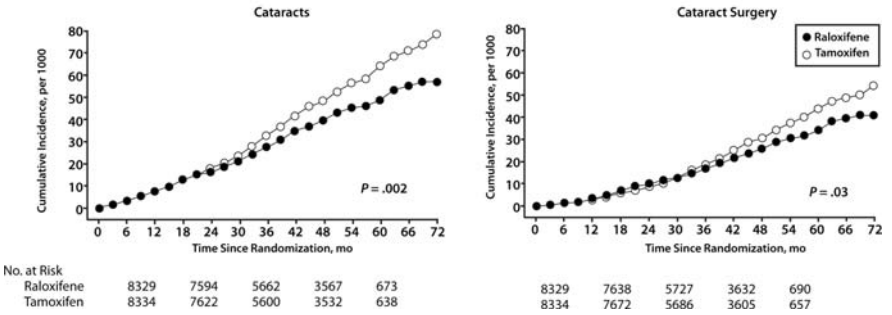

Fig. 3.4 Cumulative incidence of cataracts and cataract surgery (From Vogel et al.[24] Copyright © 2006. American Medical Association, All rights reserved)

were similar in both groups (104 in the tamoxifen arm, and 96 in the raloxifene arm). The incidence of cataracts was significantly increased in the tamoxifen arm (See Fig. 3.4).[24] Patient-reported outcomes for physical functioning, mental health and depression were similar for both groups. Types of symptoms within each group differed, however, with women in the tamoxifen arm reporting more vasomotor symptoms, leg cramps, and bladder control problems, and women in the raloxifene arm reporting more musculoskeletal symptoms, dyspareunia, and weight gain.[26] Overall, the results of the STAR trial confirm previous reports of the benefit of raloxifene in reducing breast cancer risk and indicate that raloxifene is an appropriate alternative to tamoxifen to lower the risk of invasive breast cancer among postmenopausal women at increased breast cancer risk.

Summary

Based on the results of these studies, two SERMs, tamoxifen and raloxifene, are approved for breast cancer risk reduction in women at increased risk. Both are similar in the degree of reduction of risk of invasive cancer, and both are active only for ER-positive tumors. It has become clear that both SERMs exhibit estrogenic as well as anti-estrogenic effects and produce a complex mix of benefits and risks. The two drugs have similar effects on preservation of bone mineral density. Tamoxifen is associated with a statistically significant increased risk for stroke (RR 1.49), pulmonary embolus (RR 1.88), and deep vein thrombosis (RR 1.87).[27] Raloxifene was associated with a two- to threefold

increased risk of VTEs in the MORE and CORE trials. In the STAR trial, rates of VTEs were 30% lower for the raloxifene arm than the tamoxifen arm, but the rates for stroke were identical. A small but marginally significant increase in cataracts and cataract surgery is associated with tamoxifen. The rate of endometrial cancer is increased with tamoxifen in each of the prevention trials with a combined relative risk of 2.4 (CI 1.5–4.0).[28] Raloxifene appears to spare the endometrium and is not related to an increase in either cataracts or stroke. Raloxifene is available only for postmenopausal women. The benefits of tamoxifen include a reduction in the risk of noninvasive as well as invasive breast cancer and its availability to both pre- and postmenopausal women. Overall, quality of life was similar for both drugs, although the pattern of specific symptoms reported differed by drug. For both drugs, hot flashes remains a significant problem. There was no increase in other cancers or in all cause mortality seen in any of the trials.[28] The adverse events associated with tamoxifen and raloxifene appear to be confined to the period of active treatment and do not persist after discontinuation of the drug.

In 2002, the U.S. Preventive Services Task Force (USPSTF) concluded that the balance of benefits and harms of tamoxifen and raloxifene may be favorable for some high-risk women and recommended that their use be considered in the context of each woman's level of risk for breast cancer, her potential risk for harm, and her personal preferences.[29]

Currently, new third- and fourth-generation SERMs are currently under investigation for the treatment and prevention of breast cancer. Arzoxifene is a third-generation SERM of the benzothiophene class with bioavailability and potency superior to raloxifene. Acolbifene is a fourth-generation SERM of the benzopyrans class. Both have demonstrated activity in metastatic breast cancer. Preclinical studies suggest greater bone preservation and fewer adverse effects on the uterus than tamoxifen and raloxifene.[30]

Unresolved Issues

Duration of Use

Both the BCPT and the STAR trials chose a study duration of 5 years. There are little data in the prevention setting to address the benefit of extending the duration of treatment beyond 5 years or of a sequential exposure to tamoxifen and raloxifene. Data from the Royal Marsden study indicates that the protective effect of tamoxifen persists for several years after the drug is discontinued, and all of the trials have shown that the adverse events associated with these drugs are restricted to the period of active treatment. Until further follow up is available, it appears that the 5-year schedule should be maintained.

Optimal Age of Use

Risk/benefit models indicate that the greatest clinical benefit with the least adverse side effects is achieved when tamoxifen is used in younger, premenopausal women who have the least risk of endometrial cancer and VTEs and who have the highest risk of atypical hyperplasia and LCIS, and in women without a uterus.[25] Although raloxifene is not indicated for premenopausal women, the risk of adverse events is also related to increased age. Clearly, the decision of what age to begin treatment of a chemopreventive agent must be balanced with the individual woman's risk and the presence of comorbid conditions.

Use in Women with a Hereditary Predisposition to Breast Cancer

None of the breast cancer prevention trials have specifically targeted women with *BRCA1* or *BRCA2* mutations or other breast cancer predisposing genetic syndromes. A small sub-study of the BCPT investigated *BRCA1/2* mutation status in women who developed breast cancer while on the trial. No protection was seen in the eight women on the tamoxifen arm who carried a deleterious *BRCA1* mutation. There was a 62% reduction in breast cancer among the 11 women with a deleterious mutation in *BRCA2*.[31] Although these findings are consistent with the observation that *BRCA2*-related breast cancer is more likely to be ER-positive than *BRCA1*-related breast cancer, the numbers are too small to make any recommendations regarding chemoprevention within this group of women.

Underrepresentation of Minority Women

Questions about the applicability of the breast cancer prevention trials have been raised by the low representation of minority women in all of the trials.[32] Issues such as low literacy, limited resources, lack of health insurance, and distrust toward the health care system have been cited as barriers to participation.[33] Physicians surveyed regarding the participation of Asian American women in breast cancer prevention trials cited language barriers, a lack of patient knowledge about research concepts, a lack of culturally relevant information about breast cancer, and fear of experimentation as deterrents to participation.[34] The National Medical Association has made several recommendations to improve minority participation in clinical trials (See Table 3.1).[35]

Table 3.1. NMA consensus panel recommendations

1. Educate and encourage minority physicians to participate in clinical trial research
2. Apply political pressure for the passage of laws that promote racial equality in healthcare
3. Initiate awareness programs in medical schools to encourage future minority physicians to become active in biomedical research
4. Promote outreach programs to educate and recruit minority participants
5. Advocate ethical practices in clinical research

Underutilization of Chemopreventive Agents for Breast Cancer

Based on the Gail model criteria for risk of breast cancer, over 10 million (15.5%) of the more than 65 million women in the United States between the ages of 35 and 79 would be eligible to take tamoxifen.[36] However, far fewer have chosen to take tamoxifen in the preventive setting. The decision to use tamoxifen is a highly individual decision, based on each woman's personal risk profile, the age-related risk/benefit ratio, and associated concerns over its toxicity. The approval of raloxifene, with its more favorable toxicity profile, may help to address this concern among postmenopausal women. Ultimately, the ability to personalize the choice of breast cancer chemoprevention agents to individual environmental, genetic, and physiologic risk factors will improve both efficacy and acceptance.[37]

Other Agents

Aromatase Inhibitors

Aromatase, an enzyme of the cytochrome P-450 family, is found in several tissues and is responsible for the conversion of adrenal androgen to estrogen in postmenopausal women. Aromatase inhibitors, which can reduce estrogen production by over 95%, are active in the metastatic and adjuvant setting of breast cancer treatment. Treatment with aromatase inhibitors in the adjuvant setting has shown a greater reduction in contralateral breast cancer than traditionally seen with tamoxifen.[38] The effectiveness of aromatase inhibitors in preventing invasive breast cancer in high-risk women is the subject of three ongoing trials. The International Breast Cancer Intervention Study-2 (IBIS-2) is randomizing 6,000 postmenopausal women at increased risk to anastrazole or placebo for 5 years. The National Cancer Institute of Canada Clinical Trials Group is comparing exemestane to placebo in 4,500 postmenopausal women at increased risk. The Aromasin prevention study in Italy is investigating the use of exemestane vs placebo for 5 years to prevent breast cancer in postmenopausal women with *BRCA1/2* mutations.[39,40] In addition to breast cancer outcomes, these trials will assess the tolerability and adverse effects of this class of drugs in the preventive setting.

Non-steroidal Anti-inflammatory Drugs (NSAIDs)

Epidemiological, case-control, and prospective studies have suggested a role for NSAIDs as anti-cancer agents.[41] Interest in NSAIDs as anti-cancer agents stems in part from the inflammation theory of carcinogenesis, which links inflammatory processes to the release of free radicals and reactive oxygen species which can cause cellular and genomic damage and promote cell proliferation.[42] NSAIDs target cyclooxygenase (COX), the key enzyme in arachidonate metabolism and the biosynthesis of prostaglandin H2. COX exists in two main isoforms, COX-1 and COX-2, the latter being overexpressed in a high percentage of breast tumors. Preclinical studies suggest an antitumor role of COX inhibitors in breast cancer. Potential mechanisms include COX-2 dependent induction of apoptosis as well as COX independent inhibition of proliferation, angiogenesis, and induction of cell death.[43] A prospective observational cohort study within the Women's Health Initiative found a 21% reduction in the incidence of breast cancer among postmenopausal women who reported regular use of NSAIDs (two or more tablets/week) for 5–9 years.[44] Although promising, recent concern over the cardiovascular toxicity of some NSAIDs has delayed their development as chemopreventive agents.[45]

Retinoids

Both naturally occurring and synthetic retinoids, which are derivatives of Vitamin A, have a role in the regulation of cell growth, differentiation and apoptosis through binding to retinoid receptors, which are present on both normal and malignant breast epithelium. Fenretinide, the synthetic amide of retinoic acid has been widely studied in preclinical models of breast cancer due to its selective accumulation in the breast and its relatively low toxicity profile.[46] A potential mechanism of fenretinide-mediated apoptosis includes the generation of reactive oxygen species. Other potential mechanisms include the inhibition of growth-stimulating factors and the induction of

growth-inhibitory factors. A Phase III trial begun in 1987 in Milan, Italy, randomized 2,972 Stage I breast cancer patients to fenretinide 200 mg/day vs no treatment for 5 years. After a median follow-up of 97 months, there was no difference in contralateral breast cancer occurrence or recurrent disease between the two groups. However, among premenopausal women, there was a statistically significant reduction in both ipsilateral and contralateral breast cancer in the fenretinide arm. The younger the women were, the greater was the benefit of fenretinide, suggesting a differential effect of the drug by endogenous hormone status.[47] Additional Phase III trials are underway in young women at increased risk for breast cancer.

Vitamin D

For decades, epidemiologic and dietary studies have supported a role for Vitamin D in the protection against cancer. In the Women's Health Study, which followed 10,578 premenopausal and 20,909 postmenopausal women prospectively, higher intakes of calcium and Vitamin D at baseline were associated with a decreased risk of premenopausal breast cancer.[48] No effect was seen in postmenopausal women.[49] Obtained from food sources and from exposure to UV radiation, Vitamin D is converted to its active form, $1,25(OH)_2D_3$ (calcitriol) at the tissue level (See Fig. 3.5).[50] Through interaction with the Vitamin D receptor (VDR), calcitrol plays an active role in calcium homeostasis, cell growth and differentiation, cell adhesion, and apoptosis.[50] Calcitriol's chemopreventive actions

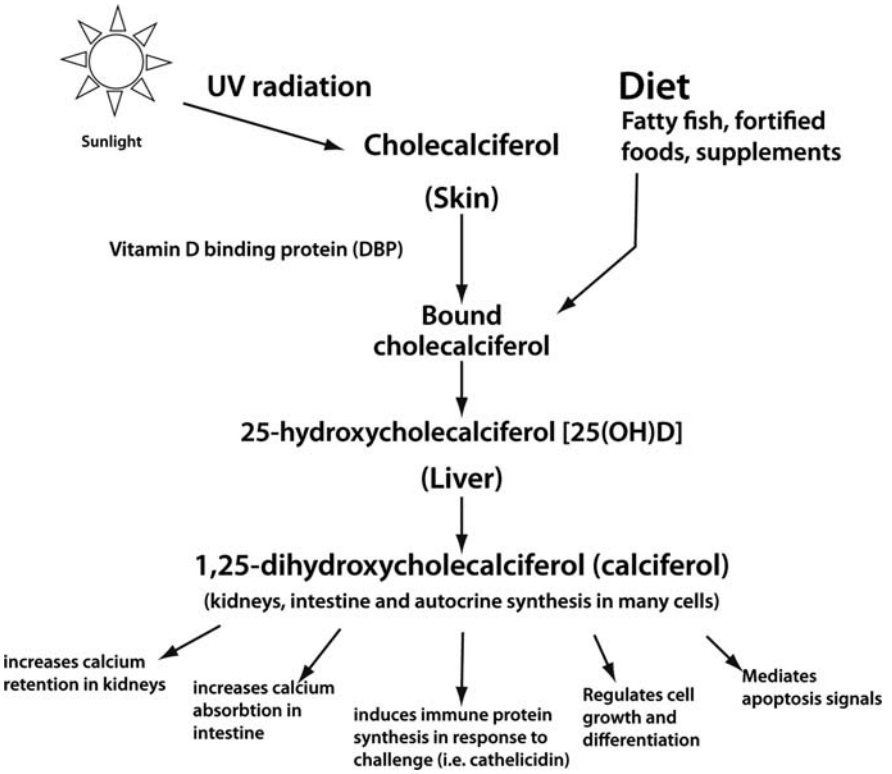

Fig. 3.5 The vitamin D Endocrine system (Reproduced from Ingraham et al.[50])

are thought to be due to its roles in cell cycle regulation, the control of apoptosis, and maintenance of cell adhesion.

Dietary Antioxidants

Oxidative stress has long been implicated in the development of many cancers, including breast cancer. Naturally occurring dietary antioxidants are an appealing alternative for breast cancer risk reduction because of their abundant presence in certain food groups and their presumed relative lack of toxicity. The molecular basis of the chemopreventive activity attributed to dietary antioxidants derives from their effect on several pathways, including transcription factors, growth regulators, adhesion molecules, apoptotic genes, angiogenesis regulators, and cell signaling molecules.[51] Table 3.2[51–55] presents a brief review of some of the effects of key dietary antioxidants in breast cancer.

Table 3.2 Natural breast cancer chemoprevention agents under investigation

Agent	Source	Potential mechanisms
Sulforaphane	Cruciferous vegetables	Phase 2 detoxification enzymes Suppression of cytochrome P450 enzymes Induction of apoptosis Cell cycle inhibition
Genistein	Soy isoflavones	Occupation of estrogen binding sites μμ sex-hormone binding globulin Cell cycle arrest Induction of apoptosis
Resveratrol	Red grapes	Induction of apoptosis Cell cycle arrest Reduced telomerase production Phase 2 detoxification enzymes
Polyphenols	Tea, pomegranates	Induction of apoptosis Cell cycle arrest
Curcumin	Turmeric	Induction of apoptosis Reduced telomerase activity
Lycopene	Tomatoes	Inhibition of cell proliferation Cell cycle arrest

Future Directions

Personalizing Chemoprevention

There is a growing movement in medicine to personalize treatment to certain genetic profiles of the individual. In the breast cancer prevention trials described above, eligibility was determined by broad categories of risk, and all women randomized to treatment arms received a standard dose of drug. Pharmacokinetic studies of tamoxifen metabolism have shown that the cytochrome P450 enzyme CYP2D6 controls the rate-limiting step in converting tamoxifen to its active metabolite, endoxifen. Polymorphisms in the *CYP2D6* gene demonstrate significant variability in their ability to metabolize tamoxifen leading to variable degrees of clinical activity. In addition to experiencing

less clinical benefit, women with poor metabolizing alleles are less likely to experience the vasomotor symptoms associated with tamoxifen.[56] Furthermore, some of the selective serotonin reuptake inhibitors (SSRIs) that are used to treat the vasomotor symptoms associated with tamoxifen are potent inhibitors of the CYP2D6 enzymes, and thus decrease the conversion of tamoxifen to its active metabolite. Considerable debate is underway to determine if genetic testing for *CYP2D6* alleles is indicated for women considering taking tamoxifen for prevention.

Similarly, genetic variability has been described both for NSAID metabolism and for the ability to induce several enzymes involved in prostaglandin synthesis and function.[42] Ultimately, to experience the full benefit of chemopreventive agents, it will be important to identify those individuals whose genetic profiles indicate that they are most likely to benefit and those most likely to suffer adverse consequences.

Biomarkers for Risk Prediction

There is great interest in identifying breast cancer biomarkers, both to incorporate into risk prediction models and to serve as intermediate markers of response to prevention interventions. Effective biomarkers should be present in a significant proportion of at-risk individuals, must be easy to measure, must reflect premalignant or preinvasive stages of disease, and must change with effective intervention.[38] Table 3.3 lists some key characteristics of neoplasia and potentially related biomarkers. Examples of breast cancer biomarkers that have been evaluated include serum hormone levels, growth factor levels, mammographic density, breast magnetic resonance imaging volume, and proliferation markers. Translational studies characterizing premalignant tissues by gene expression arrays, proteomic analyses, metabolic profiles, and other new technologies will contribute to our understanding of the neoplastic process and lead to the identification of additional biomarkers for use in chemoprevention trials.[57]

Combination Regimens

The evolution from normal breast epithelium to cancer is a long and complex process which involves multiple pathways which may exhibit both synergistic and antagonistic features. Combinations of chemopreventive agents may provide the advantages of targeting multiple sites along a pathway and multiple pathways, of overcoming resistance to a single agent, and of permitting lower doses of each agent, thus minimizing toxicities.

Table 3.3 Characteristics of neoplasia and associated molecular biomarkers (Reprinted with permission of Clinical Cancer Research. Kelloff/Lippman, 2006)

Characteristics of neoplasia	Possible molecular targets
Self-sufficiency in cell growth	Epidermal growth factor, platelet-derived growth factor, MAPK, PI3K
Insensitivity to antigrowth signals	SMADs, pRb, cyclin-dependent kinases, MYC
Limitless replicative potential	hTERT, pRb, p53
Evading apoptosis	BCL-2, BAX, caspases, FAS, tumor necrosis factor receptor, DR5, IGF/PI3K/AKT, mTOR, p53, PTEN, *ras*, interleukin-3, NF-κB
Sustained angiogenesis	VEGF, basic fibroblast growth factor, $\alpha_v\beta_3$, thrombospondin-1, hypoxia-inducible facot-1α
Tissue invasion and metastasis	Matrix metaloproteinases, MAPK, E-Cadherin.

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Chapter 4
Surgical Management of Inherited Susceptibility to Breast Cancer

Francis Freisinger and Susan M. Domchek

Abstract Individuals at increased risk for the development of breast cancer have several options for clinical management, including prophylactic mastectomy and risk-reducing salpingo-oophorectomy. Over the past decade, increasing data have become available regarding the efficacy of and the adverse effects from these surgical approaches. In this chapter we review the available literature, including the indications for such surgeries, as well as their risks and benefits.

Keywords Prophylactic surgery · Mastectomy · Salpingo-oophorectomy · Risk reduction

Key Issues

- Increasing data are available regarding the efficacy and outcomes, including impact on quality of life, of prophylactic oophorectomy and prophylactic mastectomy in individuals with a strong family history of breast and/or ovarian cancer.
- Prophylactic mastectomy significantly reduces the risk of breast cancer. Women should be counseled as to their options of screening (with MRI as a component) vs prophylactic mastectomy (with information provided on the range of reconstruction options).
- Prophylactic oophorectomy significantly reduces the risk of ovarian cancer and breast cancer in *BRCA1* and *BRCA2* mutation carriers, although the magnitude of the risk reduction, particularly for breast cancer, may be mutation dependent.
- Limited data with short term follow up suggest that prophylactic oophorectomy may improve overall survival, however, the procedure is associated with the induction of abrupt menopause in premenopausal women, and the long term effects on cardiovascular and bone health are postulated but as yet unknown.

Introduction

Prophylactic surgery for hereditary breast cancer has a long history. Oophorectomy was first proposed both as therapy and prevention for breast cancer in 1889 by a German surgeon, Albert Schinzinger.[1,2] Prophylactic mastectomy for women from high-risk families had been practiced

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for decades, but became more common in the 1960s with the introduction of breast implants that allowed surgical breast reconstruction.[3]

The discovery of the *BRCA1* and *BRCA2* mutations in the early 1990s led to commercially available genetic testing that has significantly aided risk prediction by identifying women at the highest risk for breast cancer who are most appropriate for consideration of prophylactic mastectomy. In hereditary breast cancer families with a detectable deleterious mutation in *BRCA1* or *BRCA2*, it is now possible to identify individuals who are or, perhaps more importantly, who are not, at significantly elevated risk for the development of breast and ovarian cancer.

In parallel to the advances in breast cancer genetics, our knowledge of the benefits and potential harms of surgical prophylaxis options has progressed significantly. Although we currently do not have, and almost certainly will never have data from randomized controlled trials assessing bilateral prophylactic mastectomy (BPM) or risk reducing salpingo-oophorectomy (RRSO) compared to screening, various studies have convincingly demonstrated their efficacy in decreasing the risk of breast cancer and ovarian cancer respectively.

In this chapter we focus on the indications, efficacy, and risks of BPM and RRSO for women who have been diagnosed with *BRCA1* or *BRCA2* mutations or who have a significant family history due either to unknown factors or associated with rare genetic syndromes.

Prophylactic Surgery

Surgical prophylaxis involves taking "healthy" women and exposing them to procedures with morbidity and potential mortality risks. In general, to justify such measures, a number of preconditions should apply. In an unambiguous and highly idealized situation these would include a near certainty of developing disease; a disease that is difficult to treat, with high mortality; a lack of effective non-surgical alternatives; and an operation which is highly effective in preventing the disease with low associated morbidity.[4]

These conditions are partially met in previously unaffected women with documented *BRCA1* and *BRCA2* mutations. As we describe below, RRSO is highly effective at preventing ovarian cancer in this population, and also significantly reduces breast cancer risk. Ovarian cancer is very difficult to treat unless detected in its earliest stages. Even with intensive screening, it is often diagnosed too late. One analysis that consolidated data on 6,000 women from 12 individual studies found that 63% of ovarian cancers detected by screening were Stage 2C or greater.[5] In terms of preventive options, oral contraceptive pills decrease the risk of ovarian cancer by approximately 50% and therefore substantial residual risk of ovarian cancer remains. In addition, these medications appear modestly to elevate breast cancer risk.[6–10] On the other hand, penetrance is well below 100%, and thus the majority of women who elect RRSO would never develop ovarian cancer. Ovarian cancer prevalence estimates for *BRCA1* carriers by age 70 vary from 39 to 42%. The risk is lower for *BRCA2* carriers, with estimates ranging from 11 to 27%.[11–14] In addition, there is a significant degree of morbidity associated both with the RRSO surgical procedure itself and the premature menopause that reduction of endogenous estrogen induces.

In addition to providing a high level of protection against ovarian cancer, RRSO also appears to confer significant protection against *BRCA1* and *BRCA2* related breast cancer. Overall, there is broad consensus in favor of RRSO for *BRCA1* and *BRCA2* mutation carriers once child bearing is complete.

In contrast, the decision to pursue BPM is made on an individual basis. This is true even though the breast cancer penetrance in *BRCA1* and *BRCA2* mutation carriers is higher than that for ovarian

cancer. Calculations vary widely, depending on population and ascertainment method. Estimates for the risk of breast cancer by age 70 range from 48 to 81% in *BRCA1* carriers and 45 to 84% in *BRCA2* carriers.[11–14]

Although there is strong evidence that BPM is highly effective at preventing breast cancer, a number of factors make the decision more complicated than for RRSO. First, beyond physical morbidity, there are unique emotional and psychological issues associated with the procedure. Second, there are reasonable, albeit less effective, alternatives to BPM for breast cancer prevention. Third, while the mortality rate of late stage breast cancer is high, there is a good chance of cure or long-term disease-free survival if the cancer is detected at an early stage. This is true even though, compared with non-carriers, breast cancer in *BRCA1* mutation carriers has several features typically associated with an adverse prognosis. These include a younger age of onset, a higher pathological grade at diagnosis, and a high rate of "triple negative" breast cancers (estrogen receptor, progesterone receptor and HER2/neu negative). Treatment is more likely to require chemotherapy. Despite these features, recent estimates suggest that survival is no different for *BRCA1* carriers than for non-carriers.[15] Indeed, *BRCA2* mutation carriers may actually have a better prognosis, when the disease is detected early, than non-carriers.[16] Fourth, and in contrast to ovarian cancer, screening using MRI and mammography has high sensitivity and detects many, although by no means all, breast cancers while they are still at an early stage with a high cure rate.[17,18] As a result, many *BRCA1/2* mutation carriers elect not to undergo BPM and instead choose RRSO along with breast surveillance.

Bilateral Prophylactic Mastectomy (BPM)

BPM is the surgical removal of both breasts with the goal of reducing the risk of developing breast cancer. It cannot eliminate risk entirely, since no procedure can remove all mammary tissue, which is widely distributed over the entire antero-lateral portion of the chest wall and axilla. In addition, metastatic cancer that is occult at the time of mastectomy can also rarely develop. Various surgical approaches have been developed that balance the need to remove as much breast tissue as possible with optimal cosmetic results. Total or simple mastectomy involves amputation of the entire breast but spares lymph nodes.[19] The pectoralis muscles are preserved, although the fascia is generally resected. A second approach used with prophylactic surgery is subcutaneous mastectomy, which involves removal of breast tissue from overlying skin. There is variation depending on individual anatomy and surgical technique, but typically 90–95% of breast tissue can be resected. Two recently introduced variants are skin-sparing and nipple-sparing mastectomies.[20,21] In the former, overlying breast skin is retained, but the technique increases removal of ductal tissue by sacrificing the nipple-areolar complex and using thinner skin flaps.[22] Nipple-sparing mastectomy preserves the nipple-areolar complex.[23] It differs from traditional subcutaneous mastectomy in that the technique leaves much thinner skin and nipple-areolar complex flaps.

BPM is Highly Effective

BPM was used for decades before the publication in the late 1990s of studies evaluating the technique. Since then, several studies have provided consistent evidence of its effectiveness in the prevention of breast cancer (Table 4.1). Hartmann et al. in 1999 provided the first data quantifying the protective effect of BPM.[3] The study retrospectively examined 639 women at high- and moderate-risk of breast cancer based on family history who had undergone BPM at the Mayo Clinic after 1960, with a median follow up of 14 years. Using both a risk model[27] to predict the expected number of breast cancers in the moderate-risk group and using sisters as controls in high-risk probands, the

Table 4.1 Studies evaluating bilateral prophylactic mastectomy

Study	Design	Number of women with BPM	Number of women without BPM	Mean follow up time	Breast cancer risk impact (95% CI)	Comments
Hartmann 1999[3]	Retrospective cohort	214	403	14 years (median)	90% (70.8–97.9) adjusted risk reduction	Using high risk subset. not restricted to BRCA1/2 mutation carriers
Meijers-Heijboer 2001[24]	Prospective cohort	76	63	2.9 years	0 (0–0.36) HR	BRCA1/2 mutation carriers
Hartmann 2001[25]	Retrospective cohort	26	NA	13.4 years	89.5–100% (41.4–100%) risk reduction	BRCA1/2 mutation carriers compared with expected breast cancer incidence derived from risk models
Rebbeck 2004[19]	Prospective cohort	105	378	6.4 years	0.05 (0.01–0.22) HR	BRCA1/2 mutation carriers analysis excluding women with oophorectomy had HR of 0.09 (95% CI 0.02–0.38)
Geiger 2005[26]	Retrospective case-cohort	276	196	10.3 years (BPM group), 6.2 years control group	0.005 (0.001–0.044) HR	Population based study of women with at least one qualifying breast cancer risk factor

HR = Hazard Ratio

study estimated an approximately 90% reduction in breast cancer events in moderate-risk women after BPM, and a 90–94% reduction for high-risk women. Risk of death from breast cancer was reduced by 81–94%. Of note, many of the women in this study (90%) had undergone a subcutaneous mastectomy. Six of the Hartmann cohort eventually developed cancer at their chest wall.

A 2001 study by the same authors updated the results to be specific for *BRCA1* and *BRCA2*.[25] Of the 214 women originally estimated to be at high risk, 176 were tested and 26 were found to have *BRCA1* or *BRCA2* mutations. Breast cancer risk reduction for these women was estimated to be from 89.5 to 100%.

Meijers-Heijboer et al. published the first prospective study of BPM[24] in which 139 Dutch female carriers of *BRCA1* or *BRCA2* mutations without a history of breast cancer were followed. Of the 139 women, 76 underwent BPM, and the remainder elected surveillance. During a 3-year follow up period, none of the women developed breast cancer after their mastectomy, while eight breast cancers were detected in the surveillance group, equivalent to a 2.5% yearly incidence of breast cancer. Of concern, four of these cancers were detected in the interval period between screening sessions. A potentially significant confounder was the greater percentage of women in the BPM group who had already undergone RRSO (58% vs 38%). Thus the BPM group may have had a lower pre-existing risk of developing breast cancer. Seven of the eight breast cancers were estrogen and progesterone receptor negative, were of high grade (grade III), and occurred in patients under age 50. Four were associated with positive lymph nodes at diagnosis. Using the clinical features of the cancers and based on prior estimates of mortality associated with *BRCA1* germline mutations,[28] the authors calculated that 35–50% of women who developed primary breast cancer while under surveillance would die within 10–15 years, and that overall 10–20% of women choosing surveillance would die of breast cancer within 20 years. This study had a number of limitations,[29] including only 3 years follow up and suboptimal surveillance, which consisted initially of an annual mammogram, semi-annual clinical breast examination and monthly self examination. MRI was introduced as a surveillance tool at the institution where the study was conducted 3 years after the start of the 8-year study.

A larger prospective multicenter study by Rebbeck et al. was published in 2004.[19] The study followed 483 women positive for *BRCA1* or *BRCA2* mutations, of whom 105 chose BPM and 378 were controls matched for gene (*BRCA1* versus *BRCA2*), center and year of birth. The analysis was adjusted for age at RRSO where applicable. Two cases (1.9%) of breast cancer occurred in the BPM group, while 184 cases (48.7%) occurred in the control group during the 6.4-year follow up period. The study estimated a 95% reduction in breast cancer risk in women who had prior or concurrent RRSO and 90% in women without. Both cases of breast cancer post-BPM occurred in women who had had subcutaneous rather than simple mastectomies.

Pathology Often Finds Occult Cancer

Occult premalignant and malignant lesions are frequently found in follow up pathologic review of prophylactic mastectomies, although the prevalence varies widely with population and series. Occult invasive cancer is typically found in 1–6% of specimens,[3,30–32] and premalignant lesions are common. Series find up to 18–50% prevalence of atypical ductal hyperplasia, atypical lobular hyperplasia, and ductal or lobular carcinoma in situ (DCIS/LCIS).[24,33–35] Because of the relative rarity of occult invasive cancer, sentinel node lymphadenectomy (SNL) is not generally indicated. A recent decision analysis[30] found that the prevalence of occult invasive cancer would have to be 28% to justify SNL for all women undergoing BPM, and as a result advised against this practice.

Adverse Effects of BPM

Immediate functional deficits resulting from BPM include loss of sensation, as well as the ability to breast-feed. Simple mastectomy is usually well tolerated. While a significant number of women experience both early and late surgical complications from breast reconstruction, the most significant adverse effects of BPM are psychosocial and emotional.

An early study evaluating the outcome of 572 women 14.5 years after BPM found that 70% expressed overall satisfaction with their decision, in large part because of lower anxiety. The majority of patients in this series reported either no change or favorable effects in levels of emotional stability, self-esteem, sexual relationships and feelings of femininity.[36] Most of the women in this study (89%) underwent subcutaneous mastectomy with subsequent breast implants. Only 4% of this group had a simple mastectomy without reconstruction, while 7% underwent simple mastectomy with reconstruction.

While many subsequent studies also observed that women were satisfied with their decision to undergo BPM, several noted a negative impact on sexual relations. One 2006 study[37] found that negative outcomes were associated with inadequate pre-operative counseling, although 60% of women were satisfied overall. Other studies echoed this,[38] finding that the majority of women (84%) were satisfied with their decision overall. Diminished quality of life was associated with dissatisfaction with sex life. A confounding factor may be that women who elect BPM on average at baseline have higher levels of sexual dysfunction and general distress than women who choose surveillance.[37,39]

A recent prospective study[39] evaluated body image, sexuality, emotional reactions (anxiety, depression), and quality of life before and after BPM. The study followed 90 women in Sweden. Like other studies, it found a reduction in cancer-related anxiety after surgery, but confirmed a significant negative impact on sexuality and body image, with 48% of women feeling less sexually attractive and 44% expressing dissatisfaction with the scars. As in other studies, overall quality of life before and after the surgery was unchanged. Of note, 25% of the women also underwent RRSO, which is known to negatively affect sexuality.

It is worth noting that adverse psychosocial effects of BPM may be underestimated by the closed-end questions used in the cited studies. One study by Altschuler (2008)[40] found that 70% of women expressed negative comments in open-ended responses but simultaneously indicated satisfaction in closed-ended questions.

Breast Reconstruction

Breast reconstruction is intended to mitigate the adverse psychological and aesthetic effects of BPM. Key elements include restoration of the breast mound and reconstruction of the nipple-areolar complex.[41] There are numerous approaches to reconstruction, and the choice depends on a woman's preferences, her individual breast anatomy and the skills and experience of the surgical team. The key choices are whether to use an artificial implant or autologous tissue. Currently, 70% of breast reconstruction procedures are implant-based, while the remainder are autologous tissue-based.

Most commonly, artificial implants are placed in two stages. Initially a saline-filled tissue expander is placed underneath the pectoralis muscle and gradually expanded using weekly injections over 1–2 months. Later, the expander is replaced with a permanent implant, usually in an outpatient procedure. Implants are either silicone or saline. A single step implant procedure is also available. Autologous tissue implants can come from several sites and can be transferred either as a pedicle flap (with its own vascular supply) or a free flap (which requires microvascular surgery to reattach blood vessels). The most commonly used is the transverse rectus abdominis myocutaneous (TRAM) flap, taken from the infraumbilical abdomen. A more recently developed flap, based on

the deep inferior epigastric perforator (DIEP) arteries, transfers skin, soft tissue and blood vessels from the abdomen, but spares most of the rectus muscle. Autologous tissue reconstruction is more expensive and requires a longer surgery, but may offer a more natural post-surgical appearance.[41]

Complications Are Common

There are various complications associated with reconstruction, aside from infection and bleeding. For artificial implants, these include early complications such as extrusion of the implant and late complications such as capsular construction, leak, and infection. Autologous tissue flaps face risks of necrosis of the transferred tissue and problems at the donor site. The true frequency of complications is difficult to estimate, and published studies reflect a wide variation in technique and population.[41]

A Dutch study[42] followed 358 women in Holland after BPM. Of the women who chose breast reconstruction, 93% received silicone implants. Typically, skin-sparing mastectomy was used. Nipple reconstruction took place 6 months after the initial procedure. One or more complications were reported in 50% of the women who underwent breast reconstruction. The most common early complications included infection, necrosis, and bleeding. Of these, 36% were serious enough to require surgery. The most common late complications included capsule formation and poor cosmetic appearance, with the majority of these complications leading to repeat surgery. Some other studies have found similarly high complication rates. A 2005 study of 269 women[43] found that 64% of post-mastectomy reconstructions led to complications, of which the most common were pain (35%), infection (17%), and seroma (17%). The majority of women in this series elected to have prosthetic implants. A more recent study[44] showing more favorable results followed 54 women for 42 months and reported a complication rate of 18% of which 7% were early and 11% late. Secondary corrections were necessary in only 11% of women. None of the women expressed regrets regarding their decision to undergo breast reconstruction.

Risk Reducing Salpingo-Oophorectomy (RRSO)

RRSO is the surgical removal of both ovaries and the fallopian tubes. It is sometimes combined with simultaneous total abdominal hysterectomy (TAH). There is strong evidence from multiple studies that timely RRSO in *BRCA1* and *BRCA2* mutation carriers leads to a reduction not just in the risk of ovarian cancer but also a 50% reduction in breast cancer risk (See Table 4.2). There are also data suggesting a benefit for overall short-term mortality and for both ovarian and breast cancer specific mortality.[51]

Multiple studies have been published regarding the efficacy of RRSO. Rebbeck et al. in 1999 selected 43 *BRCA1* positive subjects who had undergone RRSO,[45] had no history of breast or ovarian cancer, and had not had BPM. They were matched with *BRCA1* positive controls that had not undergone RRSO. The study found an adjusted hazard ratio (HR) of 0.53 (95% CI 0.33–0.84) for breast cancer. The risk reduction increased with time, with an HR of 0.28 for women followed for 5–10 years. Use of hormone replacement therapy did not affect the reduction in breast cancer risk after surgery.

Subsequent to this, two major studies regarding RRSO were published in 2002. Kauff et al. prospectively followed 170 *BRCA1* and *BRCA2* positive women for a mean duration of 24 months, of whom 58% chose RRSO after genetic counseling, with the remainder electing surveillance.[46] The authors observed a 75% reduction in the combined risk of breast and gynecological cancer for the RRSO group. They calculated that 94% of the RRSO group would be cancer free after

Table 4.2 Studies evaluating effect of risk reducing salpingo-oophorectomy (RRSO) in *BRCA1* and *BRCA2* mutation carriers

Study	Design	Number of women with RRSO	Number of women without RRSO	Mean follow up time	Breast cancer risk impact (95% CI)	Gynecological cancer[a] risk impact (95% CI)	Comments
Rebbeck 1999[45]	Retrospective cohort	43	79	9.6 years	0.53 (0.33–0.84) HR	NA	BRCA1 only
Kauff 2002[46]	Prospective cohort	98	72	24.3 months	0.32 (0.08–1.20) HR	0.15 (0.02–1.31) HR	
Rebbeck 2002[47]	Retrospective cohort	259[b]	292[2]	8.8 years	0.47 (0.29–0.77) HR	0.04 (0.01–0.16) HR	
Rutter 2003[48]	Case-control	133	2153	NA	NA	0.12 (0.06–0.24) OR	
Eisen 2005[49]	Case-control	166	3139	NA	0.46 (0.32–0.65) OR	NA	
Kramer 2005[50]	Prospective cohort	33	65	16.5 years	62% risk reduction (3–85%)	NA	BRCA1 only
Domchek 2006[51]	Prospective cohort	155	271	2.5 years	0.36 (0.29–0.67) HR	0.11 (0.03–0.47) HR	Mortality was primary endpoint. All unaffected
Finch 2006[52]	Prospective cohort	1045	783	3.5 years	NA	0.20 (0.07–0.58) HR	Substantial (4.3%) incidence of peritoneal cancer post-oophorectomy
Chang-Claude 2007[53]	Retrospective cohort	55	1601	NA	0.56 (0.29–1.09) HR	NA	
Kauff 2008[54]	Prospective cohort	509	283	40.3 months (mean)	0.53 (0.29–0.96) HR	0.12 (0.03–0.41) HR	BRCA1 specific risk reduction not statistically significant

[a]Ovarian, fallopian tube or primary peritoneal cancer
[b]Breast cancer risk determined from subgroup of 99 women with RRSO and 142 women without
HR = Hazard ratio, OR = Odds ratio

5 years vs 69% for the surveillance group. The study found a strong nonsignificant trend towards protection against breast cancer, with a 68% risk reduction based on a hazard ratio of 0.32 (95% CI 0.08–1.20). For gynecologic cancers (ovarian, fallopian tube, and primary peritoneal cancers) there was a hazard ratio of 0.15 (95% CI 0.02–1.31). A large 2002 case control study by Rebbeck et al. also demonstrated a significant reduction in breast and ovarian cancer risk following RRSO.[47] This group retrospectively analyzed 551 female carriers of *BRCA1* and *BRCA2* mutations, of whom 259 had undergone RRSO and 292 chose surveillance. The mean follow-up was nearly 9 years for the surveillance group and 11 years for the RRSO group. Breast cancer was diagnosed in 42% of women in the surveillance group, but only 21% of the women in the RRSO group, for a hazard ratio of 0.47 (95% CI 0.29–0.77).

As expected, gynecological cancer incidence was much lower in the RRSO groups in both studies. RRSO does not completely eliminate risk, since primary peritoneal carcinoma can arise either from an occult ovarian cancer focus or de novo from peritoneal mesothelium. In the 2002 Rebbeck study, only two of the 259 subjects who underwent RRSO subsequently developed primary peritoneal cancer during the follow up period, although six in the RRSO group were found to have occult Stage I ovarian cancer at the time of surgery. The overall hazard ratio for ovarian cancer in the study was 0.04 (95% CI 0.01–0.16).

Several subsequent studies[48–54] have confirmed these findings for both breast and ovarian cancer. Kramer et al. (2005) published RRSO effects in a prospective study that examined 673 women from families with hereditary breast and ovarian cancer, 98 of whom were *BRCA1* positive and were followed on average for 16.5 years.[50] RRSO was chosen by 33 of the women and was associated with a relative risk for breast cancer of 0.38 (95% CI 0.15–0.97). The study allowed comparison of the absolute risks for women carrying *BRCA1* mutations at different ages with and without RRSO. The 10-year breast cancer risk for a 40-year-old woman with intact ovaries was estimated to be 32% vs 11% for a woman after RRSO.

Eisen et al. (2005) published a retrospective case-control analysis of 1,439 women that included sufficient cases to have the statistical power to separate *BRCA1* from *BRCA2* carriers.[49] This group found that *BRCA1* carriers with RRSO has a decreased risk of breast cancer with an odds ratio of 0.44 (95% CI 0.29–0.66) while *BRCA2* mutation carriers with RRSO had an odds ratio of 0.57 (95% CI 0.28–1.15). Benefits were greatest when RRSO was carried out prior to age 40, and benefits were evident for at least 15 years. The non-significant result for *BRCA2* was thought to be due to the relatively small *BRCA2* sample.

The significant protective effect of RRSO in *BRCA1* mutation carriers demonstrated in these studies remains poorly understood. Theoretically, *BRCA2* mutation related cancers should respond more to the hormonal ablation induced by RRSO than *BRCA1*. *BRCA1*-mutation related breast cancers are predominantly estrogen receptor negative (approximately 80–85%), while *BRCA2* mutations are mostly estrogen receptor positive (approximately 78%).[55,56]

Most recently, a large prospective study following 1,079 women for 3 years evaluated the efficacy of RRSO in the prevention of breast and gynecologic cancer for both *BRCA1* and *BRCA2* mutation carriers.[54] It was the first prospective study large enough to attempt a separate estimate of the specific benefit of RRSO for *BRCA1* vs *BRCA2* carriers. A surveillance group consisted of women who elected not to have RRSO. After exclusions, 498 *BRCA1* carriers and 294 *BRCA2* carriers with ovaries intact at the time of genetic test results were followed for 38 months of follow up. Of these, 65% of *BRCA1* carriers and 63% of *BRCA2* carriers chose elective RRSO, while the remaining carriers entered the surveillance group. The RRSO group was older on average than the surveillance group with a mean age of 47.1 vs 42.9. There was a 72% reduction in *BRCA2*-related breast cancer following RRSO based on a hazard ratio of 0.28 (95% CI 0.08–0.92). Protection against *BRCA1*-associated breast cancer did not reach statistical significance with a hazard ratio of 0.61 (95% CI

0.30–1.22). In contrast to prior studies, this study suggested a differential benefit in *BRCA2* relative to *BRCA1* mutation carriers. The study also confirmed that RRSO is protective against *BRCA1* gynecological cancer, and strongly suggested this for *BRCA2* as well although for the latter the risks were not estimable.

The study design made significant efforts to limit biases introduced by the ascertainment strategies of prior studies. A commentary in 2003 noted multiple potential confounders in existing studies of prophylactic surgery.[57] For example, it cited evidence that certain *BRCA1* mutations appear to confer an elevated risk of ovarian cancer, but a reduced risk of breast cancer relative to other *BRCA1* mutations. Families carrying these mutations would tend to have relatively more members with ovarian cancer and fewer with breast cancer. As a result they would have been more likely to seek RRSO, and estimates of breast cancer risk derived from this population would underestimate the true risk. Although difficult to do and requiring large consortia, prospective studies, particularly those which start with women unaffected and with ovaries at the time of genetic testing, can limit these biases, as it is hard to imagine that randomized trials will be done until we have an effective screening strategy.

Beyond reduction in cancer risk, RRSO was also shown by Domchek et al. in 2006[51] to reduce mortality. In a prospective cohort study of 666 women with *BRCA1* and *BRCA2* mutations, women unaffected by breast or ovarian cancer who chose RRSO were age matched to women who elected surveillance. Subjects were followed for 3.1 years after RRSO. The study demonstrated a reduction in overall mortality with a hazard ratio (HR) of 0.24 (95% CI 0.08–0.71). There was improvement in breast cancer specific mortality with an HR of 0.10 (0.02–0.71) and in ovarian cancer specific survival with an HR of 0.05 (0.01–0.46). This study was limited by small numbers and short follow-up time. More data are needed regarding this important issue.

In summary, the evidence is compelling in support of RRSO both for *BRCA1* and *BRCA2* mutation carriers. For *BRCA1* carriers, the benefits in terms of gynecological cancer risk reduction alone of more than 90% justify the procedure. While there is evidence of a reduction in *BRCA1*-mutation related breast cancer risk, the exact level remains to be quantified. For *BRCA2* mutation carriers, although their risk of ovarian cancer is generally lower, and thus the absolute benefit in terms of gynecologic cancer is also lower, there is solid evidence in favor of significant risk reduction for *BRCA2*-mutation related breast cancer of as much as 76%.

The evidence also suggests that RRSO should be done soon after a woman has completed childbearing, ideally prior to age 40. The risk of ovarian cancer is low until age 40 for *BRCA1* mutation carriers, and until age 50 for *BRCA2* carriers, with 2–3% of cases occurring before this age.[58] However, *BRCA2* carriers are not advised to delay the procedure because of their lower gynecologic cancer risk, since their breast cancer risk reduction benefit is also deferred. These complexities emphasize the importance of expert individual counseling. For example, a *BRCA2* mutation carrier who had undergone BPM could reasonably defer RRSO for a few years. Further data regarding timing of RRSO is needed in order to aid women making these difficult decisions.

Morbidity and Potential Mortality Associated with RRSO

The main negative consequence of RRSO is that of premature and immediate menopause. Menopausal symptoms due to estrogen deprivation are common post-RRSO and can be severe. These often include vasomotor symptoms such as hot flashes and sexual dysfunction. Sleep disturbances and cognitive changes are also frequently reported. Nonetheless, an observational study of 846 women in the Netherlands who had chosen between RRSO and surveillance noted a very similar general quality of life level between the two groups, with less anxiety about ovarian and breast cancer risk in the RRSO group, offset by sexual and vasomotor side effects.[59] Most women in the RRSO group stated they would undergo surgery again, while fewer in the surveillance group were content

with their choice, and a third said they were contemplating RRSO in the future. A subsequent paper from the same group[60] looked at the impact of hormone replacement therapy (HRT) in mitigating the adverse effects of RRSO. It found that HRT offered a significant, albeit incomplete level of relief of vasomotor symptoms. However, it also found that sexual symptoms, including vaginal dryness and dyspareunia, were similar between women who used HRT and those who did not after RRSO. These sexual symptoms were significantly more prevalent than in a comparison group of women who chose surveillance over oophorectomy.

There is a growing body of evidence identifying other long-term negative consequences of surgical menopause in the general population.[61] Osteopenia and osteoporosis are well known potential consequences of estrogen deprivation. Other studies have suggested increased risk of cardiovascular disease,[62] neurologic dysfunction including dementia and parkinsonism,[63,64] and psychological sequelae including depression and anxiety.[65] Appropriate screening and treatment for modifiable risk factors for these sequelae is essential in the long term follow-up care of patients after RRSO. There are also data suggesting the possibility of increased mortality in young women in the general population who undergo RRSO. A population-based cohort study[66] compared women who had undergone unilateral or bilateral oophorectomy to matched controls. It found no overall increase in mortality from the procedure. However, in a subgroup of women aged less than 45 who had not received estrogen therapy, there was a near doubling of mortality from all causes. The author emphasized that the study could not determine causality, and it remained unclear whether the increase in mortality was due to an increase in cardiovascular, bone and neurologic disease, or whether there were undetected biases in the sample.

Unanswered questions remain about the benefits and safety of hormone replacement therapy (HRT) post RRSO. Many women choose HRT for relief of vasomotor and sexual symptoms of early menopause induced by the surgery. Beyond some symptomatic relief, there are little data regarding which of the adverse outcomes mentioned above can be prevented by combined progesterone/estrogen therapy, or pure estrogen therapy for women who have undergone hysterectomy. Whether HRT diminishes breast cancer risk reduction – one of the principal objectives of RRSO – is also uncertain, particularly in the long run. One prospective cohort study[67] looked at the effects on breast cancer risk of short-term HRT use in 462 female *BRCA1/2* mutation carriers. Of these, 155 were post-RRSO and 60% used HRT. The group was followed for a mean of 3.6 years, and the study found no significant difference in the incidence of breast cancer with the use of HRT, with a HR of 1.35 (95% CI 0.16–11.58). Additional reassurance came from a recent matched case-control study[68] that compared the use of HRT in *BRCA1* carriers who had developed breast cancer after menopause with those that had not. Cases were matched for age, age at menopause and type of menopause (surgical or natural). The study suggested a statistically significant reduction in the incidence of breast cancer in the group exposed to estrogen only with an HR of 0.51 (95% CI 0.27–0.98), and no statistical difference in the group exposed to combined estrogen/progesterone.

One study[69] used a Markov decision analytic framework to assess outcomes of RRSO with and without HRT either until age 50 or for life, using sensitivity analyses for different assumptions about the effect of HRT and the impact of RRSO on cancer risk. It found that under reasonable assumptions, RRSO lengthened life expectancy by 3.3–4.7 years in women with *BRCA1* and *BRCA2* mutations, depending on age at RRSO. Use of HRT after oophorectomy was associated with relatively small changes in life expectancy (+0.2 to –0.3 years) when HRT was stopped at age 50, but a larger drop in life expectancy (–0.8 to –1.1 years) if HRT was continued for life. The conclusion was that women could consider use of HRT after RRSO until age 50, basing their decision on improved quality of life rather than a small risk of adverse impact on life expectancy.

Further study is clearly indicated to assess the potential risks of surgical menopause and the potential for HRT to offset these risks.

RRSO Complications

RRSO generally has a low complication rate if performed by experienced surgeons, with one series noting complications in 4 of 80 RRSO procedures.[46] Because both ovaries and fallopian tubes are potential sites for malignancy, it is important that resection minimizes remnant tissue, which can persist both in the hilum of the ovary and in the insertion of the fallopian tube in the cornua of the uterus. There are at least five reports of ovarian cancer occurring in an ovarian remnant post-oophorectomy, although none of these were in *BRCA* mutation carriers.[70] An important question to discuss at the time of RRSO is whether to consider simultaneous total abdominal hysterectomy (TAH).

There are several potential advantages to this approach, reviewed by Domchek in 2006.[71] These include the ability to use unopposed estrogen replacement without increased risk of endometrial cancer.[72] Studies of women in the general population undergoing natural (non-surgical) menopause have indicated that the alternative to unopposed estrogen replacement, combination therapy with estrogen plus progesterone, is associated with an increased risk of breast cancer in postmenopausal women,[73] while estrogen alone is not.[74,75] Recent data have suggested that this may also be true in *BRCA1* mutation carriers.[68] Second, uterine and cervical cancer risk may be significantly increased in *BRCA1/2* mutation carriers,[76,77] although this association has not been consistently seen and indeed some authors[78] have demonstrated that the uterine cancer risk appears to only be associated with those carriers who take tamoxifen. Third, tamoxifen therapy may be recommended for some women. Tamoxifen has been shown to reduce risk of contralateral breast cancer in *BRCA1* mutation carriers[79] but is also associated with increased uterine cancer risk.[80,81] There are considerable data supporting the use of selective estrogen receptor modulators (such as tamoxifen and raloxifene) in women at elevated risk of breast cancer as determined by risk models. Data are still inadequate to assess specifically the potential for chemoprevention in *BRCA1* and *BRCA2* mutation carriers, though there are theoretical grounds for the belief that it may be more helpful in *BRCA2* mutation carriers because of the preponderance of ER positive cancers. A 2001 study found a strong trend to risk reduction using tamoxifen in breast cancer in *BRCA2* (a 62% drop) but no effect for *BRCA1* carriers, although sample size was insufficient to reach statistical significance (a total of 8 *BRCA1* mutation carriers).[82]

Finally, TAH eliminates a small risk of fallopian carcinoma from remnants in the uterus. Whether this risk is theoretical or actual has not yet been demonstrated.

On the other hand, hysterectomy is more extensive surgery and may bring an increased risk of post-surgical complications.[83] Whether the operative risk is offset by the potential cancer risk reduction benefit is determined on an individual basis. One study[84] found that 47% of women undergoing RRSO elected to have a simultaneous hysterectomy.

Determinants of Choice of RRSO and BPM

As the above discussion suggests, carriers of high breast and ovarian cancer risk mutations are confronted by multiple decisions that are not only emotionally charged, but also extremely complex.

There is wide international variation in BPM and RRSO prevalence, reflecting divergent cultural and institutional environments. In general, far more women choose RRSO than BPM. A large multicenter study of 2677 carriers of *BRCA1/2* mutations in 9 countries who received genetic testing and counseling found that, overall, 18% of women without a history of breast cancer chose BPM, at a mean age of 40.7 years. The highest rate was in the United States (36%) and the lowest in Poland (3%). RRSO was chosen by 57% of women overall, with rates as high as 73% in Norway. MRI

screening for women who had not undergone BPM varied widely, from 95% of women in Holland to 2% of women in Israel. The choice of at least one preventive option (including chemoprevention by tamoxifen) ranged from 26% in Poland to 75% in France. In the USA, 72% of women selected a preventive option.[85] Other studies have confirmed the strong preference for RRSO over BPM. A prospective multicenter study of 537 *BRCA1/2* mutation carriers in North America and Europe, found that 55% of women elected to undergo RRSO, vs 21% who chose BPM. Of women older than 40 years, 68% chose RRSO vs 43% of younger women. *BRCA1* mutation carriers and *BRCA2* mutation carriers made similar choices.[84]

BPM in particular is a highly personal decision, with significant risk of surgical complications and adverse psychological sequelae. A recurring concern has been that some women over-estimate their personal risk of developing breast cancer, generating extreme anxiety and biasing their decision about whether to undergo BPM. Although all documented *BRCA1/2* mutation carriers are felt to be at significant risk for developing breast cancer, overestimating risk may be a particular problem for women in families without known mutations and with less dramatic family histories.[86] This was noted in a 2008 review[87] which noted that preference for BPM was associated with elevated anxiety about cancer. Notably, women with elevated anxiety also tend to overestimate their actual risk, highlighting the need for comprehensive counseling.[86] Other factors that tend to promote a preference for BPM include parenthood, physician recommendation, and the number of relatives affected with breast cancer. Interestingly, one predictor of a patient later experiencing regret for choosing BPM was when a physician had first introduced the option. Women who choose RRSO have a stronger family history of ovarian cancer or a greater number of relatives with a breast cancer diagnosis. In addition, older age tends to predict a preference for RRSO relative to BPM.

Individual family history and the presence of mutations are important variables in the woman's decision. A 2008 study[88] found that women who had a sister with breast cancer were significantly more likely (OR = 2.4) to choose BPM than those without. Having a mother or sister with ovarian cancer made the choice of RRSO more likely (OR = 1.6), while a *BRCA2* mutation made a choice of RRSO less likely (OR = 0.49).

Comprehensive counseling is key. A Dutch study in 2007[89] reported that, despite gynecologic consultation, many women were not adequately informed to make a sound decision. Baseline questionnaires of 160 *BRCA1* and *BRCA2* mutation carriers who had completed child bearing were collected, and the patients followed for 12 months. Approximately 74% chose to undergo RRSO during the period, with predictors including lower levels of education and poorer general health perception. However, it was notable and concerning that 64% of those who elected surveillance believed that ovarian cancer was "often or always" a curable disease.

Comparing RRSO and BPM

Several studies have attempted to elucidate the choices women face when diagnosed with *BRCA1* and *BRCA2* mutations using decision analysis. Estimates vary widely, but in general predict a greater gain in life expectancy from BPM than from RRSO, at least when quality of life is not taken into account. One early study in 1997[90] estimated 2.9–5.3 years of increased life expectancy from BPM and from 0.3 to 1.7 years from RRSO. Gains were minimal for 60-year-old women. Among 30-year-old women, the study calculated that oophorectomy could be delayed 10 years with little loss of life expectancy. A 2002 study[91] found that a 30-year-old woman could prolong her survival 2.6 years with RRSO alone, 3.5 years with BPM and 4.9 years with both. This study also attempted to add an estimate of quality-adjusted survival, which showed a greater benefit for RRSO vs BPM

(4.4 years vs 2.6 years). Another study[92] focused on *BRCA1* mutation carriers, comparing different combinations of screening and BPM and RRSO. It estimated an 11.7 year gain in life expectancy for a 30-year-old choosing RRSO and BPM vs screening if the highest estimates of lifetime penetrance of breast and ovarian cancer were used. Interestingly, when quality of life adjustments were made, RRSO proved to be a superior strategy for women at more moderate risk, or in younger women at high risk.

The fact that women tend to prefer RRSO with intensive screening to BPM despite possible life expectancy gains reflects the esthetic and psychological effects of BPM. As Metcalfe et al. (2005) point out, in recent years, most surgeons have recommended total mastectomy to maximize risk reduction, accepting a cosmetically inferior result to that possible with other mastectomy approaches such as skin-sparing and nipple-sparing.[93] These authors review case reports and earlier studies and estimate a risk of breast cancer after nipple and areola sparing subcutaneous mastectomy of approximately 4%. This is well below the overall population average risk of 9% faced by women who do not carry high risk mutations, although still greater than the close to zero risk conferred after total mastectomy. They argue if women were offered an option of subcutaneous mastectomy, many more would choose BPM and there would be a net reduction in the incidence of breast cancer.

Conclusion

In summary, the choices facing women diagnosed with *BRCA1* and *BRCA2* mutations, or with a very strong family history of breast and/or ovarian cancer, are complex. Decisions made are highly personal, and need to be made after detailed discussion with oncologic, surgical, and possibly psychological specialists.

We require improved data on safety, efficacy, long-term side effects and effect on quality of life of these invasive procedures. With respect to ovarian cancer risk, there is a consensus that RRSO is currently the only safe option in mutation carriers, because of the difficulties of timely diagnosis and the high lethality of all but early stage disease. RRSO provides a very high, but not complete protection against ovarian cancer. There is great hope that some day an effective and validated screening tool may be developed.

In the future, we hope to be able to individualize risk prediction for women (even those with a *BRCA1* or *BRCA2* mutation) and to provide effective non-surgical management of hereditary breast and ovarian cancer risk. In the meantime, doctors must provide comprehensive information to women and help guide them as they make these difficult decisions.

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Part II
Early Detection

Chapter 5
Clinical Breast Examination and Breast Self-Examination

William H. Goodson, III

Abstract Historically, clinical breast examination (CBE) was a used as a diagnostic tool both to recognize and to diagnose breast cancer. CBE is no longer used to diagnose breast cancer, but rather is currently used as a screening test that can identify areas that might be breast cancer. Many of the observations that students are taught regarding CBE are more appropriate to advanced cancers that were common a century ago than to the smaller cancers seen in current practice. While CBE is less sensitive than mammography, it is nonetheless the primary mode of detecting the 15% of breast cancers that are missed by mammography.

Palpation of the supine patient is the essential step to detect almost all cancers that can be detected by CBE. Underlying ribs are the surface against which tissue is palpated. Observing where ribs can or cannot be felt through breast tissue is a useful way to compare different areas of the breasts of a woman and to compare the breasts one to another woman.

Breast self-examination (BSE) is an attempt to have women identify their own cancers at an early stage. Although there are many reasons to anticipate that BSE should work, randomized trials of BSE with increasingly sophisticated procedures for retraining and sustaining BSE practice have found that, although there is increased identification of benign breast abnormalities, there is no increased identification of cancer, and no improvement in breast cancer specific survival.

Silicone models are widely used to teach both CBE and BSE skills. Nonetheless, there are no data to support claims that these complicated and time-consuming methods of specific patterns and depths of palpation are the optimal way to teach or perform CBE or BSE.

Key Issues

- CBE is a screening test, not a diagnostic test
- Although CBE is less sensitive than mammograms in breast cancer detection, CBE is essential for the detection of the 15% of breast cancers that are missed by mammograms.
- Palpation of the supine patient is the essential step in conducting a CBE.
- The motion of the breast, its position and contour, and the changes in thickness with changes in posture and arm position are best understood after realizing that the breast arises from and has its closest attachments to the skin rather than to the underlying chest wall.

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- Many of the features that students are taught to identify on CBE, such as skin dimpling, peau d'orange, hard, fixed, etc., were first described before mammography and apply to advanced breast cancers, but generally not to the earlier stage cancers that are typically encountered in current practice.
- Nipple discharge is of interest only if it is spontaneous. The origin of spontaneous discharge should be localized to aid clinical work-up.
- Routine breast self-examination has not been demonstrated to improve breast cancer survival. It does increase the detection of benign abnormalities leading to more biopsies for non-malignant lesions.

Introduction

Clinical breast examination (CBE) originated as a diagnostic tool, and this origin still skews expectations of CBE into the twenty-first century. In contemporary medicine, CBE is no longer a diagnostic tool: it is a screening tool. Students, however, are still taught to look for signs from the era before microscopy and image-based screening. Many of the words they are taught are appropriate to advanced cancer rather than to screen detected cancers.

Surgical texts from the eighteenth and nineteenth centuries describe the recognition of cancer – often called a scirrus – by CBE using the words thickening, lump, mass, fixed, dimpling, retraction, skin changes, ulceration, etc. There was even debate as to whether a mass could be cancerous if it was *not* painful. Continued emphasis on these words is misleading because most of these signs are rarely seen or felt.

In the early nineteenth century there were debates between surgeons – who insisted that a cancer had to look and feel like a cancer to be a cancer – and the predecessors of pathologists who insisted that cancer should be diagnosed with "the microscope." The implication of clinical diagnosis was that if a mass felt and looked like cancer to an experienced surgeon, then it was cancer, whereas the opinion of those using the microscope was that cancer could be diagnosed only if it looked like a known pattern of malignancy under the microscope.

Even at the end of the nineteenth century surgeons diagnosed breast cancer clinically. For example, it can be inferred that Halstead practiced clinical diagnosis since, four decades after the fact, review of tissue from his original 50 cases of radical mastectomy found that 2 women did not have cancer.[1]

In 1922 MacCarty at the Mayo Clinic compared surgical opinion to pathology from 2,100 breast masses examined preoperatively by experienced surgeons. Experienced hands were wrong 11% of the time when they stated that a mass was benign and 5% of time when they were certain a mass was cancer.[2] A year later Haggard and Douglass observed that 13 of their 126 cancers had ". . .all the clinical signs of a benign tumor." They concluded "To reach a conclusion while the tumor is most amenable to surgery, microscopic evidence alone is of diagnostic importance. . . (because) malignancy (may exist) in tumors that are small, encapsulated, movable. . .without any of the external clinical evidences of malignancy." Using just the set of women who had a biopsy, sensitivity of clinical diagnosis was 90% and specificity about 90% even when the average size of cancers was 4 cm.[3]

The US Preventive Services Task Force (USPSTF) recommended routine screening mammography for women over 50 years old, but said mammography could be "with or without clinical breast examination (CBE). . .." They further opined that ". . .evidence is insufficient to recommend for or against routine CBE beyond mammography to screen for breast cancer."[4]

Even before the USPSTF web posting, physicians seemingly extrapolated the emphasis on screening mammography into a reason to abandon screening CBE. Coleman et al. summarized sequential surveys of large populations from multiple regions of the United States.[5] Receipt of CBE in conjunction with a mammogram decreased from 95 to 85% in parallel with an overall increase in the use of screening mammograms from 25 to 45%. Although percentages receiving CBE were higher, Chagpar and McMasters found a significant trend toward a longer interval between CBE.[6] Using chart review, Campbell et al. found that persons just trained in CBE actually did CBE for only 24% of eligible women.[7]

Loss of CBE skills is costly for two reasons. First, CBE is the back up for mammography, magnetic resonance imaging (MRI), and ultrasound (US), and will likely be the back up for future imaging techniques. MRI is currently the most sensitive test to diagnose breast cancer, followed by mammography, US, and CBE. However, studies of screening MRI or mammography, when conducted as *scheduled, repeated screening events over time*, also report cancers that become clinically apparent between screenings. When these interval cancers are included in the denominator, the sensitivity of image-based screening drops. The diagnosis of interval cancers, whether noticed by the patient or found by a clinician, requires CBE unless one proposes the costly and impractical step of frequently repeated image-based screening. CBE will never replace image-based screening, but it is equally likely that the need for CBE will persist.

Second, lost CBE skills are costly because inability to make observations with CBE, inability to use the information derived from CBE, and/or lack of confidence in the clinician's own observations from CBE is a major reason for physician-caused delay in the diagnosis of breast cancer. Goodson and Moore evaluated the diagnostic steps for 454 sequential breast cancers in the San Francisco Bay Area in the late 1990s when over 70% of Bay Area women had routine mammograms.[8] Forty two women had physician caused delay in diagnosis of their breast cancer. Twenty one women, half of those with a delay in diagnosis and 5% of the whole series, had delay because the physician felt a mass but reassured the woman that the mass was benign, most often after a correctly read negative mammogram. Recognition and dismissal of a mass was significantly more common when the woman was using menopausal hormones, suggesting that the fault was not in palpating the mass but rather in making the assumption that what was palpated was benign. It is likely there would be even more cases where the clinician would have reassured the patient if there had not been a positive mammogram. Whatever one's opinions about possible adverse effects of delayed diagnosis, the plaintiff's bar has focused on this sequence of events. Delayed diagnosis after a mass has been palpated is the leading cause of money paid in breast cancer related malpractice litigation.

The Utility of CBE

The Role of CBE in Recognizing Breast Cancer

The role of CBE in the detection of breast cancer is similar with or without widespread screening mammography (Table 5.1). Before widespread mammography, 11–19% of cancers were recognized by routine CBE.[9–12] With widespread mammography, 10–11% of cancers are recognized by routine CBE.[8,13] This is consistent with the known false negative rate of all image-based screening techniques.

With widespread mammography, cancer recognition attributed to the patient has declined dramatically. However, mammography receives more credit than is warranted because, in a common –

Table 5.1 How breast cancer is recognized

Author	Setting	Years for data	Cancers n=	Patient found n=	[BSE]	[Accidental]	Total percent found by patient	CBE by clinician n=		Mammography n=	
A. Before widespread mammography											
Greenwald et al.[9]	Regional breast cancer program	1975–1978	263	235	[55]	[180]	(89)	28	(11%)	none	
Huguley and Brown[10]	State cancer management network	1975–1979	2083[a]		[431]	[1191]	(79)	358	(17%)	85	(4%)
Senie et al.[11]	Urban cancer center	1976–1978	729	598			(82)	101	(14%)	30	(4%)
Muscat and Huncharek[12]	State tumor registry	1980–1982	435[b]	279			(64)	85	(19%)	13	(3%)
B. With widespread mammography											
Reeves et al.[13]	State tumor registry	1988–1990	3197	1754			(55)	321	(10%)	1122	(35%)
Goodson and Moore[38]	Academic medical center	1992–1999	454[c]	161			(35)	52	(11%)	217	(48%)

[a]Includes 18 recognized for other reasons not tabulated
[b]Includes 58 recognized for other reasons not tabulated
[c]Includes 24 recognized for other reasons not tabulated

but usually untabulated – scenario, the patient notices a mass, calls her physician, a mammogram is ordered, a suspicious abnormality is seen, and no CBE is performed either before the imaging study or before an image guided biopsy by the radiologist. Even when the patient knows that she found the mass, she states that her cancer was found by a mammogram. The risk of such practice is inappropriate reliance on mammogram at the expense of ignoring what is felt by CBE.

Sensitivity of CBE During Screening

The sensitivity of CBE is influenced by the size of the cancer and use of competing screening mammography. With CBE alone, the rate of interval cancers ranged from 17 to 42% (sensitivity 60–83%) (Table 5.2) with a specificity ranging from 84 to 98%.[14–17]

When CBE is combined with mammography, the rate of interval cancers after the screening process declines to between 2 and 38%[18–24] (38% is from the Health Insurance Plan of New York in 1971 when mammography was likely less sensitive) (Table 5.3). The sensitivity of CBE for these cancers was from 21 to 58%, with improved specificity of 96–99%.

The decreased sensitivity of CBE reflects the ability of mammography to detect cancers before they reach a palpable size: when mammograms detect nonpalpable cancers, the apparent sensitivity of CBE decreases. This, in itself, does not indicate decreased sensitivity of CBE for a mass that would have otherwise been palpable. Rather, it is the result of a shift in the population to which CBE is applied.

The decreased sensitivity in CBE when combined with mammography may also reflect a decline in CBE skills. If CBE were done less often, then a decline in skills would be expected; and if mammography is perceived as less fallible than CBE, there might be less attention to details of CBE. The latter is likely the explanation because, even if fewer benign biopsies are done, one must still account for biopsies of benign tissue that would have been done if CBE was the only screening modality. The higher specificity suggests that mammograms are used instead of biopsy for certain palpable lesions.

The Limited Ability of Clinical Breast Examination to Identify Cancer

CBE can identify most palpable cancers; but *most* is a limiting word. MacCarty demonstrated 80 years ago that, even with large cancers, experienced hands missed 10% of cancers.[2] In the first randomized trial of clinical screening of breast cancer, Venet et al. found that the presentation of cancers was not consistent: they could be soft to cystic (38%), freely movable (61%), and/or regular (41%).[18] When looking at cases that had a biopsy, the sensitivity of CBE ranges from 75 to 82% (Table 5.4A),[25,26] so, if the final decision were predicated solely on the surgeon thinking cancer was present, 18–25% of cancers would be missed.

CBE interpretation is a subjective skill; experienced clinicians can disagree.[27] Boyd et al.[27] asked four surgeons to examine 100 inpatients (Table 5.4B). Of these women, 41 had been admitted for a breast biopsy, and 15 were ultimately found to have cancer. The other 59 women were in the hospital for unrelated surgery. The surgeons did not agree on which patients needed biopsy. Individual surgeons recommended biopsy for between 12 and 14 of the cancers. No patient was recommended for biopsy by all four surgeons, and no surgeon recommend biopsy for all 15 cancers, although biopsy was recommended for every cancer by at least one surgeon.

Table 5.2 Sensitivity of screening clinical breast examination as a single modality

Author	Setting	n=	Total cancers	Screen detected cancers	Interval cancers	Sensitivity (%)	Negative CBE	Specificity (%)
Holleb et al.[14]	Urban screening center	25,629	97	63	34	65	25,074	98
Gilbertsen and Kjelsber[15]	Urban cancer detection center	46,150	104	62	44	60	Not reported	
Murimoto et al.[16]	"Experienced" university surgeons screening "about 120 subjects per day" by CBE	8,271	18	11	7	61	Not reported	
Baines et al.[17]	First screen, Canadian national breast screening study control group, women age 50–59	19,965	90	75	15	83	17,469	88
	Women age 40–49 in same study	25,620	[a]	[a]	[a]	71	[a]	84

[a]Raw data not given

Table 5.3 Sensitivity of screening CBE used in a program with mammography

Author	Setting	Screen events n=	Total cancers	Cancers found by CBE with or without mammo-gram	Cancer found only by CBE	Cancers found by mammo-graphy alone	Interval cancers	Sensitivity of CBE (%)	Specificity of CBE (%)
Venet et al.[18]	Health insurance plan of New York	65,282	214[a]	88	59	44	82	41	Not reported
Hicks et al.[19]	Regional center of breast cancer detection demonstration project	10,117	113	37	8	48	28	33	98
Rodes et al.[20]	Regional center of breast cancer detection demonstration project	31,387	152	60	19	76	16	39	Not reported
Bobo et al.[21]	Breast and cervical cancer early detection program	752,081	3,780	2224	616	1452	83	59[b]	96
Kolb et al.[22]	Urban radiology department	27,825	246	68[c]	6	75	Not measured	28[d]	99[d]
Banjec et al.[23]	First round Canadian breast cancer screening database	143,282	964	386	44	578	Not measured	40[d]	97[d]
Ostereicher et al.[24]	Urban pre-paid health plan	61,688	574	Measured but not reported	Measured but not reported	Measured but not reported	Measured but not reported	21	98

[a]There were 279 cancers in the group invited, including 65 from those who were invited but who declined screening; results are based on the 214 cancers in the group that was invited and accepted screening
[b]196,098 had no mammogram results
[c]Examiner knew results of mammogram
[d]Did not evaluate interval cancers so sensitivity is artificially high but minimal effect on specificity

Table 5.4 Accuracy of CBE before biopsy

Author	Setting	Biopsies n=	Cancer n=	Positive CBE		Negative CBE		Sensitivity (%)	Specificity (%)
				Cancer	Benign	Cancer	Benign		
A. Clinical series									
Shapiro et al.[25]	Health Insurance Plan of New York	432	85	64	111	21	236	75	68
Hansel et al.[26]	Urban teaching hospital	444	104	85	42	19	298	82	88
Author	Setting	Examined n=		Surgeon recommended biopsy		Surgeon did not recommend biopsy		Sensitivity (%)	Specificity (%)
				Cancer	Benign	Cancer	Benign		
B. Same patients examined by four surgeons									
Boyd et al.[27]	Urban teaching hospital, four surgeons evaluate the same women[a]	100	15	12	27	3	58	80	68
		100	15	13	22	2	63	87	74
		100	15	14	14	1	71	87	84
		100	15	14	21	1	64	93	75
Author	Setting	Letters n=	"urgent" referral		"soon" referral		"routine" referral		
			n=	Percent cancer	n=	Percent cancer	n=	Percent cancer	
C. Urgency indicated in referral letter as surrogate for physician's recognition of risk									
Marsh and Archer[28]	General practitioner referral letters for breast care	496	94	43	186	6	216	2	

[a]Four surgeons examined the same 100 women (41 in hospital for breast biopsy and 59 without known breast disease) and recorded whether or not they would recommend a breast biopsy for each patient

Generalists do not do better than specialists, but neither do they do much worse.[28] Using the urgency of wording in referral letters to specialist care as a proxy for the generalist's estimate of whether cancer was present (Table 5.4C), 43% of urgent referrals had cancer so an urgent referral was usually appropriate. However, 2% of routine referrals also had cancer.

The Techniques of Clinical Breast Exam

CBE is not an interpretive exercise. It is a screen for what does or does not exist. CBE is intended to detect changes in the consistency of breast tissue and areas of asymmetry in the distribution of breast tissue. Other tests are needed if an area of asymmetry is found. CBE does not seek to determine *whether* discharge can be elicited; but it does seek to localize the source of discharge if the patient has noted a spontaneous nipple discharge. When cancer exists, CBE seeks signs of advanced cancer such as changes in the visual appearance of the breasts and/ or signs of local metastases, e.g., to regional lymph nodes. Signs of advanced cancer are rarely encountered during CBE of a truly asymptomatic patient.

The patient's personal and family history, as reviewed in Chap. 1, are relevant to estimating the prior probability that cancer will be found. Nonetheless, since the majority of breast cancers occur in women without known risk factors, history is not relevant to the technique of CBE and has no role in deciding the results of CBE.

The Choreography of CBE

There are two types of CBE: Staging CBE and Screening CBE.

For patients with a palpable mass, a suspicious mammogram, or a previously diagnosed cancer, e.g., after a core biopsy in interventional radiology, a Staging CBE is used to estimate the resectability of the lump or cancer and to seek clinical signs of metastasis. The Staging CBE is usually taught to students.

The more common use of CBE in primary care is as a screen to look for signs of cancer. Many steps of Staging CBE are irrelevant when mammography is part of the screening process.[29]

Staging CBE begins with visual inspection of the breasts while the woman is sitting. Observe skin color, rash, changes in visible pore structure, and any visible masses (this observation can also be done while the patient is supine). She then lifts her arms over her head or in some manner causes the skin to shift the position of the breast. Shifting the skin up pulls the breast up. The breast typically lifts symmetrically with no visible focal tension on the skin. If cancer is present, but typically only for advanced cancers, the breast tissue does not shift shape uniformly, or the breast tissue binds to the underlying chest wall, and the pulling maneuver causes a dimpling of the skin where asymmetric or focal tension pulls on the skin. Retraction of the skin can be demonstrated on a supine patient by placing fingers on both sides of a mass and gently squeezing the mass while pulling the skin forward with the fingers. Normal skin will pinch up in a convex curve. The skin over a cancer will often be held back toward the cancer, remaining flat as the skin is pulled forward giving the appearance of what has been called the "Plateau Sign."

After observation of the breasts, palpate the common areas of lymph node enlargement. Enlarged lymph nodes may be a sign of regional metastases, but they may also be a response to infection, hematoma, or recent surgery on the breast. Normally lymph nodes are more easily felt in women

with little subcutaneous fat in the axilla, and women who use their hands for soiled tasks, e.g., garden work, care of cats, changing diapers, etc., often have small nodes. Such nodes are usually symmetric. Lack of symmetry is a reason to be suspicious.

Typically, one examines for nodes in the supraclavicular area, in the infraclavicular space, in the axilla, and the neck.

The Central Importance of Palpation of the Supine Patient

The goal of CBE is to seek palpable changes in the breast target area, to observe the skin and especially the nipple for any abnormalities, and to find the area of the nipple or areola from which a discharge comes if the patient has noted a spontaneous discharge. These goals can all be met while palpating the breasts of the supine patient.

Pennypacker and Pilgrim[30] observed that the steps of visual inspection and examining nodes are "...less sensitive than manual palpation...." In 1982, Mahoney and Csima identified 275 of 286 breast cancers by palpation of the supine patient.[31] This was before widespread mammography, but they still concluded that screening CBE should focus on palpation of the supine patient. Goodson et al. evaluated palpation as an adjunct to mammography.[29] Mammography identified 78% of cancers. Palpation of the supine patient identified 58% of cancers. If CBE was limited to supine palpation, but in conjunction with mammography, omitting visual inspection while the patient was sitting and palpation of nodes would have missed only one of 1,401 cancers.

Breast cancer can arise anywhere in breast tissue. This includes both the usual breast area and the common sites of ectopic breast tissue, including the abdominal wall in the area of the milk line of early primates (To support this point, this author has removed a fibroadenoma at the level of the umbilicus and an invasive cancer arising from intraductal cancer at the costal margin, well below the inframammary fold). A clinician doing CBE usually assumes responsibility for the area bordered by the clavicle, the mid-axillary line, the middle of the chest, and the inframammary fold (Fig. 5.1a).

How best to assure that this target area is examined is a matter of debate. A lot has been written asserting that clinicians should examine the breast in vertical strips but the objective of palpation is to examine a specific area, not to adhere to a specific pattern (see discussion of teaching CBE and BSE).

Palpation uses two or more fingers at a time, three fingers being most common. Breast tissue is palpated with the pulp rather than the tip of the fingers. As old-time safe crackers knew, the pulp of the fingertip is more sensitive than the tip. The fingers are moved in a gently rotating pattern.

Some instructors insist that palpation should be done using only one hand. The origin of one-handed exam is not certain, but it can be traced to illustrations by Haagensen, although he does not specify that CBE be done with one hand.[32] He does, however, urge the clinician to position the patient partially on her side and to balance the breast on the chest wall so that the nipple floats above the chest wall. With silicone model training there seems to have been a logistical reason to use one hand as an adaptation to limitations of the breast model. The use of one hand seems to have been perpetuated from this research setting, although there is no published comparison of one to two handed CBE.

Using a second hand during CBE stabilizes the breast minimizing wiggle, rotation, or other motion (Fig. 5.1b). We perceive bumps not as up and down, but rather by the imperceptible slowing of the motion of our fingers that occurs when we encounter a bump by running into it from the side.[33] Thus, when the breast is mobile, it seems less likely that we will notice small points

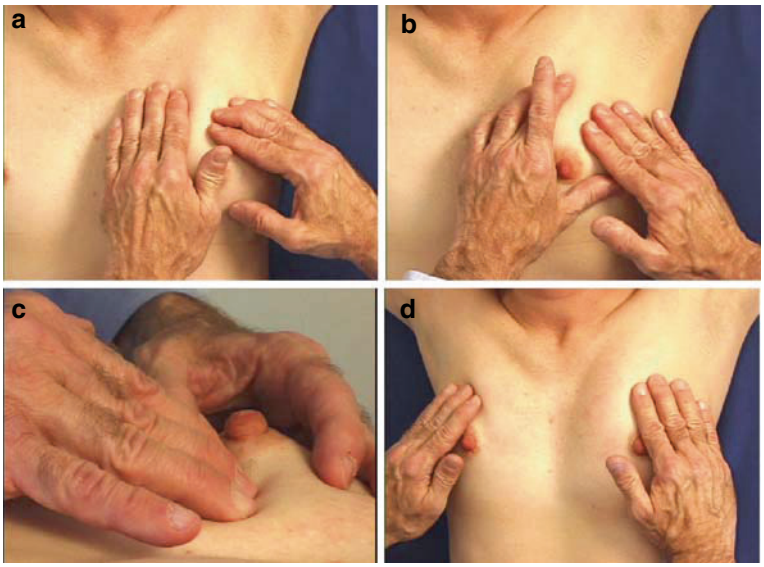

Fig. 5.1 Palpation in clinical breast examination: **a** palpation of the extreme upper inner quadrant with the left hand as part of the entire target area (note that the right hand is stabilizing the breast); **b** using the left hand to stabilize the breast and retract it medially facilitates palpation of the upper outer quadrant with the right hand; **c** palpation of an area where ribs can be easily felt through breast tissue (note that the pulp of the finger is palpating the breast tissue against the rib rather than in an arbitrary plane parallel to the floor); **d** assessing symmetry by palpating both breasts simultaneously (Reproduced with permission from www.2minutebreastexam.com where a video demonstrating palpation of the entire breast area can be viewed. Copyright 2 Minute Breast Exam LLC, San Francisco, CA)

of resistance to lateral motion of our fingers. There are strong opinions on this matter, but only a prospective trial can resolve it for certain. In an informal survey, all breast surgeons from major cancer centers used two hands.

Duration of CBE

Duration of CBE is linked to technique and opinion. Mahoney and Csima[31] concluded that the lower limit of time for a CBE is probably 2 min. This author conducts all CBEs twice and times the first exam. The initial palpation takes an average of 2 min 20 s. Rarely is any additional abnormality found on the second exam, and cancers have been found on the first exam that were both unnoticed by the patient and unrecognized by pre-visit mammograms.

The opinion supporting longer CBE duration derives from the duration of a training examination using silicone breast models which ranges from 5 to 10 min per breast model, implying 10–20 min to examine both breasts. If only one hand is used, the examiner must accommodate the rocking of the breast induced by the rotary motion of the fingers. There are no data evaluating time for CBE when the breast can be seen as well as palpated. Studies of perception have shown that sensitivity of touch increases when visual attention is focused on the area being touched.[34] This author believes that 2 min is the minimal acceptable time, but there are no data other than measurement using silicone models. As discussed below, that method does not duplicate the conditions of CBE.

The Nipple and Areola

During lactation and nursing, milk collects in the ducts just under the skin of the areola. The infant rolls its tongue under the nipple and areola to express milk. Except when distended with milk, the area behind the nipple feels soft and the surrounding edge of breast tissue at the edge of the areola creates the feeling of an edge that can be interpreted as a mass. Retroareolar masses can be felt by palpating around the areola. Irregularities in the firmer breast tissue around the areola are common, and finding a symmetric edge around the contralateral areola suggests that what it being felt is within normal limits. The nipple should be gently palpated to ensure consistency on both sides.

Inspection of the nipple is a visual process, easiest while the breast is being palpated and the light source is directly overhead. The nipples are generally of the same shape and coloring. If they are not symmetric, ask the patient if she has noted the difference, and if so, for how long. Look for crusting, scaling or dry skin, or bleeding from broken skin. If there is a rash or break in the skin, notice whether it involves the nipple, the skin of the areola, or both. Eczema of the nipple is more common than Paget's disease. Paget's disease rarely involves the areolar skin unless the nipple is also involved, whereas eczema often involves the areola but spares the nipple. Response to topical corticosteroids can be misleading and causes apparent improvement of Paget's disease as well as eczema, so observing a response to steroid creams is not a diagnostic test.

Discharge

There is sometimes a misguided impulse to elicit a nipple discharge during screening CBE, but this is counterproductive because discharge can be elicited from women with no demonstrable pathology. In studies of nipple fluid, Petrakis et al. could elicit nipple fluid from 70% of Caucasian women, 40% of African American women, and 24% of Asian women.[35]

If a discharge is spontaneous, e.g., if she has noted a spot on her gown or brassiere, it is appropriate to locate the section of the breast from which the discharge comes. Locating the source of a discharge is done in two steps. First, press at sequential positions just outside of the edge of the areola (usually with one finger at a time) until all areas around the areola have been pressed. If focal pressure causes

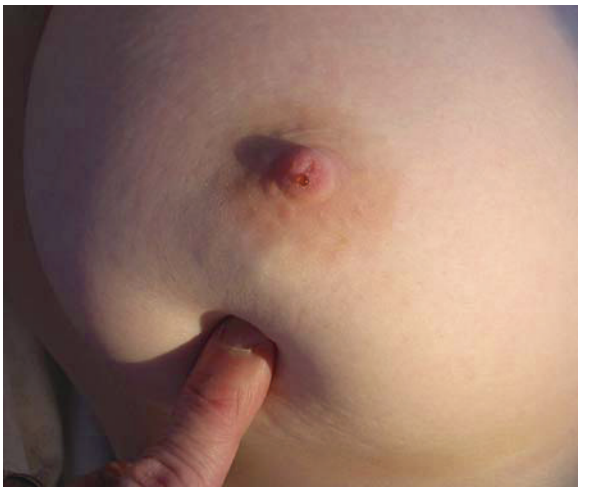

Fig. 5.2 Locating the source of discharge. Pressure on one point in the lower outer quadrant of the right breast causes discharge. The location of this point is recorded for use by the radiologist and the surgeon

a discharge like what the patient observed, record the number of centimeters from the nipple and the o'clock position as though the areola was the face of a clock with the head as the 12 o'clock position.

If pressing around the areola does not duplicate the discharge, repeat the pattern rolling a finger from the periphery of the breast toward the nipple from all locations around the areola. If a discharge is elicited, record the position (Fig. 5.2). Location information is used to plan duct excision or to aid performance of contrast ductography. It is useful to repeat the examination several days apart to assure consistent findings.

Understanding CBE

The breast is a modified sweat gland. It begins as a special cluster of cells in the chest wall skin of the fetus. This anlage thickens and extends into the subcutaneous tissue between the skin and the hard surface constituted by the underlying ribs and muscles. Blood vessels enter the breast from the mesodermal tissue, mostly from medial and lateral connections. The breast connects to the skin more closely than to the chest wall. The plane between the gland of the breast and the fascia of the muscles is a loose fatty layer that can be easily dissected with little more than a push of the hand (unless the surgeon takes the pectoral fascia), whereas removing breast tissue from skin requires step by step dissection in most women.

The effect of a primary skin rather than chest wall attachment is that the breast hangs from the skin. When a woman is upright, the breast tissue hangs down and much of the tissue can be below the level of the nipple. When a woman is supine, the breast tissue hangs to the side, often in an upward position that follows the posterior curve of the chest wall as the ribs become smaller toward the clavicle and shoulder.

Normal breast tissue is flexible and changes shape as the patient changes her position. When she is sitting, the breast falls to one area in a closed shape. When she is supine, the breast spreads out over a wider area. The ability of normal breast tissue to spread out for palpation is the same characteristic that radiologists use for spot compression films to evaluate whether they are seeing a mass as opposed to seeing a shadow of tissue that is clumped by chance related to positioning – rather than pulled into a tight mass of tissue by the fibrosis around a tumor.

Mobility is a characteristic of the normal breast, and loss of mobility indicates that something has increased the breast's attachment to the chest wall. Loss of mobility is typically seen in a reconstructed breast. The fibrous capsule around an implant reconstruction attaches to the chest wall (often intentionally behind the pectoral muscle), and a breast reconstructed with autologous tissue adheres to the chest wall, even if it is in front of the pectoral muscle. The result is that reconstructed breasts have only one position, i.e., the same position regardless of whether the patient is sitting or lying, and typically not flattening when the patient is supine.

The next general characteristic of breast tissue is the surface texture or nodularity. Such nodularity is common, and it is quite influenced by a woman's previous breast size relative to the current size. There is no rule that predicts breast nodularity, but some changes are predictable: a woman who has been pregnant, nursed and weaned a child, and lost weight back to her pre-pregnancy weight will often have more nodular breasts. The cause of the nodularity is unproven, but these changes reported by Linthal Cheattle over 80 years ago[36] still seem likely: "As age advances, and especially after lactation, these ligaments become more dense, and their terminal branches as they reach the skin enclose lobules of fat which are separated from each other. When the surface of such a breast

is palpated it gives rise to a sensation of diffuse nodularity of the underlying gland which in these circumstances cannot be felt at all."

Nodularity in the breast is not disease, and the degree of nodularity does not relate to risk of future cancer. Goodson et al. prospectively recorded nodularity with an ordinal scale for 87 women who were about to have partial mastectomies.[37] Because the criteria for adequate resection was 2 mm or greater margins, margin specimens were essentially samples of tissue from high risk breast tissue with various degrees of nodularity. They found no significant relationship between breast nodularity and the presence (or absence) of proliferative fibrocystic change, atypical ductal or lobular hyperplasia, or unrecognized in situ cancer. Although a larger sample might have found some relationship, a relationship that requires large numbers to detect would have little relevance to an individual patient.

During palpation, one feels the summation of skin, subcutaneous fat, gland tissue of the breast, fatty tissue between the breast and the chest wall, muscles of the chest wall, and ribs. The underlying ribs create the framework for a systematic evaluation of breast tissue (Fig. 5.1c).

The breast can be considered as consisting of two areas: those where ribs can be felt through breast tissue and those areas where ribs cannot be felt. Interference with palpation of ribs by breast tissue, a property called durity, reflects the resistance of the breast tissue to deformity, the total amount of tissue, and a composite of durity and the amount of tissue.[38]

For reference, the extreme upper *inner* quadrant of all but women with the largest breasts is usually such that ribs can be felt through the breast. In areas where underlying ribs can be felt, they function as a virtual second hand behind the breast and almost any mass or nodule will be felt as something that interferes with the continuity of the ribs as they are felt through the breast. In areas where nothing interferes with palpation of ribs through the breast, the examiner can be confident that there is no palpable abnormality.

The extent of area where ribs cannot be felt (where breast tissue has greater durity) varies from the entire breast, which is uncommon, to breasts where ribs can be palpated through all parts of the breast. Typically, durity is greatest in the upper outer quadrant. Usually, the areas where ribs cannot be felt are symmetric. If there is an area in one breast but not the other where ribs cannot be felt, this must be considered abnormal.

The second characteristic of areas with greater durity is surface texture. The nodularity one feels on the surface of the tissue is usually the same on both sides. If it is not, an explanation should be sought. Finally, the examiner should palpate areas where ribs cannot be felt. While it will not be possible to palpate ribs in all cases, and inability to palpate ribs does not necessarily imply that an abnormality is present, one knows they have successfully palpated deeply into breast tissue only if they feel the underlying ribs.

The Significance of Palpability

Cancers recognized between screening mammograms, usually by palpation, have a less favorable outcome. It is assumed that these cancers were too small to be seen at the time of the mammogram, and they became palpable in the interval between screenings because they grew quickly. This presumed faster growth is assumed to indicate more aggressive cancers. Although these inferences are probably correct in general, they miss the fact that palpability itself indicates a more aggressive nature of the cancer (Table 5.5). Cancers recognized between screening mammograms, usually by palpation, have higher growth rates,[39] tend to be higher grade,[40,41] and have less favorable survival.[42,43]

Table 5.5 The significance of palpability

Author	Setting	Tumor palpability	Biopsies n=	Total cancers	Invasive cancers		In situ cancers		Node positive	
A. Invasive vs non-invasive cancer										
Rodes et al.[20]	Regional cancer center	Palpable	[a]	60	56	(93%)	4	(7%)	22	(37%)
		Nonpalpable		76	54	(71%)	22	(29%)	12	(16%)
Pagana et al.[44]	Community cancer center	Palpable	[b]	112	108	(96%)	4	(4%)	46	(45%)
		Nonpalpable		60	44	(73%)	16	(27%)	6	(10%)
Bassett et al.[45]	University medical center	Palpable	143	48	46	(96%)	2	(4%)	16	(33%)
		Nonpalpable	229	72	49	(68%)	23	(32%)	11	(15%)
Reintigen et al.[46]	Urban cancer center	Palpable	[c]	345	326	(94%)	19	(4%)	163	(47%)
		Nonpalpable		208	132	(63%)	76	(37%)	25	(12%)
Perdue et al.[47]	Military medical center	Palpable	589	65	61	94%)	4	(6%)	[c]	
		Nonpalpable	372	55	28	(51%)	27	(49%)	[c]	

			Tumor size							
			0–1 cm		11–2.0 cm		2.1–3.0 cm		> 3 cm	
Author	Setting	Tumor palpality	n=	Percent (+) nodes	n=	Percent (+) nodes	n=	Percent (+) nodes	n=	Percent (+) nodes
B. Node involvement vs palpability at different tumor sizes										
Reintigen et al.[46]	Urban cancer center	Palpable	21	0	136	43	76	45	58	71
		Nonpalpable	127	5	74	18	10	40	4	25

Table 5.5 (continued)

Author	Setting	Tumor palpality	n=	Node positive (%)	Stage I (%)	Median 9 year recurrence (%)
C. Palpability vs survival						
Lung et al.[48]	Regional tumor institute	Palpable Nonpalpable	111 55	23 11	49 58	24 15
						10 Year survival
Lopez et al.[49]	State cancer detection demon-stration project	Palpable Nonpalpable	60 76	37 16		61 85
						5 year survival
Sener et al.[50]	Urban university medical center	Palpable Nonpalpable	677 372			87 94

[a]576 biopsies combined
[b]482 biopsies combined
[c]Not reported

That palpable cancers are more likely to be invasive is a tautology of the fact that identification of in situ cancers has been a major result of screening mammograms.[20,44–47] Of palpable cancers, four to seven percent are in situ, whereas 27–49% of nonpalpable (image-detected) cancers are in situ (Table 5.5A). Because invasive cancers are generally more aggressive and have a less favorable outcome, it can be argued that invasiveness rather than palpability that is associated with poorer prognosis.

However, Reintigen et al. compared the presence of lymph node metastasis in palpable vs nonpalpable invasive tumors of the same size.[46] Palpable tumors were more likely to have positive nodes than nonpalpable tumors of the same size in three of four size categories. In addition to a higher percentage of positive nodes and higher staging in general, palpable tumors have lower survival at 5, 9, and 10 years from diagnosis.[48–50] Palpable tumors, the objective of CBE, are very relevant to the future health of patients.

The Significance of Asymmetry

Symmetry is assessed by examining both breasts simultaneously (Fig. 5.1d).

The breasts of many women are asymmetric. Typically, the left breast is larger both by estimates of breast volume and by measurements of the distance from the sternal notch to the nipple.[51,52] Asymmetry is clinically detectable in nearly half of women, and about 10% have marked asymmetry.[52] Focal asymmetry should always be assessed further.

Generalized asymmetry is usually physiologic. In generalized asymmetry, if there is more tissue in the upper inner quadrant of the breast, there will be proportionately more tissue in the upper outer quadrant, the lower outer quadrant, and possibly even noticeably more tissue in the lower inner quadrant; and this can tentatively be attributed to individual variation especially if it is the left breast that is larger. If asymmetry has been observed *and recorded* in the past, then further evaluation is unnecessary. If stability is uncertain, further evaluation or, at a minimum, short term follow up is appropriate.

General asymmetry may indicate increased risk. Kopans et al. found that mammographic breast asymmetry with no other mammographic abnormality and without palpable changes was not associated with cancer, but 3 of 40 women with palpable density associated with mammographic asymmetry (without other mammographic signs) had cancer.[53]

In a case control study, Scutt et al. used measurements from mammograms to estimate the absolute fluctuation of symmetry between breasts.[54] Fluctuating asymmetry, which they likened to developmental asymmetry, was more common in women with cancer than in controls, but cancers did not develop preferentially in the larger breast.

Changes Caused by Previous Surgery

Surgery can leave both more dense areas of scar and/or less dense areas where tissue has been removed. Some surgeons favor a skin-only closure assuming that hematoma and/or seroma will fill the space, the body will infiltrate the hematoma with connective tissue, and fat will replace the connective tissue. This may happen sometimes, but scars tend to contract so skin-only closures can cause retraction, a defect or a dent in the breast. The dent is easy to recognize, but the firmness of the edges of the defect can suggest a mass adjacent to the scar. To assess this during CBE, stabilize the defect with a finger in the defect and palpate around the edge of the defect with the other hand. An

adjacent mass usually has another edge that is encountered while palpating around the circumference of the defect.

At the other extreme, previous surgery leaves a palpable scar or density that would be suspicious for cancer if there were no history of surgery. It is important to describe meticulously the scar recording its position, distance from the nipple, and dimensions. It is usually safe to assume that a scar which becomes apparent immediately after biopsy or treatment of cancer is a benign process. This assumption, however, requires confirmation, usually in the form of fine needle aspiration to demonstrate benign cells and/or careful reobservation after several months.

Knowledge that a mass appeared soon after surgery and is stable can be very reassuring, but such knowledge will only be available if previous examiners have kept detailed records. Both to help one's self, and as a courtesy to subsequent examiners, meticulously record post operative changes as soon as they are noted, especially after breast conserving treatment of cancer.

What Cancer Feels Like

Cancer is most often recognized as an irregularity in breast tissue that does not change during palpation. Although there are cancers that feel like well-defined nodules with sharp edges, they are less common.

Usually it is not the cancer itself but rather the fibrosis elicited by the interaction of the cancer cells with stromal cells that is palpated. Cancer extension into surrounding tissue causes it to become fixed. This is not to be confused with fixation to skin or the underlying chest wall seen in advanced cancer. It is rather that motion of the cancer drags surrounding tissue with it. Haagensen described this phenomenon: "The hardness of the tumor and its relative fixation in the area of breast tissue in which it lies, making it impossible to move it without carrying along the surrounding breast tissue, are the features which most often suggest that it is a carcinoma."[32]

Sanfillipo et al. emphasized the importance of a discrete mass:

> . . .[a] true mass ordinarily has margins on all sides and is asymmetric with the other breast. Women with chronic cystic breast disease [sic] can have diffusely granular breasts or ones with regular firmness. . .When the density is different in quality or quantity from the general consistency, it must be considered a dominant mass. . .simultaneous palpation of both breasts can help determine if the breasts are pathologically asymmetrical. If there is still doubt, it is an acceptable practice in premenopausal women is to repeat the examination after 2 or 3 weeks, when they are in a different phase of the menstrual cycle. If the previous finding is the result of hormonal stimulation, it may be resolve by this time.[55]

Early cancer is like getting a bit of chewing gum or a bad tangle in long hair. Neither the chewing gum nor the core of the tangle are felt. Instead, the hair simply does not move right. It does not have the flexibility of the hair that is uninvolved. This is like the "wiggle test" described by experienced pathologists during gross examination of a breast biopsy. Non-malignant tissue, even when very dense, will flex in a uniform, evenly distributed fashion. Benign tissue will flex around the cancer with the cancer itself being relatively inflexible.

The reason to know that cancer moves with surrounding tissue – that it draws normal tissue along with it as it is moved by examining fingers – is to emphasize that many if not the majority of small cancers are felt, not as lumps, but rather as irregular areas where the tissue simply does not move or is not symmetric. Biopsy (open biopsy or fine needle aspiration by a trained person) is necessary to know whether a cancer exists.

Breast Self-Examination

The idea of patient participation in the recognition of breast cancer began in the late nineteenth century when physicians observed that the prognosis was related to the number of "infected" nodes at the time of surgery. Simultaneously, physicians noted that women had often noticed changes in their breast long before they sought medical care.

It was argued that if women were induced to seek help as soon as they noted changes in their breasts (notwithstanding the severity of treatments then in use), they would have longer survival from their cancers. It was this spirit that led to formal efforts to change women's behavior such as Cancer Weeks that were begun in several major cities shortly before World War I.

Cancer Weeks were somewhat successful. As noted, in 1923 Haggard and Douglass reported that only half of their breast biopsies had found cancer whereas, just 10 years earlier, almost all of their biopsies had found cancer.[3] Attention was being spent on masses that were not malignant, and one can infer there was a parallel increase in false positive breast examinations leading to the benign biopsies.

Building on these successes, Haagensen in 1952 published a comprehensive description of BSE – including parallel strip patterns to assure examination of the entire breast area that has been essentially unchanged since[56] (Fig. 5.3a). This dovetailed with cancer awareness campaigns sponsored by the American Cancer Society and the advice to see a physician if one felt a "lump or thickening in the breast."

Over several decades, published series compared the outcome of cancers found by BSE to cancers found in women who did not practice BSE[9,11,57,58,59–61] (Table 5.6). The average cancer size was smaller when it was detected in women who practiced regular or monthly BSE, women who practiced BSE had lower stage of cancer at diagnosis, and it appeared that women who practiced BSE had better survival from breast cancer. Women who practiced BSE less frequently than monthly

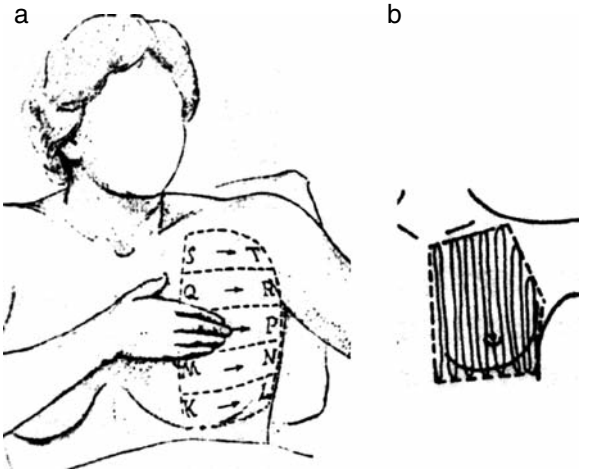

Fig. 5.3 Strip patterns for breast examination. Strip patterns have been suggested to insure that the entire breast area is examined, but such patterns may not be necessary during CBE if the examiner uses another method to examine the entire breast area: **a** transverse strips as originally described by Haagensen; **b** vertical strips as modified by Saunders and Pennypacker (**a**. Reprinted with permission from Haagensen.[56] Copyright © 1952, American Medical Association. All Rights reserved. **b**. Reprinted from Saunders KJ, et al.[69] Copyright © 1986 American Cancer Society. This material is reproduced with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

Table 5.6 Projected benefits of BSE

Author	Setting	Method cancer detection	n=	Mean tumor size (cm)	Percent negative nodes	Percent Stage 0 or I	Survival (%) 5 Years	8 Years
Foster et al.[57]	Breast cancer network demonstration project	BSE monthly	61	1.97	60	54[a]		
		BSE < monthly	68	2.47	32	33		
		No BSE	117	3.59	9	17		
Greenwald et al.[9]	Regional breast cancer program	Routine BSE[b]	79	2.39	65	21		
		No BSE	178	3.00	59	17		
Huguley et al.[58]	State breast demonstration project	BSE	1400			30		70
		No BSE	683			19		51
Senie et al.[11]	Urban cancer center	BSE	944	2.82	57			
		No BSE	459	3.54	50			
Feldman et al.[59]	Urban medical center	BSE monthly or several times a year	408	2.5	48	54		
		BSE never or rarely	588	3.3	38	39		
Foster et al.[60]	Breast cancer network demonstration project	BSE monthly	194	2.1		22[a]	75[c]	
		BSE < monthly	228	2.4		14		
		No BSE	410	3.2		4	57	
Auvinen et al.[61]	National program in a European country	BSE monthly				57	80	
		BSE < monthly				57	80	
		No BSE				54	79	

[a]Expanded series in 1984 with clinical stage in 1978 and pathologic stage in 1984
[b]Includes routine CBE
[c]Monthly and less than monthly BSE were not different, so pooled

had size and staging intermediate between regular BSE and no BSE. Interestingly, in two studies in which survival was estimated, there was no difference between regular and less than regular BSE. The explanation probably is that even among women who practice regular BSE, a large portion of the cancers are still identified as an accidental recognition at a time different from their regular BSE.[58,60]

The fact that a woman who practices BSE may still find her cancer by chance suggests that the benefit of BSE training is primarily linked to a greater awareness of self at all times, not simply during a scheduled self-evaluation. It may also be that learning to do BSE teaches the trainee how to be less apprehensive about looking at herself. Based on these suggestions of success, epidemiologists, notably Anthony Miller in Toronto, Canada suggested that "In most parts of the world. . . [BSE would be] a potentially valuable method of early diagnosis which need not be expensive in resources."[62] However, Miller et al. also recognized the basic problem of lead-time bias and that ". . .survival or case fatality comparison between BSE-detected and symptomatic cancers are inevitably biased, and comparisons of survival between all breast cancers diagnosed in populations offered or not offered BSE containing programmes do not overcome this problem. The only unbiased measure of effectiveness is a comparison of the number of women who die of breast cancer in the BSE population compared with a control population. . .."

Largely through the leadership of Miller and his colleagues, the World Health Organization launched two prospective, randomized, population-based trials of BSE, the first in what is now the Former Soviet Union and the other in Shanghai, China.

Even before the World Health Organization trials began, the UK Trial of Early Detection of Breast Cancer began a study of BSE by enrolling 236,103 women from 8 centers in 8 annual cohorts beginning late in 1979[63] (Table 5.7). Two of the centers offered screening that included annual clinical breast examination and biannual mammography. In two centers, the women were taught breast self-examination as a single event without subsequent follow-up training. Four centers had no specific breast cancer detection program. Initially, all women between age 45 and 64 were invited. Thereafter, women were invited to enroll when they reached 45 years of age. After an average of 14 years follow-up, there was a 27% decrease in breast cancer mortality among women treated in the centers that offered screening with clinical breast examination and mammography. There was no decrease in mortality for women whose only intervention was being taught BSE (Fig. 5.4a). The limitation of this study was that the women were taught BSE is a single session, and there was not an intense program to encourage the performance of BSE. Retraining efforts were, however, a component of the World Health Organization sponsored trials.

Due to political disruption surrounding the collapse of the former Soviet Union, the part of the Russian BSE trial in Moscow has not been completed. However, the St. Petersburg and Shanghai trials have both been completed. They had similar designs and similar results.

The St. Petersburg portion of the Russian trial began in 1985 under the direction of Vladimir Semiglazov.[64] In the Soviet Union, healthcare was primarily through regional polyclinics from which patients were referred to cancer centers for further diagnosis and treatment. Twenty eight regional polyclinics were randomized as entire units for patients to be taught BSE or to have routine care such that 120,471 women were randomized to receive BSE training, or not, on the basis of which polyclinic they attended. The frequent criticism of this study is that, 4 years into the study, a random sample of 100 women from each of the clinics receiving BSE training found that only 40% or less were doing monthly BSE. What is overlooked is that this was recognized in 1989, and the reeducation programs were instituted at that time. In follow up surveys over the next 6 years, 70% or more did BSE at least five or more times a year. After 12 years of the trial, there was no improvement in breast cancer survival for women trained to do BSE (Fig. 5.4b). There were, however, significantly more biopsies for women who had been trained in BSE. Miller et al.[62] specified

Table 5.7 Prospective trials of BSE

Author	Setting	Design	Start date	Study groups	n=	Performance measures				
						Breast cancer deaths up to 10 years	Breast cancer deaths up to 16 years	Relative risk of breast cancer death	(95% CI)	
UK trial of early detection of breast cancer group[63]	Eight centers in England and Scotland	Two centers annual CBE and biannual mammogram; two centers BSE taught; four centers usual care	1980	Screen BSE[a] Usual Care	67,888 81,847 165,558	282 529 1001	360 661 1312	0.79 1.05 1	(0.07–0.88) (0.95–1.15)	
						BSE 5 or more times per year	Self-referral for "lump"	Biopsies n=	Cancer n=	Mortality per 100,000 women
Semiglazov et al.[64]	28 Regional and industrial polyclinics in St Petersburg, Russia	Randomized to teach BSE or usual care	1984	BSE Usual care	57,712 64,759	76%[b] N/A	4,340 2,438	1138[c] 797[c]	493 446	272 253

Table 5.7 (continued)

Author	Setting	Design	Start date	Study groups	n=	Performance measures: Detected 3-mm simulated mass in breast model[d]	Biopsies n=	Cancer n=	Died of breast cancer
Thomas et al.[65]	Clinics located in textile factories	Randomized all workers and retirees according to their factory	1989	BSE Usual care	132,979 133,085	61% 42%	3620[c] 2395[c]	864 896	135 131

[a]Attendance at BSE training ranged from 14 to 56%
[b]Random sample of 400 women from each polyclinic found that after first 4 years (using only BSE reminder cards) monthly BSE dropped from 53 to 18%.Thereafter, instituted reeducation every 3 years
[c] $p < 0.05$ in St. Petersburg
[d]Measured in random sample immediately after training. No assessment of frequency of BSE

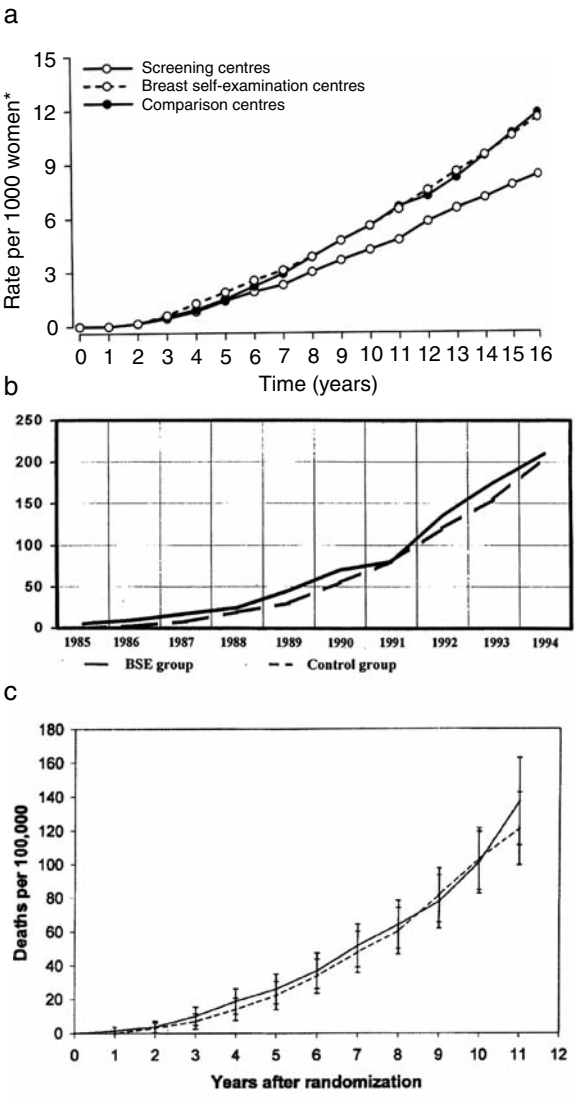

Fig. 5.4 Breast cancer mortality in prospective trials of breast self-examination. None of the trials has shown a decrease in mortality: **a** UK Trial of early detection of breast cancer[63] showing no difference in control vs. BSE groups (the third group (*lower line*) with better survival had annual CBE and biannual mammograms.); **b** St. Petersburg trial[64] showing no difference in survival; **c** Shanghai trial[65] showing no difference in survival (**a**. Reprinted from UK Trial of Early Detection of Breast Cancer Group, Copyright © 1999, with permission from Elsevier.[63] **b**. Reprinted with permission from Semiglasov VF, et al.[64] and **c**. Reprinted from Thomas DB, et al.[65] with permission of the National Cancer Institute)

that subjects be over age 40 because "A disadvantage of educating young women is that harmless benign breast disease is commoner in young women than in old and identification of this by BSE might lead to an unnecessary increase in biopsies." Apparently, this happens in women of all ages.

The Shanghai trial began accrual in 1989.[65] In China at that time, healthcare was organized around one's place of work (as in Russia, political disruption during the trial might have had some effect) and 256,064 women from 250 textile factories were randomized to receive, or not receive, BSE training on the basis of where they worked or had worked in the case of retirees. There were intensive group meetings and reeducation sessions to encourage continued practice of BSE throughout the study. Of note, however, is that, even with this training, the proportion of women who correctly identified simulated masses in silicone breast models never exceeded about 70%, while a

random sample of women from control clinics found 40% of simulated masses. Thus, the untrained women were still capable of finding masses, and the trained women, though better, were not even twice as successful. They did not estimate BSE frequency but it is unlikely that a more intensive program of reeducation could be carried out, so this is probably the best that could be expected. Again, there was no survival benefit associated with BSE training (Fig. 5.4c) and, again, there were significantly more biopsies for benign disease in the BSE-trained group.

As these three trials began at about 5-year intervals, there was a stepwise increase in reeducation efforts. Otherwise, they have similar designs whereby BSE was taught to all eligible women on the basis of their usual place to receive healthcare.

From these studies one can conclude that BSE is not a reliable *stand-alone screening technique for early detection of breast cancer when other technology is available*. However, BSE in conjunction with heightened clinical awareness is still the option available – perhaps the only option – in some settings. For example, a recent public awareness program in Malaysia based primarily on BSE has reduced the average size of breast cancers at diagnosis.[66] Thus, it is difficult to deride BSE for all settings and, as noted, training in self-awareness might be the major benefit of BSE for women whose cancers are not identified by mammograms.

Teaching CBE and BSE

Starting in the 1970s, the Psychology Department at the University of Florida attempted to parse BSE into component parts to improve the design of BSE training (Table 5.8). Four studies have become the basis for many efforts to teach BSE and CBE.

Adams et al. tested the concept that silicone breast models might be used to analyze and to teach palpation skills.[67] Based on sales data, their breast model was designed "...to approximate closely the size and physical characteristics of the young, well-supported, 'B-cup' breast of a young woman. The [embedded, simulated] lumps were judged to simulate accurately the characteristics of firm, well-fixed tumors."

Simulating the "well supported, 'B-cup' of a young woman" meant that the model was and is unlike the post menopausal woman for whom cancer is a greater risk. Because the model was transparent, subjects reached through a screen with one hand so that they would not be able to see the simulated masses that were visible through the transparent models being used.

This limitation of their model had two consequences: they restricted testing to palpation with a single hand, and they separated visual from touch perception. Separation of visual and touch sensations might correctly model BSE, but sense of touch improves when one looks at the area being touched,[34] so the study does not accurately model CBE in which the clinician can and should focus vision on the breast while CBE is done.

Hall et al. tested the ability of subjects to detect breast masses in six human "stimulus subjects."[68] The stimulus subjects were women with longstanding breast masses (presumably benign). Each stimulus subject had been examined by a panel of three clinical experts, and the experts all agreed on the existence and location of 13 masses in the 6 women.

Twenty women with no previous physical examination training were asked to touch the breasts of the stimulus patients and to identify whatever masses they could find. Half were then trained in a 30-min session using silicone breast models with simulated masses and all research participants re-examined the same stimulus patients. Prior to training, both groups identified 25% of the masses on the pre-test. After half of the group was trained, trainees increased their ability to find masses to 48%. Also after training, both examination duration and the number of "false positives" increased

Table 5.8 Studies cited in support of the basic claims of the VS3P method of breast exam and use of silicone breast models

Author	Subjects	n=	Study measure	Pre intervention results	Intervention		Post intervention results	Comment
Adams, et al.[67]	Unpaid volunteers	16 (one man)	Detection of simulated masses in transparent silicone breast models		26 sequential trials to examine four models presented in random order; repeated daily until stable results for four subjects		50% detection if 2.4 cm diameter	Detectability depended on lump size; results stable after mean 1.5 daily sessions
Hall et al.[68]	Women with no previous training in breast examination	20	Detection of masses by palpation of six, separate volunteer women who had long standing breast masses	Found 25% of masses	30 min supervised training using patterned search on a silicone model containing simulated breast masses		Found 48% of masses	Training also associated with increased false positives and longer exam
Saunders et al.[69]	Undergraduate women	28	Percentage of the breast exam target area palpated during self-exam[a]	Subjects palpated 28% of chest target area	Subjects read instructions for three search patterns (concentric circles, radial spoke, vertical strip); applied patterns with no further instruction	Concentric circles	39% of target area	Possible bias since authors themselves scored area covered by observation
						Radial spoke	45% of target area	
						Vertical strip	68% of target area[b]	

Table 5.8 (continued)

Author	Subjects	n=	Study measure	Pre intervention results	Intervention	Breast model type	Post intervention results	Comment
McDermott et al.[70]	Internal medicine residents and faculty	79	Effect of model characteristics on finding simulated masses in silicone breast models manufactured to be either more soft/less nodular or less soft/more nodular	Found 51% of simulated masses	Half hour video then instruction with silicone model and patient-instructor	less soft/more nodular model	61% of simulated masses[c]	Sensitivity improved with both types of models, but training decreased specificity (p=0.02) with less dense/more nodular models (data not shown)
				Found 65% of simulated masses		More soft/less nodular model	74% of simulated masses[c]	

[a]Target area bordered by the clavicle, midline of the chest, mid-axillary line, and the fifth rib (i.e. the inframammary fold)
[b]Significantly more than the other two other search patterns, $p<0.01$
[c]$p < 0.02$ compared to pretest for the group using that model type

significantly. For both trainees and controls their initial experience involved touching an intimate part of the body of an unfamiliar human so that their pretest sensitivity reflected both unfamiliarity with the skill and social inhibitions to touching a stranger in an intimate fashion. Thus this study demonstrates that a silicone model can be used to teach breast examination, but the study does not inform whether using silicone models is the optimal way or even a necessary way to enhance the breast examination skills of persons who have some knowledge of breast examination or who have previously been involved in physical diagnosis. Superiority of the model is not established – only that it can be used.

Saunders et al. evaluated whether a woman could visualize how to apply written instructions for the pattern she would use to touch all of her own breasts.[69] For the pretest, subjects without training were asked to examine their breasts. Researchers doing the study observed the percentage of the usual target area of CBE that untrained women touched. Subjects then read a written description of one of the three patterns of breast self-examination, and the same observers watched women examine their own breasts using the described pattern. There was no other instruction, and there was no trial with feedback before the evaluation. Using the vertical strip method (Fig. 5.3b), subjects examined 64% of the target area compared to 38–48% of the target area with the concentric circle or radial spoke methods.

This study is cited to claim superiority of the vertical strip method for both BSE and CBE. However, results were based on a single session in which the training in all three patterns of palpation was accomplished by having the subject read a document without training on a model or patient and without feedback. Second, the area covered was scored by visual observation, and these observations were made by the first two authors without apparent methodology to minimize bias. Interestingly, subjects spent 30% more time doing the radial spoke as well as the vertical strip methods (compared to the concentric circle method) suggesting that these patterns are less intuitive than concentric circles and that using more time was an artifact of difficulty in applying the instructions. The fact that similar time was taken to do the radial spoke and vertical strip methods supports the interpretation that a person without training in medical observation often has more difficulty examining the breast using these techniques than if instructed to move their hand in concentric circles.

Although there was an increase in the percentage of the target area palpated by subjects after training, at best only two-thirds of the target area was covered. Complete coverage was not achieved suggesting a need for feedback, and the study does not provide data that either the vertical or horizontal strip technique is superior to any alternative systematic effort to cover the entire breast area. A clinician, for example, can see the target area and consciously decide to cover the areas, just as the observers in this study were able to check off the areas of the breast that were examined by their subjects.

McDermott et al. evaluated the effect of using various silicone models that were manufactured with different characteristics.[70] Sensitivity increased after training with both more dense/less nodular models and less dense/more nodular models. Specificity, however, decreased in the more nodular models. This would indicate that it is more difficult to decide that a simulated mass does not exist in a more nodular model. It is likely that clinicians know intuitively this decreased specificity, and compensate for it by raising their threshold for concern, since physician-caused delay in diagnosis of breast cancer is more common when breasts are softer and more nodular.[38]

Using three pressures in CBE to palpate at three depths shows up in subsequent papers, but the references are to the papers just cited.[67–70] The study designs involved positioning simulated masses at different depths. The authors then taught subjects to focus on the different levels they had manufactured in order to find simulated masses at three depths. They did not assess the three pressures as independent variables. Nevertheless, these papers are frequently cited as the basis for

Table 5.9 Training clinicians to recognize simulated masses in silicone breast models[a]

Author	Subjects	n=	End points of study	Intervention	Sensitivity (%)		Specificity (%)			Comment
					Pre test	Post test	Pre test	Post test		
Fletcher et al.[71]	Untrained women	311	Identify 18 simulated masses in 6 silicone breast models	None	None	40	None	66		Duration of EXAM correlated positively with Sensitivity, negatively with Specificity
	Physicians, of which 81% had prior CBE Training	62		None	None	58	None	52		
									Percent of routine visits when CBE done in following 6 months	
Campbell et al.[7]	Nurses and residents randomized to training vs no training	43 trained	Identify 18 simulated masses in 6 silicone breast models	1 h training in six components of CBE combined with volunteer patient and silicone model; post test 4 months later	57	63	56	41	24[b]	Trainees did fewer CBEs in clinic follow-up[b]; controls decreased mean exam time by almost 1 min
		46 Control			57	56	56	68	38[b]	

Table 5.9 (continued)

Author	Subjects	n=	End points of study	Intervention	Sensitivity (%)		Specificity (%)		Referral to specialist	Comment
					Pre test	Post test	Pre test	Post test		
									Refer to specialist	
Smith et al.[72]	Managed care, primary care physician panel	985	Identification of simulated masses in a silicone beast model 6 months after training	In-office, 1 h training session	24	83			2.2	Sensitivity 74% in six month follow-up of random sample
		"controls"[c]							1.9	
							False positives (%)			
Trapp et al.[73]	Nurses attending a conference	34	Identify 18 simulated masses in 6 silicone breast models	3.5 day training in CBE	None	76	None	6		Median 9.8 min per breast model; longer search correlated with higher sensitivity
Vetto et al.[74]	Statewide sample of primary care providers	205	Detection of five simulated lumps in a silicone breast model	1.5 h self-assessment, 2 h training including patient-instructor	55	82	23	14		Percent using five or more minutes increased from 23 to 44%
Steiner et al.[75]	Non-randomized residents with (R-1) and without (R-2) training	50 trained	Identify single 3 mm nodule in silicone breast model	1 h self-study followed by 2 h training with silicone model and surrogate patient	None	84	None	21		Longer exam, vertical strip search pattern, varying Pressure correlated with sensitivity
		65 Control			None	46	None	13		

[a]Sensitivity, specificity, and false positive are in silicone breast models
[b] $p<0.05$, authors′ statistics
[c]"Controls" were a comparison group drawn from practices not trained for this study

palpating at three depths during BSE and CBE training using what has been called the "vertical strip three pressure" or VS3P method using silicone models.

BSE and CBE trainees using the VS3P method have greater success at finding simulated masses in silicone models[7,71–75](Table 5.9). However, follow up of trainees when they return to the clinic has been disappointing.

As expected, Smith et al. found increased sensitivity to simulated masses in silicone breast models after in-office training of individuals and small groups from a managed care provider panel.[72] In follow up, trainees referred 2.2% of women to specialists compared to a 1.9% referral rate from untrained clinicians. However, since the difference is only 0.3% and they present no historical or other data to suggest pre-training similarity of the trained and untrained clinicians, the data do not constitute a study of the effect of VS3P training on clinical practice.

In the one study with actual clinical follow up, Campbell et al. found that trainee specificity using silicone models decreased in parallel with increased sensitivity on the silicone models.[7] Controls did not reduce specificity. When the trainees returned to the clinic, however, they actually did fewer CBEs. The authors found that trainees did CBE for only 24% of women who might have been expected to have a routine CBE, whereas controls did a CBE for 38% of eligible women ($p<0.05$ according to the authors).

The suggestion from the work of Campbell et al. is that the complexity of the VS3P method is so daunting that clinicians do not start. A key issue may be the clinician's time: the VS3P method requires 5–10 min to palpate one simulated breast and that is unlikely to be much faster with real patients.

A recently tested alternative approach is to emphasize that the entire breast area must be palpated and to ask clinicians to record CBE results, not as a check box, but rather using a dedicated form to record what they have felt.[76] There was no training in any CBE technique. Using this simple intervention, Goodson et al. doubled the call rate of CBE into the range reported for large screening studies. Clinicians found the number of cancers that would be expected from SEER data and using the form was associated with fewer rather than more biopsies. Although further study is needed, the potential advantage of this approach is that it does not seek more clinician time which is likely why VS3P trainees do fewer CBEs.

The Everpresent False Positive Screening Evaluation

By one estimate, if a woman has routine CBEs for 10 years, there is a 13% chance that she will have a false positive CBE,[77] or 300 false positive exams for 10,905 exams. During the same time, 30 cancers were found on the basis of palpation (7 by routine CBE and 23 by the patient noting an abnormality).

All screening results in false positive screens. A century ago, when women were asked to seek advice for any mass they noted, the portion of false positive biopsies went from negligible to half of all biopsies. When clinicians or patients are trained using silicone models, there is an increase in the number of false positives. When surgeons examine the same patients, they vary in the number of patients for whom they perform biopsies and they vary in which patients from the same population for whom they recommend a biopsy.

The issue is not whether there are false positives, but whether the supposed harm of a false positive screen test is as significant as some authors claim. Studies have compared the anxiety of women who have had a false positive screen to women who had a screen and were told they were okay.

The appropriate reference point, however, is to compare the anxiety of women who have cancer and wonder if it might have been detected earlier if they had had a routine CBE, or if the bar for mammography had been set to be more sensitive, even at the sacrifice of some specificity. Until the anxiety of these women is compared to the anxiety of women who had false positive screening evaluation, the issue of anxiety with false positive screening is not adequately addressed.

When to Say When

Because CBE is a subjective test, the best that any clinician can do is to be careful. Unlike mammograms or other breast images that can be reviewed later by an "expert," CBE is only in the hands of the person who is there at the time. The encouragement we found in our study using a dedicated paper form to record the CBE was that, with an attention-focusing device as simple as a dedicated form, clinicians found the cancers that were anticipated to be present.

The time to say "When" is when the clinician has focused his or her attention and looked carefully at the patient. This should be done at least once a year, but an on-line survey has found that less than one quarter of respondents receive a basic CBE even yearly.

As a profession, we are not yet ready to say "When."

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Chapter 6
Mammography

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Abstract Mammography is one of an array of breast imaging modalities used to evaluate women with clinical breast symptoms, and its utility for this is well established. However, it is as a screening tool that mammography makes its unique contribution to the detection and treatment of early breast cancer, and it is in this role that it has received the most visibility and, sometimes, controversy. Despite its limitations, namely decreased sensitivity in premenopausal women and in women with radiographically dense breast parenchyma, mammography remains the only imaging modality that is proven to reduce breast cancer mortality.

This chapter discusses mammography from its historic perspective as well as from the perspective of the larger topic of screening for preclinical disease. Structured mammography screening programs are generally most effective for the detection of non-palpable breast cancer while minimizing false positive studies. Digital and film screen mammography are different acquisition methods of the same imaging study and, overall, demonstrate equivalent effectiveness in the detection of breast cancer. Radiographic breast density and its relevance to breast cancer detection and breast cancer risk has been extensively studied and continues to be a topic for research and debate. Digital breast tomography is an application of digital mammography that is still a research tool but shows promise for increasing the accuracy of mammography. The American College of Radiology (ACR) has developed a lexicon to report mammograms, based on standardized criteria for interpretation of mammographic findings. Research has demonstrated that when radiologists adhere to these criteria, they increase their cancer detection rate. The evaluation of mammography findings is presented, using the ACR lexicon.

Keywords Preclinical disease · Breast cancer screening · Tomosynthesis · BIRADS · Contrast enhanced mammography

Key Issues

- Mammography remains the only breast imaging modality which, when used within a screening program, is documented to reduce breast cancer mortality. Overall, mammograms can detect 80% of nonpalpable breast cancers. Mammography is less effective in women with radiographicly dense breast tissue, premenopausal women, and in women with BRCA mutations, particularly the Type 1 mutation.

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- The structure of a mammography screening program is essential to its effectiveness. In the United States, there is federal oversight of mammography facilities to ensure adherence to FDA requirements for performance of mammography.
- The expertise of the interpreting radiologist has significant impact on the effectiveness of mammography screening. Research has demonstrated that subspecialist breast imaging radiologists have a higher cancer detection rate, with a lower false positive rate, than general radiologists.
- The false positive and false negative rate of mammography remains a concern. Digital breast tomosynthesis is an application of digital mammography that shows promise for improving the accuracy of mammography.
- The American College of Radiology has developed a lexicon for reporting of mammograms, based on standardized criteria for interpretation of mammographic findings. Research has demonstrated that when radiologists adhere to these criteria they increase their cancer detection rate. We present an overview of mammography findings, described utilizing the ACR lexicon.

History of Mammography

Radiography has been used to evaluate the symptomatic breast for many decades, beginning in the early twentieth century.[1] In 1913 Salomon described his results radiographing mastectomy specimens and documenting radiographic soft tissue findings as well as the presence of calcifications. More recently, the application of radiography to screening for breast cancer has been a major advance for women's health. Screening asymptomatic women for breast cancer was first studied in the 1960's with the Health Insurance Plan Project (HIP) study in 1963, in which 62,000 women ages 40–64, randomized to invitation vs no invitation to screening with mammography and physical exam. At 10 year follow-up, breast cancer mortality was 29% lower in the screened group. In the 1960's and 1970's the Breast Cancer Detection Demonstration Project (BCDDP), a cohort study enrolling 50,000 women who were given 5 rounds of annual mammogram and physical exam, observed a cumulative breast cancer mortality which was 20% lower than a control group composed of unscreened women in the Surveillance, Epidemiology, and End Results (SEER) population. These projects further advanced knowledge of imaging preclinical disease. The development of mammography-specific screen film systems in the 1970's and mammography-specific machines in the 1980's accelerated the field of screening mammography. The standards of care established over these decades serve not only to inform the current practice of mammography but also guide the research that is investigating other imaging modalities for preclinical cancer detection.

The cumulative results of randomized controlled trials performed in the 1970's and early 1980's showed a significant reduction in breast cancer mortality among women who were screened.[2] Interestingly, these trials all preceded the major advances in mammography image quality that resulted from dedicated screen film systems and dedicated mammography machines. It is reasonable to wonder how much greater the impact of screening is with the current equipment that can detect more and smaller cancers.

Screening Mammography Today

Overview

Screening mammography has been practiced and much discussed for over 4 decades. Although the topic may feel familiar, as we explore other modalities for screening for breast cancer, the

same principles for screening mammography will apply and therefore it is worth a review of this topic.

Screening by definition involves a systematic method for testing asymptomatic people for preclinical disease.[3,4] The rationale is that treatment of disease detected in a preclinical stage will improve outcome as compared to detection and treatment instituted after the disease becomes symptomatic. This rationale has been validated by the results of seven randomized screening trials performed in Europe and North America.[2] But as we become more aware of the heterogeneous nature of breast cancer it is obvious that the topic continues to merit discussion. The breast cancers detected at mammographic screening may be biologically distinct from the breast cancers diagnosed clinically as interval cancers.[5,6] Screening methods for breast cancer detection continue to be studied to determine the best means for diagnosing these different types of cancer.[7,8]

The analysis of screening effectiveness is complex. The effectiveness of a test for symptomatic clinical disease can be analyzed by a binary model: disease is either present or absent.[3] However, since screening for a disease can occur anywhere during the evolution of the disease from asymptomatic to symptomatic, the analysis of its effectiveness is much more complex. All screening tests have false positive results, and all false positive results result in some morbidity to the patient. Thus, the morbidity from inevitable false positives must be balanced against the benefit of early detection, so understanding the evolution of the preclinical phase of the disease being tested for is critical to understanding how to balance the morbidity of the process of screening against its benefit.

An important difference for a screening test, as compared to a diagnostic test for symptomatic disease, is that increasing sensitivity of the screening test does not necessarily provide greater benefit.[9] Increased sensitivity of the screening process may detect more abnormalities but these may be clinically insignificant pseudodisease. Thus the net effect may be increased morbidity (from the detection and treatment of clinically insignificant findings) without improving overall outcome. This has important implications for interpretation of screening mammograms, where increasing sensitivity of interpretation must always be balanced against increased false positives. This must also be remembered as we investigate other methods for early detection of breast cancer. That is, another modality may be able to detect cancers that are not visible on mammography but this must be shown to increase benefit to the patient as compared to using on mammography alone.

Radiation Risk and Mammography

There has never been a case of breast cancer attributable to mammography. However, concern over radiation dose has been longstanding. In 1974, the Breast Exposure: Nationwide Trends Program allowed the FDA to evaluate radiation dose in mammography, which spurred the development of the low dose systems which are the precursors of the units used today. This concern is based on data identifying excess breast cancers in three groups of women who received significant chest wall or breast radiation (doses in excess of 100 rad) at a very young age: North American women treated with radiation for post partum mastitis, North American women with tuberculosis who received multiple chest fluoroscopic exams, and Japanese women who survived the atomic bomb.[10] At that time, the doses used in mammography were 3–4 rad per view; today, the dose is 0.1–0.2 rad per view. There is no evidence to suggest that even the higher doses of older mammography exams pose any radiation risk to women over 30. Because of concern for potential radiation risk to breast tissue of young women, as well as the very low yield for finding cancer in this age group, mammography is generally not indicated for women under 30.

Screening Mammography in Practice

The goal of any screening mammography practice is to find as many non-invasive or small in size, node negative invasive breast cancers as possible, while creating as few false positive findings as possible, in order to maximize benefit and minimize morbidity in this asymptomatic population. To this end, screening mammography performs far better when it is part of a structured program. In screening mammography, which by definition is performed in asymptomatic women, the pre-test probability of disease is about 0.5%. To detect these few malignancies while protecting the other 99.5% of women who do not have cancer from unnecessary morbidity and anxiety requires a structured screening program. Based on the results of published audits of screening practices, standards for evaluating the performance of a screening mammography practice have been developed. These standards include evaluation of numbers of screening callbacks and false positive studies, cancer detection rate, positive predictive value of biopsy recommendation, as well as characteristics of screen detected cancers: size, stage, and node positivity.[11–14]

Success of a screening program depends on many factors. Among them is the interpretive expertise of the radiologists. Multiple published audits of screening practices have found that subspecialist breast imagers detect about twice the number of cancers per thousand women as compared with general radiologists, with half the recall rate.[15–17] This suggests that screening effectiveness, including minimizing morbidity and the cost of false positive findings is optimized in a subspecialist setting. Subspecialist breast imagers further reduce morbidity by their expertise at performing minimally invasive percutaneous image-guided needle biopsy, which significantly minimizes the need for open surgical biopsies.[18–20] This effectiveness is sometimes accompanied by some increase in inconvenience for women as, by definition, these subspecialist settings will be more centralized and less numerous than non-specialist settings. In addition, there is concern that restricting mammography to subspecialist settings would result in inadequate service capacity in the United States.[21]

Screening recommendations vary from country to country. In the United States, for the general population of women, screening mammography is recommended annually beginning at age 40 [although recent recommendations from the US Preventive Task Force (see reference 4 in Chapter 5), which to date have not been accepted by other national agencies, suggest that screening begin at age 50]. In many countries in Europe, screening is performed at less frequent intervals. Long term follow-up of breast cancer incidence and outcomes from these different approaches is producing data that may help us better understand the biology of breast cancer.[7,22] Younger women (aged 40–49) may experience greater impact from screening in spite of their lower incidence of breast cancer, due to their longer life expectancy.[23]

Unique to screening mammography is the existence of federal oversight. Mammography became more widely utilized during the 1970's and 1980's as a result of technical advances, specifically the creation of specialized screen film systems and the development of dedicated mammography units, which significantly improved the diagnostic quality of mammographic images while also significantly reducing radiation dose. With the increased use of mammography came concern over radiation dose, and this concern prompted the evaluation of mammography facilities. These evaluations uncovered wide variations in quality of the performance of mammography, due to great variation in the adoption of specialized mammogram units and film screen combinations, and due to variation in the quality of mammographic positioning and image processing.[24] Concern over this variability in quality led the American Cancer Society (ACS) to approach the American College of Radiology (ACR) to create a program to establish quality standards for mammography sites.[25] The ACR Mammography Accreditation Program created standards for clinical image quality, phantom image quality, glandular dose, and processor performance. The intent of the program was not merely to survey quality but also to educate mammography sites about how to implement quality assurance programs to optimize the success of their mammography programs. This voluntary program began

in 1987 and, by 1991, one half of mammography units in the United States had applied for this accreditation. The importance of this program was validated by the early results, which showed that about one third of the applications failed the evaluation, more than half for clinical image quality. Of the sites that failed initially, almost all passed the evaluation on the second attempt, a testament to the effectiveness of the educational component of the program. In 1990, the legislation that provided for Medicare reimbursement for screening mammography included a requirement for quality standards for sites performing mammography on Medicare eligible women. An additional bill passed in 1991, the Women's Health Equity Act, includes the Breast Cancer Screening Safety Act which requires every mammography site in the country to be accredited through a national accreditation program such as the ACR MAP and to undergo additional federal or state inspection. Under the Mammography Quality Standards Act (MQSA), signed into law in October 1992, the FDA was given responsibility for implementing these regulations.

Breast Imaging Reporting and Data System (BIRADS)

As standards for performance of mammography were being developed, it was noted that there was also widespread variation in physician interpretation and reporting of mammograms.[26,27] Reports often did not clearly describe findings nor clearly communicate what, if anything, should be done about the findings.[28] Since, in the asymptomatic screening patient, the findings on the mammogram dictate all further action, clear communication of findings and clear recommendations for further management are mandatory. In 1993, the ACR created the Breast Imaging Reporting and Data System (BIRADS), a dictionary of standardized descriptors for the reporting of findings, and the MQSA mandates that all reports include one of the specified final assessment categories.[29] The final assessment category that is mandated on all mammography reports is intended to communicate, without equivocation, precisely what the radiologist is recommending on the basis of the mammogram findings. In addition to standardizing terminology for improved communication, research has demonstrated that training in the use of BIRADS categories improves radiologist performance in screening mammography, specifically improving feature analysis, increasing consistency for final assessment of findings, and, most significantly, improving biopsy rate for malignant lesions with no significant increase in biopsy rate of benign lesions[30,31] (Table 6.1)

Each assessment category has a definition of terms. Categories 1 and 2 are self explanatory. Category 3 (probably benign) by definition means that the finding has <2% chance of malignancy, which does not warrant biopsy but for which short interval follow-up is recommended.[32,33] Usually this is performed as follow-up at 6 months, 12 months, and 24 months. Category 4 includes all findings with >2% chance of malignancy so it is a very broad category. Recently the BIRADS lexicon has expanded category 4 to include subcategories for low, intermediate, and higher suspicion. However, there is no corresponding data that evaluates risk of various types of mammographic findings, so these subcategories are based on empiric assessments. Category 5 is any finding considered

Table 6.1 Final assessment categories

Assessment category 0, the study is incomplete and further evaluation is necessary
Assessment category 1, negative, no evidence of malignancy
Assessment category 2, benign finding with no suspicion for malignancy
Assessment category 3, probably benign finding for which short interval follow-up is recommended
Assessment category 4, suspicious finding for which biopsy is recommended
Assessment category 5, highly suspicious finding for which biopsy is recommended
Assessment category 6, biopsy proven cancer

>95% likely to be malignant. Category 6 applies to mammograms performed in women who have a known malignancy prior to or during treatment, for instance as follow-up to chemotherapy before definitive surgery.

Film Screen vs Digital Mammography

A mammogram is the image obtained after an X-ray is passed through the breast and recorded on a receptor. The X-ray beam is attenuated by the tissue components of the breast to a greater or lesser degree, and the resulting image is a record of these interactions. Malignant tissue commonly attenuates an X-ray differently than normal breast parenchyma, and so can be detected on the mammogram. The receptor that acquires the X-ray image can be a film screen cassette (film screen mammography) or a digital receptor (digital mammography).[34] Digital mammography has several advantages over film screen mammography, especially regarding image management and storage. In film screen, the mammogram film serves as the medium for acquisition, display, and storage of the image. In digital imaging each of these functions can be optimized individually. Radiation dose of digital mammography also tends to be lower than film screen mammography. The first digital mammogram unit obtained FDA approval for sale in the US in 2001, so this is relatively new technology.

A number of studies have examined the question of cancer detection on film screen as compared to digital mammography.[35–37] The question of superiority of cancer detection was addressed in the DMIST trial, which studied the results of nearly 50,000 women who had film screen and digital mammograms performed and interpreted independently on the same day.[38] Overall, the same number of cancers was found in each modality, suggesting that overall film screen and digital mammography are equivalent. Each modality missed some cancers that the other found. Digital mammography performed slightly better in radiographically dense breasts, and conversely in fatty breasts film screen performed slightly better (although not to statistical significance) than digital.[12] As of this writing, fewer than half of all mammography units in the United States are digital. For the many women who will be having their mammogram on a film screen unit, the results of the DMIST trial should be reassuring. Expert interpretation is much more important than the method of acquiring the mammogram.

Regardless of the method of image acquisition, all mammograms are performed the same way. Two standard views are obtained, craniocaudal and mediolateral oblique. In these views, the technologist uses a compression paddle to spread out the breast tissue as much as possible given the individual woman's breast size and texture. This compression accomplishes quite a lot: radiation dose is reduced, scatter radiation is reduced which sharpens image quality, and the posterior breast tissue is held in place so that it can be included in the image. Most women find this compression uncomfortable but quite bearable for the 1–2 s that are required to obtain the image. For the few women who experience severe discomfort, there are a number of techniques that can help make the exam more tolerable.[39]

Special Situations: Imaging Implants

Breast augmentation is increasing rapidly in the US. Mammography is performed slightly differently on women with implants and generally will require at least eight views, craniocaudal and mediolateral oblique views with and without displacement of the implant.[40] The routine views image the implant itself (although not the posterior extent) and some posterior breast tissue at the margins

of the implant. The implant-displaced views are performed to image the breast tissue overlying the implant. For these views, the technologist displaces the implant posteriorly and superiorly and places as much of the mobile breast tissue as possible on the image receptor, compressing and imaging the tissue with the implant displaced out of the field of view. Usually, much of the breast tissue can be imaged. Some posterior tissue will always be obscured by the implant but, interestingly, research has failed to show any negative impact on breast cancer diagnosis.

Breast Density

The radiographic density of breast parenchyma, apart from the interpretation challenges that it presents, has also been studied as a marker of risk for development of breast cancer. The first study correlating breast parenchymal patterns with breast cancer risk was published in 1976.[41] Since then multiple studies have generally supported this correlation, although it is obvious that the relationship is complex. Interestingly, in his earliest work over 30 years ago, Wolfe brought up issues that have continued to be pertinent: he recognized that reproducibility of standardized assessment of parenchymal patterns would be difficult, and he theorized that the cancers that develop in fatty as opposed to dense breasts might be biologically different.[42] The reason for this relationship is not yet known, and is being extensively researched. Radiographic density of the breast is due to the presence of varying amounts of the epithelial cells, stromal cells, collagen, and fat that make up the breast. The relative amounts of each of these tissue components vary greatly and are felt to be influenced by endogenous and exogenous factors that also influence breast cancer risk.[43]

The relationship of radiographic breast density to breast cancer risk is complex, with interesting exceptions. For instance, obesity is a known risk factor for breast cancer yet is strongly associated with decreased breast density. Asian women have a lower risk of breast cancer than Caucasian women but typically have dense breast parenchyma.[44] Although there is no research to support this, there are some who consider breast density to be a risk factor that justifies screening with modalities other than mammography, such as ultrasound or MRI. Note that any attempt to quantify breast cancer risk based on parenchymal density is limited by the intrinsic difficulty of accurately and reproducibly quantifying parenchymal density on a conventional two-view mammogram.[45]

Interpretation of the Mammogram

To the outside observer, interpretation of a mammogram may seem much more art than science but, in fact, interpretation criteria for mammography are well established and based on decades of research and follow-up.[46–48] As this chapter is not intended as training for image interpretation, the following simply represents an overview of the approach to interpreting a mammogram, to provide the clinician with a framework for understanding the process of analysis of the mammogram images. As discussed above, the findings on the mammogram are evaluated and described using the American College of Radiology (ACR) Breast Imaging Reporting and Data System (BIRADS).[29]

The initial approach to the mammogram is assessment of adequacy of positioning and technique to ensure that the images are interpretable. Second, breast density is assessed. The American College of Radiology (ACR) Breast Imaging Reporting and Data System (BIRADS) identifies four major groups for classifying breast density[49] (Fig. 6.1):[1] predominantly fat (<25% glandular tissue);[2] fat with some fibroglandular tissue (25–50% glandular tissue);[3] heterogeneously dense (50–75% glandular tissue);[4] extremely dense (>75% glandular tissue).

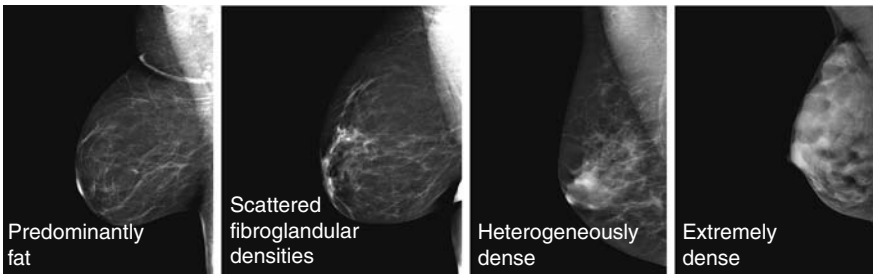

Fig. 6.1 Right mediolateral oblique (MLO) views demonstrate the four different categories of breast density in four patients: **a** predominantly fat; **b** scattered fibroglandular densities; **c** heterogeneously dense; **d** extremely dense

Masses

Following this, comparison with prior films is performed, if available, and an assessment of symmetry is made. If there is an asymmetry (a one-view finding), a focal asymmetry (a two-view finding without definite mass margins) or a mass visualized, spot compression and 90° lateral views are performed. If the asymmetry persists as a mass on additional views, the mass size, location, shape, margins, density, and associated features are described. The shape of a mass is described as round, oval, lobular, or irregular. The mass margins are described as circumscribed, obscured, microlobulated, indistinct, or spiculated. If a mass is identified, ultrasound is performed for further characterization. If, however, the asymmetry disappears with additional views and has the appearance of confluent glandular tissue, no further work up is required (Figs. 6.2, 6.3, 6.4 and 6.5).

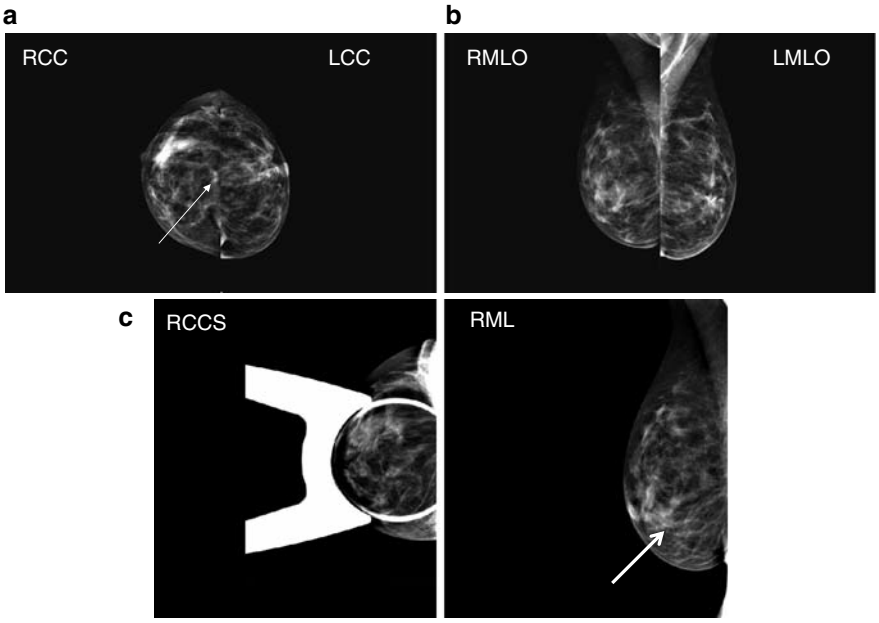

Fig. 6.2 **a** In the deep central right breast, visible on the craniocaudal (CC) view, 6 cm from the nipple, there is a 1 cm asymmetry. Spot compression CC and 90° lateral views were requested. **b** Spot compression and 90° lateral views of the right breast were obtained. The asymmetry compresses into normal fibroglandular tissue with changes related to reduction mammoplasty. No mass is identified

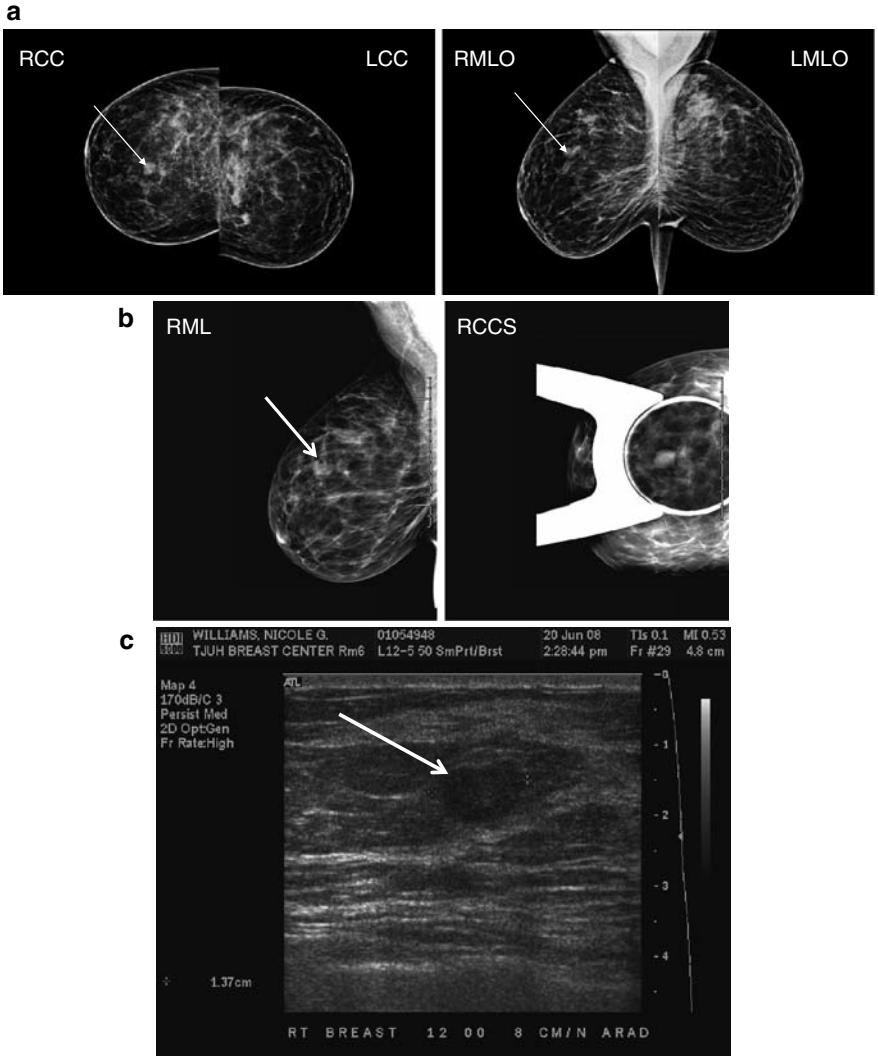

Fig. 6.3 **a** There is a 1.4-cm mass at the 12:00 position, 8 cm from the nipple. Spot compression and 90° lateral views, in addition to right breast ultrasound were requested. **b** Additional views confirm the presence of an oval mass with circumscribed margins at the 12:00 position of the right breast. **c** Targeted right breast ultrasound demonstrates a circumscribed lobulated mass. Ultrasound guided core biopsy yielded pathology results of fibroadenoma

Calcifications

Once symmetry is established, both breasts are evaluated for calcifications. The distribution of calcifications is assessed, if present. Terms used for distribution include diffuse/scattered, regional, grouped/clustered, linear, and segmental. Diffuse/scattered calcifications are distributed randomly throughout the breast and are usually benign. Calcifications in a regional distribution occupy >2 cc of tissue and do not conform to a ductal pattern. A cluster of calcifications implies that there are at least five calcifications in 1 cc of tissue. Segmental calcifications conform to a ductal distribution. Once distribution is established, a description of calcification appearance is made. Typically benign

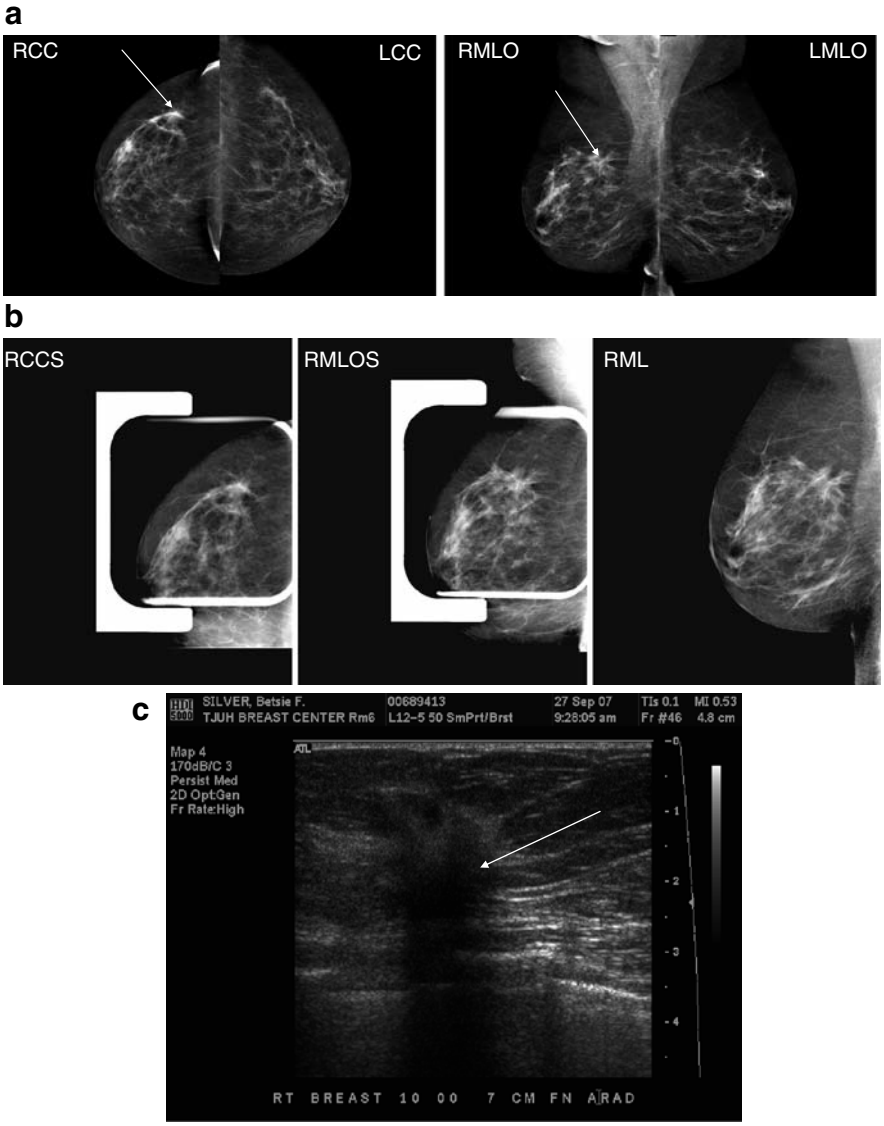

Fig. 6.4 **a** In the upper outer right breast approximately 7 cm from the nipple, there is a focal asymmetry. Spot compression and 90° lateral views were requested. **b** Spot compression and 90° lateral views of the right breast were obtained. The focal asymmetry persist as an irregular mass with spiculated margins, at the 10:00 position, 7 cm from the nipple. **c** Targeted ultrasound to the area of concern demonstrates an irregular hypoechoic mass with indistinct margins and posterior acoustic shadowing. Ultrasound guided core biopsy was performed yielding invasive lobular carcinoma

calcifications include vascular, popcorn, large rod like, round, lucent centered, milk of calcium, sutural, and dystrophic. Calcifications of intermediate concern include amorphous or coarse heterogeneous types. Those with higher probability of malignancy include fine pleomorphic or fine linear branching calcifications. To characterize calcifications, a 90° lateral view is performed to exclude milk of calcium as an etiology. Spot magnification views are performed to evaluate and describe accurately the appearance (Figs. 6.6 and 6.7).

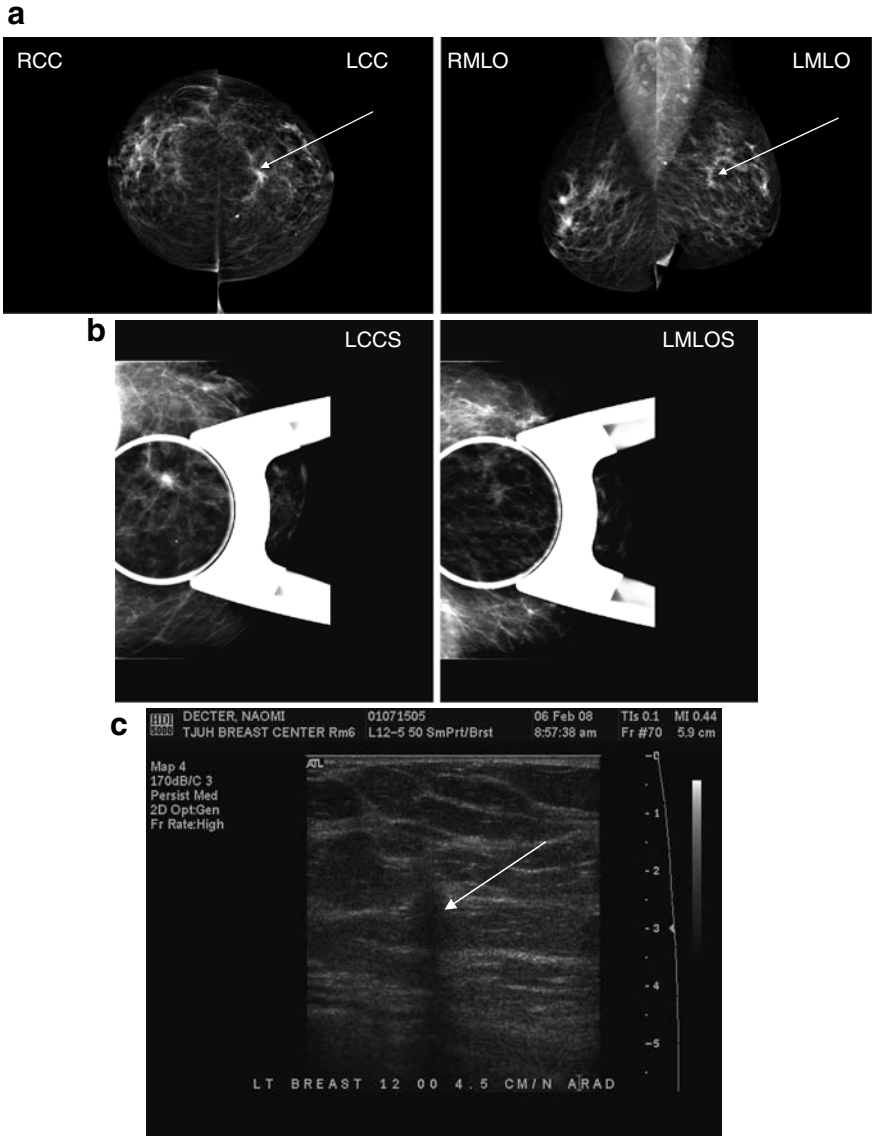

Fig. 6.5 **a** There is a 1-cm irregular mass at the 12:00 position of the left breast. Spot compression and 90° lateral views in addition to left breast ultrasound were requested. **b** Spot compression views confirm the presence of an irregular mass with spiculated margins at the 12:00 position. **c** Targeted ultrasound demonstrates an irregular hypoechoic mass with indistinct margins and posterior acoustic shadowing. Ultrasound guided core biopsy was performed yielding invasive ductal carcinoma

Emerging Technologies in Digital Mammography: Digital Tomosynthesis and Contrast Enhanced Mammography

Some of the limitations of mammography are due to the limitations of displaying a three dimensional structure on a two dimensional image. The shadows by overlapping tissues can both obscure cancer (false negative result) and can create pseudomasses (false positive result). The greater the amount

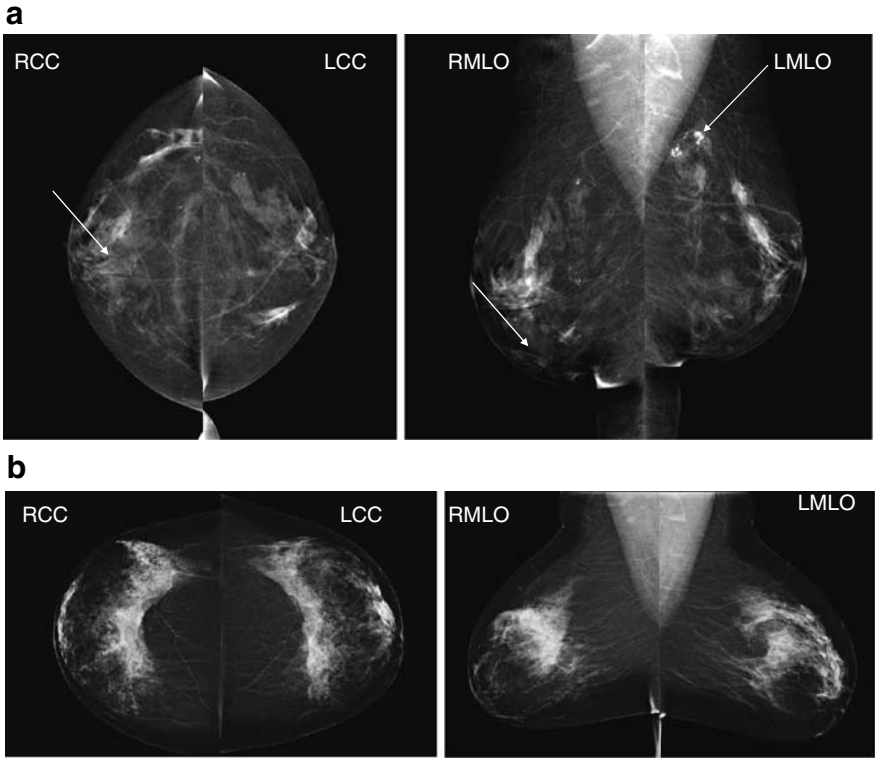

Fig. 6.6 **a** There are multiple calcifications in both breasts, some of which are dystrophic and some which contain lucent centers. They have a benign appearance. Obtaining a relevant history is important. **b** No calcifications are seen on the patient's prior mammogram but the breast size is significantly reduced on the more recent examination (**a**). The calcifications are therefore secondary to fat necrosis from breast reduction surgery

of radiographically dense tissue in the breast, the greater the likelihood of both false positives and false negatives from summation shadows. This helps explain the observation that cancers that present in the interval between screenings are more common in women with dense breasts. Digital breast tomosynthesis is an application of digital mammography that seeks to address these limitations.

Tomosynthesis is the acquisition of multiple thin-section two dimensional images of the body which are reconstructed into a three dimensional image. In breast digital tomosynthesis, the conventional digital mammography platform is modified such that the breast is positioned and compressed as for a conventional mammogram, in craniocaudal and mediolateral oblique projections, but the X-ray tube is modified to acquire multiple images as it travels through an arc above the breast. Depending on the design, about 10–20 images will be obtained over about 10–20 s. Each individual projection is very low dose, about 10% of a normal single-view mammogram, so the total dose is comparable to a conventional mammogram. These images are reconstructed into a data set for interpretation and viewed in conventional orientations on a workstation, as individual images or as a cine loop.[50] Since each tomographic image represents only a thin section of the breast, it will, in theory, not suffer from problems with overlapping tissues.

If tomosynthesis is introduced as a screening tool, recall rates are expected to reduce and there will be an expected increase in positive predictive value for biopsy recommendation and higher cancer detection rates.[50] A recently published study from Sweden compared breast cancer visualization on

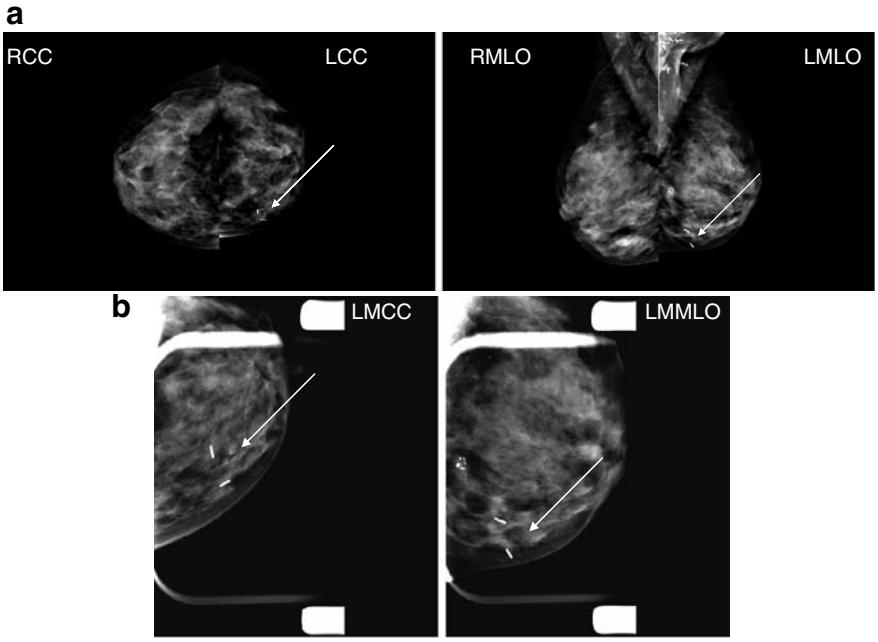

Fig. 6.7 **a** Routine annual mammogram was performed on this patient with a history of lumpectomy for ductal carcinoma in situ (DCIS) 15 years previously. Calcifications are demonstrated adjacent to the lumpectomy site in the inferior medial left breast. Spot magnification views were performed. **b** Spot magnification views demonstrate a segmental grouping of pleomorphic calcifications adjacent to the lumpectomy site. Stereotactic core biopsy was performed yielding DCIS

one-view tomosynthesis to digital mammography. A total of 36 patients were selected with subtle signs of breast cancer on digital imaging. One-view tomosynthesis was performed in the projection that the lesion was least visible on two-view digital imaging. Cancer visualization and BIRADS classification were compared for each patient using tomosynthesis and digital technique. Forty breast cancers were found in 37 breasts. Cancer visualization was better with tomosynthesis and BIRADS classification was upgraded in a significant number of cases when compared with digital imaging ($p < 0.01$).[51]

Poplack et al. recruited 98 women to their tomosynthesis study who were being recalled from a screening mammogram. The interpreting radiologist compared the image quality of tomosynthesis with mammography and assessed the need for recall when tomosynthesis was added to digital screening mammography. Recalls were regarded as unnecessary when tomosynthesis did not show an abnormality or allowed a lesion to be clearly defined as benign. With the addition of tomosynthesis, their recall rate was reduced by 40%.[52]

Available data are limited and disadvantages include longer exposure time and therefore increased patient discomfort and increased motion artifacts, as well as increased radiation dose. Breast tomosynthesis, however, appears to have great potential, but much work is needed before its optimal role in the clinical setting is achieved.

Contrast enhanced mammography is being studied with digital tomosynthesis as well as with conventional digital mammography. Contrast enhanced mammography involves administration of an iodinated contrast agent intravenously in conjunction with conventional mammography. There are two techniques used, the temporal subtraction technique and the dual energy technique. The

temporal subtraction technique involves high energy image acquisition pre- and post-administration of intravenous contrast. The dual energy technique involves acquisition of both low and high energy images after intravenous contrast administration. Kinetics of a lesion can only be determined with the temporal subtraction technique although this technique is prone to motion artefact.[53]

Several small studies have demonstrated the sensitivity of contrast enhanced mammography for the detection of breast carcinoma to lie between 80 and 85%.[54–56] False negatives included cases of both DCIS and invasive carcinoma. When comparing true positives with false negatives, higher intratumoral microvessel density was identified in the true positive group. Progressive enhancement was the most common kinetic curve observed in malignant lesions compared to the washout curves more frequently visualized on MRI. Kinetic differences are thought to be attributed to breast compression.

In the pilot studies performed, contrast enhanced mammography has been compared to conventional mammography. Comparison with MRI may be more useful. Contrast enhanced mammography is less expensive than MRI, and if the sensitivity is similar, it may be used to replace MRI in certain clinical settings.[57]

Available data are again limited but contrast enhanced mammography may be useful in certain settings, including characterizing mammographically equivocal lesions, detecting occult lesions in patients with dense breasts and determining extent of disease in patients with biopsy proven carcinomas.

Conclusion

Mammography has been the most important tool for early detection of breast cancer and has had considerable impact in that role. In addition, the lessons learned from the implementation of mammography as a screening tool can provide guidance as other imaging modalities are evaluated for their potential usefulness in the detection of preclinical breast cancer. The structure of a screening mammography program and the expertise of the radiologists interpreting the mammograms are critical to the success of a screening mammography program.

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Chapter 7
Current Status and Future Prospects in Breast Carcinoma of Positron Emission Tomography

Sandip Basu, Joshua I. Rosenbaum, and Abass Alavi

Abstract In this communication, we explore the current status and future prospects of positron emission tomography (PET) imaging in breast carcinoma. While the use of FDG-PET in the evaluation and management of cancer patients continues to increase, its precise role in the management of breast carcinoma is not yet clearly defined. Currently the most useful applications are in monitoring response to therapy (especially neoadjuvant chemotherapy for locally advanced breast cancer), diagnosis of recurrent and metastatic disease, and defining tumor biology based upon FDG uptake in the lesion. PET has a limited role in diagnosing the primary malignancy, especially in patients with small tumors and those with lobular carcinoma, but can prove useful in certain specific and difficult situations (e.g., in patients with dense breast tissue, significant fibrocystic changes, fibrosis after radiotherapy, and inconclusive results from MR imaging and other imaging modalities). FDG-PET has a relatively low sensitivity for detection of diseased axillary nodes, but the predictive value of a positive PET is very high. We have found that quantitative FDG-PET parameters help define and predict tumor biology. FDG uptake in the index lesion correlates well with tumor aggressiveness, and partial volume correction of the standardized uptake value substantially improves its accuracy especially in lesions less than 2.5 cm in diameter. In this review, we discuss the clinical utility of PET vis-à-vis existing modalities.

Keywords FDG · PET · Breast carcinoma · Tumor biology · Infiltrating ductal carcinoma · Lobular carcinoma · Axillary node · Sentinel node · Metastasis · Positron emission mammography (PEM) · Local recurrence · Locally advanced breast carcinoma · Triple negative breast carcinoma

Key Issues

- There is currently no defined role for FDG-PET in the detection of primary breast cancer. Based on criteria adopted in various studies, the sensitivity of PET imaging ranges from 68 to 94% with specificity between 84 and 97%.
- FDG-PET does not appear to have a sufficiently high negative predictive value to justify forgoing axillary evaluation.

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- FDG-PET has been highly successful in monitoring response to treatment, tumor recurrence, and restaging.
- There is growing evidence of the potential of this modality to define tumor biology, predict disease aggressiveness and assist in radiotherapy planning.

PET in Breast Cancer: What Are the Oncologist's Needs?

To evaluate the efficacy of any new diagnostic modality, the ultimate aim is to improve patient management. To achieve this goal in the setting of breast carcinoma, the utility of PET can be judged regarding (1) detection and characterization of primary breast lesions, (2) accurate locoregional and distant tumor staging, (3) evaluation of response to therapy, (4) assessment of tumor biology and disease prognosis, and (5) determining the frequency that patient management is changed based on PET imaging. Currently the best established and most promising roles appear to be in the (1) prediction of response to neoadjuvant and adjuvant chemotherapy, (2) detection of metastatic disease, and (3) detection of locoregional and distant recurrences. Information gained from PET in each of these settings can guide treatment planning. Other important areas in which PET imaging is of potential value but requires further assessment are (1) defining tumor biology and forecasting prognosis and (2) radiotherapy planning. We shall critically assess the role of PET imaging in the abovementioned settings based upon the results of various pertinent clinical trials.

Detection and Characterization of the Primary Breast Tumor

One of the critical breast carcinoma characteristics is the size of the primary lesion. At present, film screen or digital (which increasingly is replacing film screen) mammography is the primary imaging modality used to detect early clinically occult breast cancer. In addition to screening and diagnosis, mammography represents the primary imaging modality for determining the extent of disease within the affected breast. The local extent of breast cancer is currently established clinically by physical exam and radiographically by mammography, followed by pathologic confirmation of tumor size in the surgically resected specimen. Despite advances in mammography, there are limitations in its ability to determine efficiently and accurately the local extent of breast cancer. Many patients require multiple surgical excisions to achieve negative margins and, despite these procedures, some go on to mastectomy. The 40% local recurrence rate after excision alone speaks to the inability of current methods to detect multifocal and multicentric disease.[1]

The primary tumor has been reported to be detected by FDG-PET in different series with a sensitivity of 64–80%. Avril et al.[2] studied the accuracy of FDG-PET in characterizing breast masses in 144 women referred for surgery for abnormalities discovered on physical exam or mammography. The sensitivity of PET was primarily dependent on two factors: tumor size and histopathology. First, size of the lesion: sensitivity did not exceed 90% until tumors were at least 2 cm in diameter. FDG-PET performance is suboptimal in small lesions with low metabolic activity. This is related to the scanner resolution and is known as the partial volume effect. In general, lesions larger than 1 cm can be detected by FDG-PET with both sensitivity and specificity in the range of 96–100%. Second, histopathology: infiltrating ductal carcinoma has a greater FDG uptake than infiltrating lobular carcinoma and therefore is detected with a significantly higher sensitivity, independent of tumor size.

Using technology that was current at the time but inferior to current technology, investigators found that the sensitivity in the detection of primary breast cancer ranged from 64.4 to 80.3%, and specificity from 75.5 to 94.3%, depending on the criteria they used.[2] Breast carcinomas were identified with an overall sensitivity of 64.4% using Conventional Image Reading or CIR and 80.3% using Sensitive Image Reading or SIR. The increase in sensitivity with SIR resulted in a noticeable decrease in specificity, from 94.3 (CIR) to 75.5% (SIR). Only 30 (68.2%) of 44 T1 (0–2 cm) breast carcinomas were detected, compared with 57 (91.9%) of 62 T2 (2.1–5.0 cm) tumors. A higher percentage (65.2%) of invasive lobular carcinomas were missed compared with invasive ductal carcinomas (23.7%). However, positive PET scans provided a high positive-predictive value (96.6%) for breast cancer in this study.[2] Similar results were observed in 137 patients in a study by Cermik et al. from the University of Pennsylvania, with >90% sensitivity in the detection of T2 and T3 lesions (Table 7.1). Their data demonstrated that both the primary tumor standardized uptake value, or SUVmax, and the sensitivity of FDG-PET increase as the size of the lesion detected increases[3] (Fig. 7.1).

Table 7.1 The tumor size and SUVmax of invasive primary cancers and the sensitivity of FDG-PET according to pathological tumor (pT) stages (Reproduced with permission from Cermik et al.[3] with kind permission of Springer Science + Business Media)

(n = 137)	T1mic & T1a	T1b	T1c	T2 & T3
Tumor size (mm; mean ± SD)	3.4 ± 1.8	8.6 ± 1.7	14.3 ± 3.0	31.7 ± 11.3
SUVmax (mean ± SD) (range [95% CI])	1.6 ± 0.8 (1.1–2.0)	1.8 ± 0.8 (1.5–2.4)	3.4 ± 2.4 (2.5–4.4)	5.6 ± 4.5 (4.3–7.4)
Sensitivity	53(8/15)	63(15/24)	80(36/45)	92(49/53)

T1mic, microinvasion ≤1 mm; T1a, tumor >1 mm but ≤5 mm; T1b, tumor >5 mm but ≤10 mm; T1c, tumor >10 mm but ≤20 mm; T2, tumor >20 mm but ≤50 mm; tumor >50 mm

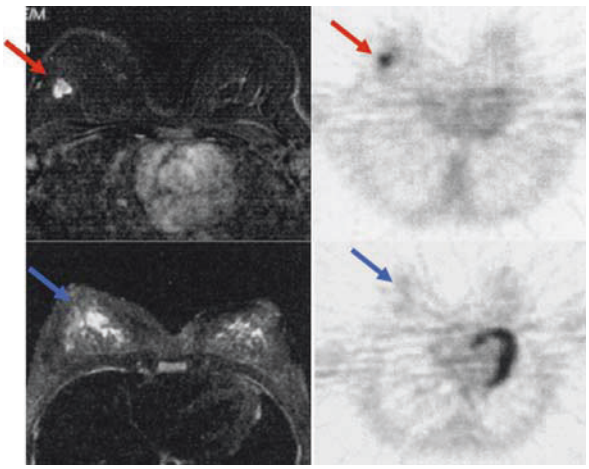

Fig. 7.1 An example of a true positive and a false negative FDG-PET scan. MRI images are on the *left*, and FDG-PET images on the *right*. The *upper arrows* point to a 2.8-cm invasive ductal cancer that is easily seen with PET as an intensely hypermetabolic focus in the right breast, whereas the *lower arrows* point to a 5-cm invasive lobular carcinoma that is not visible on PET (From Avril et al.[2] Reprinted with permission of the American Society of Clinical Oncology)

Summary

There is currently no defined role for FDG-PET in the detection of primary breast cancer. Based on criteria adopted in various studies, the sensitivity of PET imaging ranges from 68 to 94% with specificity between 84 and 97%. The sensitivity for small tumors (<1 cm) is low. The overall specificity of FDG-PET is relatively high, with false-positive results in some benign inflammatory conditions and fibroadenoma.[4] Hence, current studies do not support the use of FDG-PET as a screening modality for asymptomatic women, or to exclude breast cancer in patients with suspicious breast masses or abnormal mammograms, due primarily to the low sensitivity of whole body FDG-PET in the detection of small tumors. This conclusion may change as dedicated mammographic PET imaging instruments are increasingly employed (Fig. 7.2 and 7.3).

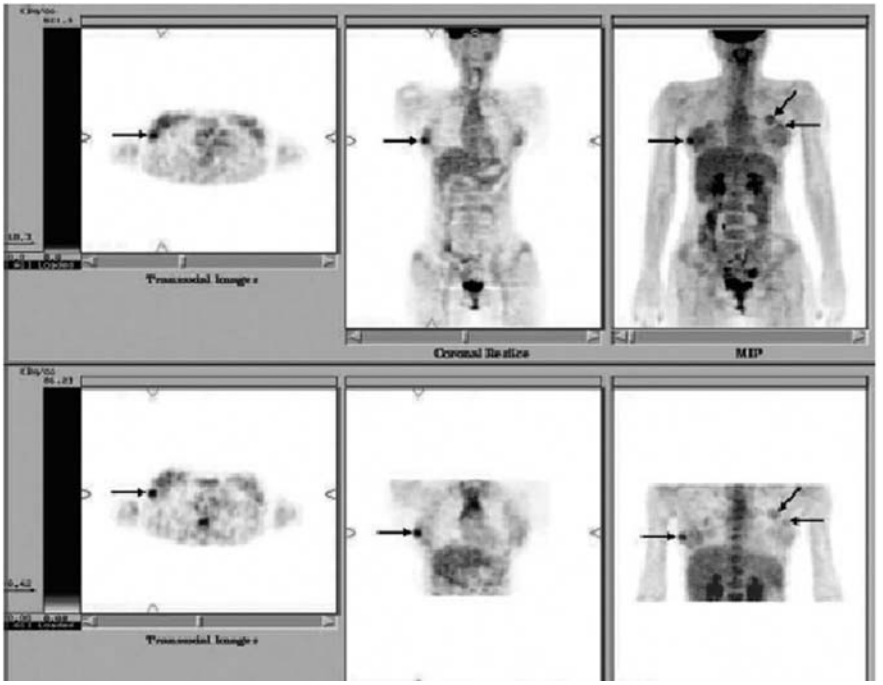

Fig. 7.2 FDG-PET (early and delayed) in a 16-year-old female with a history of medullary carcinoma of the thyroid. Multiple foci of FDG uptake of varying degrees are noted in both breasts. Prominent among them were three foci (*arrows*): one in the right inferolateral (SUVmax 2.95), left upper inner (SUVmax 1.96), and left upper outer (SUVmax 1.55) quadrants. Delayed limited PET demonstrated lower SUVmax values for all three lesions. Final histopathological diagnosis: Multiple Juvenile Fibroadenoma (Reproduced with permission from Basu et al.[5])

Positron Emission Mammography (PEM)

Recently, attempts have been made to develop dedicated breast imaging devices. Such instruments have the potential to improve the ability to detect primary tumors with high sensitivity compared to the conventional whole body imaging. A dedicated breast PET scanner was first proposed by Weinberg in 1993. He coined the term "positron emission mammography". Improvement in the

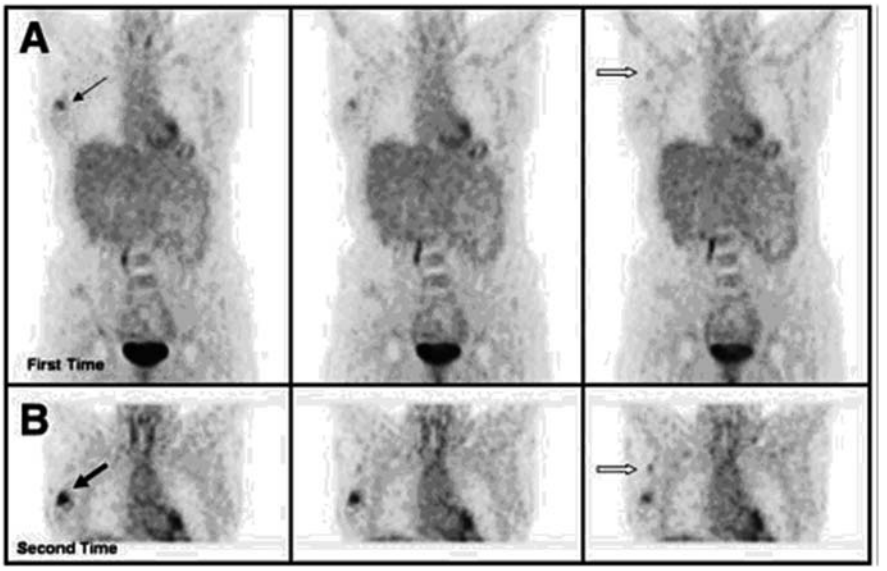

Fig. 7.3 Patient with invasive ductal carcinoma of right breast was examined with dual time point PET: **a** coronal slices were obtained at first time point; **b** corresponding delayed images. Both image sets show the primary lesion an axillary metastasis, with greater uptake intensity on delayed images. Pathologic review confirmed a 2.5-cm invasive ductal carcinoma with axillary metastasis (From Mavi et al.[6] Reprinted with permission from the Society of Nuclear Medicine)

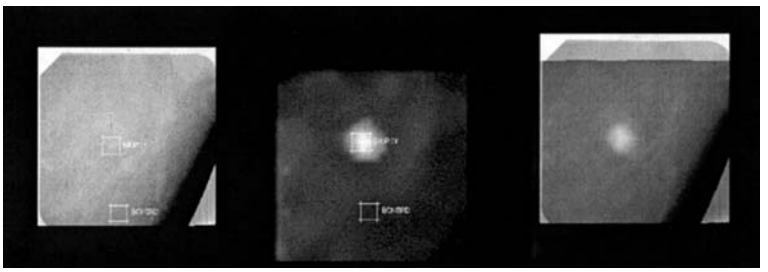

Fig. 7.4 The images shown represent (*from left to right*) digital X-ray with biopsy site and background regions, reconstructed PET image, and overlaid PET/X-ray images. Pathologic review of the biopsied lesion demonstrated invasive ductal carcinoma (Reproduced with permission from Levine et al.[8] with kind permission of Springer Science + Business Media)

instrumentation has taken place since this initial report to provide superior image quality and detect breast cancer in several centers around the world[7] (Fig. 7.4).

The role of PEM is being assessed by several groups. Dedicated breast imaging provides scans that are of high quality and allows lesion detection and characterization with high accuracy. This is in contrast to assessment by whole body imaging, where differential attenuation of gamma rays by adjacent tissues such as the chest wall, lungs and the spine substantially degrade image quality. Preliminary data from the University of Pennsylvania[9] indicate that lesions in the breast are more clearly defined by PEM than by whole body scanning. PEM may ultimately prove to be the test of choice in characterizing breast lesions detected by other modalities. The main challenge with

prototype PEM devices is that lesions near the chest wall are difficult to image. Future designs may require imaging the patient in an upright position similar to that adopted for conventional mammography.

Axillary Lymph Node Staging with FDG-PET

Axillary node status is a major determinant of prognosis in breast carcinoma. While axillary node dissection (ALND) has been the traditional standard approach for axillary node assessment, approximately 40% of patients who undergo the procedure suffer complications including lymphedema (of which about 5% becomes permanent), upper arm anesthesia, seromas, delayed wound healing, and neurovascular injury. In patients with tumors 2 cm or smaller, the incidence of axillary metastases is only 3–20% and therefore 80% of patients could be spared ALND.[2,3,4,7] This has led to sentinel lymph node biopsy (SLNB) as a standard of care in subjects with small tumors and clinically node-negative axillae. When SLNB results are positive, complete ALND is undertaken. A non-invasive technique to identify accurately positive lymph nodes before surgery would offer a significant advantage over current approaches.

One of the earliest reports of FDG-PET in determining the status of axillary lymph nodes in breast carcinoma[3,10] was published by Greco et al. at the National Cancer Institute in Milan. The sensitivity for detecting axillary metastases was 86% in patients with small primary tumors, increasing to 98% in patients with larger tumors, without a decrease in specificity. In this study, sensitivity was high even in patients without palpable adenopathy. The negative predictive value of a normal PET scan was 95%, which is comparable to sentinel node biopsy.[10] Unfortunately, the results have not been replicated, with investigators observing that FDG-PET may fail to detect limited nodal metastases.[11] Another study reported increasing sensitivity with increasing N stage, reaching 100% with pN3 disease.[3]

In patients with locally advanced breast cancer, PET accurately determines the extent of disease in the loco-regional lymph nodes and is therefore recommended in this group of patients (Fig. 7.5).

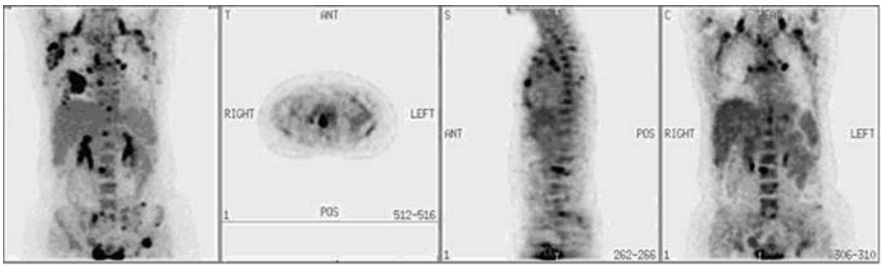

Fig. 7.5 FDG-PET images anterior 3D projection and in the transverse, sagittal, and coronal planes show multiple lymph node and skeletal metastases. Images also demonstrate bilateral axillary lymph node metastases and bilateral primary breast lesions (Reproduced with permission from Cermik et al.[3] with kind permission of Springer Science + Business Media)

Summary

With an increasing number of trials and larger sample sizes, more recent studies consistently suggest that FDG-PET may not have a sufficiently high negative predictive value to justify forgoing ALND [9,12,13,14,15]

Characterization of Tumor Biology

Biological characteristics of the tumor such as whether the tumor is invasive or not and its receptor status are considered important prognostic factors in patients with breast cancer. Our group examined the potential of FDG-PET in predicting tumor biology in a prospective study involving 174 patients with newly diagnosed breast carcinoma who had undergone dual time point FDG-PET before therapeutic intervention.[16] The patients were divided into three groups: 64 patients with primary tumors and axillary lymphadenopathy (Group I), 18 patients with axillary and distant metastases (Group II), and 92 patients (Group III) without either lymph node or distant metastases. The uptake was higher in Group II, followed by Group I and Group III lesions. The results are consistent with more aggressive tumor biology in Group II.

In addition, we compared the imaging characteristics of triple (estrogen receptor [ER-]/progesterone receptor- [PR-]/heregulin 2 [HER2-]) negative and ER+/PR+/HER2- breast carcinomas. Primary lesions in patients with triple negative breast cancer were identified in all cases as areas with focally enhanced FDG (sensitivity100%). In patients with ER+/PR+/HER2- breast carcinoma, the mean initial and delayed uptake was significantly lower (P=0.0032, P=0.002, respectively). Grade 3 tumors had greater uptake than lower grade tumors (P=0.022).[17]

Detection of Recurrent Disease

Despite efforts to select patients carefully for optimal management of breast cancer, some will develop local recurrence, with a reported rate of approximately 5–10% at 5 years[18] and 10–15% at 10 years.[19] Local recurrence is most likely related to the presence of residual disease after excisional biopsy and radiation or mastectomy, and is more common with the presence of multifocal/multicentric disease.[20,21,22]

In contrast to the limited utility of FDG-PET in detecting primary breast cancer, it has proven useful in detecting locoregional and distant recurrence. Several studies (Table 7.2) have demonstrated that FDG-PET is more sensitive than conventional imaging for the detection of recurrent disease. The advantage of FDG-PET is that it provides an accurate assessment of both local and systemic disease and hence allows restaging.

Table 7.2 Studies comparing the ability of FDG-PET to detect loco-regional and distant recurrences in patients who have previously undergone primary treatment for breast cancer (From Eubank.[23] Reproduced with permission from Elsevier.[24,25,26,27,28,29,30,31,32,33])

Study	Number of patients	Confirmed positive/negative cases	FDG-PET sensitivity (TP/TP+FN)[a](%)	FDG-PET specificity (TN/TN+FP)[a](%)
Bender et al.[2]	75	60/15	95 (41/43)	96 (213/221)
Moon et al.[3]	57	29/28	93 (27/29)	79 (22/28)
Lonneux et al.[4]	39[b]	33/6	94 (31/33)	50 (3/6)
Kim et al.[5]	27	17/10	94 (16/17)	80 (8/10)
Lin et al.[6]	36	11/25	85 (23/27)	96 (85/89)
Liu et al.[7]	30[b]	28/2	89 (25/28)	50 (1/2)
Suarez et al.[8]	38[b]	26/12	92 (24/26)	75 (9/12)
Gallowitch et al.[9]	62	34/28	97 (33/34)	82 (23/28)
Siggelkow et al.[10]	57	31/26	81 (25/31)	96 (25/26)
Kamel et al.[11]	60	43/17	89 (24/27) LRR 100 (26/26) DM	84 (16/19) LRR 97 (30/31) DM

[a]Values calculated on patient analysis except in [*2*] and [*6*], in which values are calculated on lesion analysis.
[b]Patients were mostly or all asymptomatic with elevated tumor markers.

Promising Role of FDG-PET in Locally Advanced Breast Cancer (LABC)

LABC is defined as a primary tumor size greater than 5 cm, inflammatory breast cancer, tumor involving the skin or chest wall, and/or fixed axillary lymph node metastases. Overall, it has a poor prognosis and a high incidence of distant metastases during follow-up.[34] FDG-PET upstages LABC from Stage III to IV in approximately 10% of patients, allowing more accurate treatment. The potential role of FDG-PET as the first test for the management of LABC appears well defined and established.[35,36,37,38] In general, because of its ability to accurately stage patients who have advanced breast cancer, FDG-PET has a significant impact on choice of treatment and management in this population.

Detection of Skeletal Involvement in Breast Cancer by FDG-PET

The skeleton is the most common site of distant metastases of breast cancer, with around 69% of the patients who die of the disease having evidence of skeletal metastases before death.[39] There are ongoing studies to determine which radiotracer is most appropriate for breast cancer, 18F-fluoride or FDG, since FDG is taken up directly into the tumor tissue of skeletal metastases and not into surrounding reactive bone, which is an advantage of FDG over 18F-fluoride. On the other hand, concerns have been raised with regard to the utility of FDG-PET in the detection of sclerotic bone metastases which have limited metabolic activity. Thus, osteolytic metastases usually show enhanced FDG uptake, while osteoblastic metastases show low FDG uptake and may even be undetectable on PET images.[40–43] FDG-PET shows a lower sensitivity for sclerotic bone metastasis compared to ^{99m}Tc-MDP or 18F-Fluoride PET imaging, especially in breast or prostate carcinoma, which is likely due to the relatively acellular nature of sclerotic lesions and lower volume of viable tumor tissue within these lesions.[43]

On the other hand, the detection of bone marrow metastases by FDG-PET is superior to anatomic imaging. FDG-PET can demonstrate changes in the metabolism of bone marrow metastases before they are visible on skeletal scintigraphy or CT. FDG uptake reflects the immediate tumor activity of bone metastases, whereas radiographic morphology changes vary greatly with time among patients.[43]

Assessment of Treatment Response in Breast Cancer

FDG-PET plays a pivotal role in assessing treatment response for primary and metastatic breast cancer.

Changes in tumor glucose consumption occur early in the course of chemotherapy and ultimately predict treatment outcome. The use of FDG-PET to monitor treatment response in breast cancer patients holds promise to reduce ineffective treatment and unnecessary side effects and to facilitate the evaluation of new therapeutic approaches. Investigators at UCLA and the Northern California PET Center compared the accuracy of PET and conventional imaging techniques to predict disease-free survival in 61 women who had completed therapy. Women with a negative PET scan showed a significantly longer disease-free survival (DFS) than women with a positive PET scan, with differences between negative and positive conventional imaging being less predictive of DFS, mainly due to the large number of false negative results for metastatic disease with conventional imaging. This study suggests that PET can be useful in detecting metastatic disease and evaluating response to therapy[44] (Fig. 7.6 and Table 7.3).

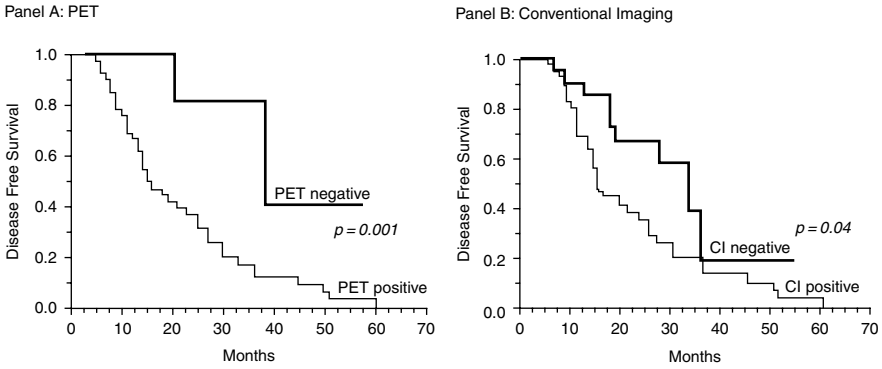

Fig. 7.6 Prediction of disease-free survival by PET and CI Kaplan-Meier estimates of disease free survival (Vranjesevic et al.[44] reproduced with permission from the Society of Nuclear Medicine)

Table 7.3 Studies examining treatment monitoring in different oncological settings for breast carcinoma

Oncological treatment	Study
Neo adjuvant therapy of LABC	Wahl et al.[45] Schelling et al.[46] Smith et al.[47]
Multiple metastatic sites – uniformity of response	Gennari et al.[48]
Bone-dominant breast cancer	Stafford et al.[49]

Summary

FDG-PET has been a highly successful modality in monitoring response to treatment, tumor recurrence, and restaging. One must be aware of the phenomenon of "Metabolic Flare", which is the transient increase in FDG activity 7–10 days after initiating hormone therapy due to partial estrogen-like agonist effects of Tamoxifen, which should *not* be interpreted as evidence for disease progression. Metabolic flare also occurs after RT/systemic therapy, due to the accumulation of inflammatory cells at the treatment site. The utility of FDG-PET in monitoring treatment response to breast cancer has been proven both after neoadjuvant therapy of LABC and in other settings which have demonstrated its superiority over conventional imaging techniques.

Imaging of Estrogen and Progesterone Receptor Functionality in Breast Cancer

In addition to FDG, several novel tracers have been utilized to image breast cancer. One that is of special interest in breast cancer is fluorine labeled estradiol (FES). As outlined in other chapters, the assessment of ER in breast cancer is important for many reasons. ER+ tumors are usually better differentiated and less aggressive than ER- tumors, and ER+ (but not ER-) tumors are likely to respond to hormonal therapy. Assessment of ER functionality can stratify the prognosis of patients independent of disease stage and can assist physicians in planning treatment directed at blocking ER in both pre- and postmenopausal women using tamoxifen or decreasing circulating estrogen in postmenopausal women using aromatase inhibitors. Several ligands that selectively bind

ER are being studied for use with PET. The ligands, including fluoroestradiol (FES), fluoromoxestrol, fluoro-11β-methoxy-fluoroestradiol and fluorotamoxifen are being studied to assess estrogen receptor functionality. Among these, FES has been most widely investigated and preliminary data indicate that FES shows promise to detect ER+ tumor sites and predict response to hormonal therapy.[50,51,52,53,54]

The main advantage of FES-PET over ER assessment of excised tumor tissue is that it provides in vivo evidence for receptor status and can quantify the degree of ER functionality in the tumor. Hence, the potential utility of FES-PET in clinical practice is to (1) determine the efficacy of antiestrogen therapy, (2) predict prognosis, and (3) differentiate between metabolic flare and treatment response (Fig. 7.7).

PET/CT in Radiation Therapy Simulation

The role of PET/CT in radiation treatment planning for breast cancer will require a randomized controlled trial to determine its efficacy. The potential of combined anatomic and functional imaging to improve outcome is particularly important for LABC in the era of image-guided RT planning. The accurate assessment of internal mammary nodes is of great importance in RT planning. Two large randomized trials demonstrated a survival benefit to the postmastectomy chest wall and regional nodal radiation that included the internal mammary nodes.[55,56] On the other hand, recent meta-analyses suggest a high incidence of late cardiac death in patients treated with radiation therapy compared with those treated by surgery alone.[57] The use of coregistered FDG-PET and CT images generated by modern hybrid scanners can improve radiation therapy outcome by demonstrating metabolically active disease sites in a single examination.

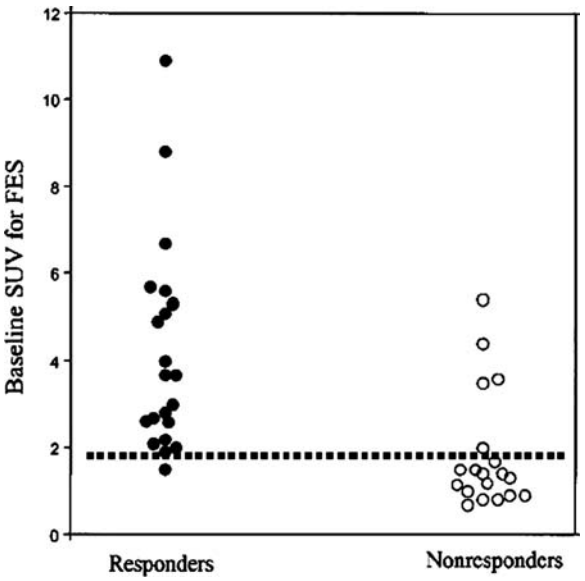

Fig. 7.7 FES uptake predicts response of advanced breast cancer to Tamoxifen. Shown here are examples of the utility of FES scan in predicting response to hormonal therapy. Both groups had ER+ primary tumors, but one group with a positive and the other with a negative FES scan. The FES positive group shows excellent response to hormonal therapy (Reproduced with permission from American Society of Clinical Oncology for Mortimer et al.)[52]

Changes in Patient Management Resulting from PET

To be considered cost effective, the information gained from PET must impact patient management. Physicians treating breast cancer patients who were surveyed with regard to how PET findings altered disease stage and clinical management decisions indicated that PET changed clinical stage in 36% (28% upstaged, 8% downstaged) and changed treatment in just under a third of patients.[58]

Summary of the Role of PET in Breast Cancer: We Are Still Learning

PET is a useful test in women with breast cancer, but it has limitations. PET may miss breast cancer in women with small tumors, and cannot replace conventional radiological techniques and histological lesion assessment. PET can be used to evaluate the axilla for metastatic disease, but is less sensitive than sentinel node biopsy or axillary dissection. On the other hand, PET is more sensitive than conventional imaging for detecting metastatic disease, and should be considered when staging or restaging women with suspected or known distant metastases. PET is quite useful in evaluating response to therapy, both in the neoadjuvant and adjuvant setting.

Future Potential

Modern dedicated breast PET scanners have both higher sensitivity and spatial resolution than conventional whole body scanners. As more studies evaluate the role of these dedicated scanners, the role of FDG-PET in assessing primary breast cancer may change. New PET tracers have the potential to classify tumors on a molecular level and to identify tumors that respond best to new therapies. PET has the potential to refine prognostic indices and stratify treatment. Overall, PET has a number of advantages in refining and improving the management of breast cancer in appropriate settings.

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Chapter 8
Breast MRI

Barbara C. Cavanaugh and Sorcha McNally

Abstract Breast MRI has become one of the most useful breast imaging modalities. It is the most sensitive modality for detection of invasive ductal carcinoma. It will likely play an increasing role in screening women at high risk for developing breast cancer. Although there remains significant variability in specificity of MRI interpretation, as practice patterns become more established and standardized, variability should decrease.

Keywords Magnetic resonance imaging · Breast cancer screening · Screening high risk women

Key Issues

- Contrast enhanced breast MRI is very sensitive for detection of invasive breast cancer, and in some studies has been found sensitive for detection of ductal carcinoma in situ.
- Contrast enhanced breast MRI is used in a variety of clinical settings. The American Cancer Society recommends screening with breast MRI for women with a 20% or more lifetime risk of developing breast cancer. Other indications include evaluation for multifocal and multicentric ipsilateral disease; for contralateral disease in patients with newly diagnosed cancer (particularly lobular carcinoma); for evaluation of therapy response in patients treated with neoadjuvant chemotherapy; for evaluation of patients with axillary or distant metastases when the breast primary is not known or for axillary metastases with an unknown primary. Additional indications can include screening in patients with a history of cancer treatment with breast conservation therapy and problem solving for equivocal findings on other imaging modalities, although evidence of benefit in these settings is yet to be determined.
- Optimal performance of breast MRI is highly technique dependent. Patient positioning, imaging parameters, optimal spatial and temporal resolution, and timing of dynamic phase imaging are of crucial importance in the performance of breast MRI.
- Any site that performs breast MRI examinations should be able to perform MRI guided procedures.
- Reporting should be performed by a radiologist with breast MRI experience using the ACR BI-RADS MRI Lexicon.

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- A disadvantage of contrast enhanced breast MRI is lack of specificity. MR spectroscopy and Diffusion Weighted Imaging may improve accuracy. These remain research tools but a number of studies have shown promising results.

Introduction

Among the first investigators to describe breast MRI, El Yousef et al. performed breast MR using a 0.3-Tesla coil without IV contrast.[1] Much has changed since then. Initially, non contrast breast MRI evaluated the integrity of silicone implants. The introduction of paramagnetic IV contrast and the improvement in resolution afforded by higher field strength magnets provided differentiation in the MRI appearance of normal vs. malignant tissue, and allowed MR imaging to occupy an increasingly important role in breast imaging. While there is controversy among clinicians regarding the utility of breast MRI, patients perceive breast MRI to be very valuable. Zakaria et al.[2] surveyed women who had had a breast MRI exam for high risk screening or staging of breast cancer and found that the women considered the exam to be an important component of their care, even when the clinician's assessment of the contribution of MRI to their care was negligible or even negative.[2]

Use of breast MRI is increasing in the United States. A recent survey indicated that nearly three-quarters of radiology practices offer contrast enhanced breast MRI. There is variation among practice patterns, and nearly one-third of practices still do not offer MRI guided breast biopsies.[3] In order to standardize the level of care offered at different practices, the American College of Radiology has developed guidelines for interpretation of breast MRI in a manner similar to the guidelines developed for mammography and ultrasound.[4] As breast MRI becomes more widespread for screening, thereby affecting otherwise healthy people, the ethical and socioeconomic implications of the quality of care that is offered become magnified and guidelines for standards of care become more important.

Reported sensitivity of breast MRI varies by investigator, and has been reported as high as 100% for the detection of invasive ductal carcinoma.[5] The variation in sensitivity may be related to sample size and variations in patient selection and study design. Sardanelli et al., in a large sample of women undergoing mastectomy, correlated whole breast pathologic exam with presurgical mammography and contrast enhanced MRI, and reported MRI sensitivity for invasive cancer as 89%, and for DCIS, 40%.[6] As whole breast pathology was used as the gold standard for the presence of malignancy, these figures may be the most accurate assessment of MRI's sensitivity. Specificity of breast MRI varies widely, with the reported specificity ranging from 37 to 97%.[5] In general, the highest specificity and sensitivity will be achieved in a subspecialist breast imaging setting. Since the purpose of breast MRI is to identify lesions that cannot be palpated or visualized on mammography or ultrasound, it follows that there will be findings that need to be biopsied using MRI guidance. Therefore it is imperative that a practice that offers women breast MRI also offer them a means to biopsy the lesions that are found using MR guided biopsy.

Indications for Breast MRI

Initial Staging for Newly Diagnosed Breast Cancer

Breast MR detects cancers that are occult on mammography, ultrasound, and physical examination. In the patient with newly diagnosed breast cancer, multifocal or multicentric disease will be detected in up to 30% of women, resulting in altered treatment.[7,8] Thus the incorporation of MRI

into breast cancer staging generally increases the number of mastectomies performed.[9] Staging with breast MR detects contralateral breast cancer.[10–12] Lehman et al., in a large multicenter trial, found contralateral cancer in 3% of newly diagnosed breast cancer patients.[13] The impact of staging MRI on long term outcome has been uncertain.[14] The equivalence of outcomes of patients treated with breast conserving treatment vs mastectomy is well established.[15] The patients who were included in these studies were evaluated with mammography and targeted ultrasound when appropriate, and with clinical examination. Presumably the mammographically, sonographically, and clinically occult cancers that we can now document with MRI existed in similar numbers during these trials. This suggests that changing treatment based on information obtained from breast MRI will not alter long term outcome.[14]

However, it certainly seems logical that increased detection and treatment of multifocal and multicentric disease should decrease the rate of local recurrence in women treated with breast conserving treatment. Fischer et al. described a significant decrease in local recurrence and in incidence of contralateral breast cancer in women who received staging MRI prior to treatment with breast conservation.[16] However, Solin et al. demonstrated no difference in local recurrence rate or incidence of contralateral cancer among women with or without MRI prior to breast conserving treatment.[15] Currently, although there are no definitive data to support a benefit, many clinicians advocate staging MRI in women with newly diagnosed cancer and associated invasive lobular histology, dense breasts, and age under 40. Further research should help determine the role of breast MRI in staging women with a new diagnosis of breast cancer.

Patient with Axillary Node Metastases and Unknown Primary Malignancy

In the patient presenting with axillary metastases and a primary not identified by physical exam, mammogram, or breast ultrasound, breast MR can detect a primary lesion in up to 86% of cases.[17,18] This provides tremendous benefit to patient care. In the report by Orel et al., 41% of patients were able to be treated with breast conserving treatment.[18] Identification of the primary tumor also allows for improved treatment planning.

Monitoring Response to Neoadjuvant Chemotherapy

Neoadjuvant chemotherapy is employed primarily to shrink large tumors to allow breast conserving treatment. Women who have a complete pathological response to neoadjuvant chemotherapy have favorable survival. Consequently, it is desirable to monitor response to chemotherapy in order to attain optimal pathological response. Thus there is a need for an imaging tool that accurately evaluates tumor response.

Mammography has been used to assess response to neoadjuvant chemotherapy but was not useful in predicting pathologic response.[19] MRI is more sensitive than mammography or ultrasound at detecting residual disease following neoadjuvant treatment,[20–22] although MRI can either over- or underestimate residual disease in nearly one-third of patients.[22,23] While MRI can accurately assess tumor extent during and after chemotherapy, a negative MRI does not confirm complete pathologic response, as up to 30% of patients will have residual tumor at resection following a negative MRI. Therefore, complete pathologic response can only be confirmed with surgical excision and pathologic review.

Multiple investigators have studied breast MR during and after chemotherapy to determine if there are specific changes in the MR features of the tumor that allow prediction of tumor response to treatment. Features predictive of pathological response have not been established. Different investigators used different methodologies. While a pretreatment MRI was always performed, the timing of subsequent exams varied, being performed at some point during and/or after treatment. MR imaging has been performed after completion of treatment,[24,25] after the first cycle of chemotherapy,[26] after the second cycle,[27] after the second cycle and after completion,[28] and after the third cycle and after completion.[23] Different investigators evaluated different features on MR imaging: quantification of washout kinetics to assess change,[28] residual tumor volume,[25] and largest tumor diameter as measured at late phase of enhancement.[27]

While decrease in size and loss of suspicious (washout) vascular kinetics correlate with pathologic response to chemotherapy, there is not yet a defined set of changes that allow for prediction of complete pathologic response. In addition, the studies evaluating MR and chemotherapeutic response have had small sample sizes. Therefore, there are no specific criteria on MR imaging that can guide clinical decision making.

Evaluation of Equivocal Clinical and Imaging Findings

In the face of a challenging mammogram, ultrasound, or physical exam, it is tempting to perform another modality to assist in decision making. MRI is often the additional modality that is used. However, the negative predictive value of MRI is not high enough to be useful in this setting, and there are no data to support the use of MRI in this role.[29,30]

The standard of care for mammography and breast ultrasound requires that for a finding to be followed rather than undergo biopsy, it have a <2% chance of being malignant.[31] For MRI to be useful in excluding the need for biopsy based on these criteria, the negative predictive value would need to be 98%. The overall NPV of MRI is closer to 85%. MR is particularly non contributory in the setting of mammographic calcifications.[32] Therefore MRI is not useful to exclude the biopsy of lesions that are considered suspicious on mammography or breast ultrasound. In short, MR is not a substitute for a careful and complete breast imaging evaluation with mammography and/or breast ultrasound. If there is a lesion of concern that is amenable to biopsy, whether image guided percutaneous biopsy or surgical excision, this is the best management.[29] That said, there are situations where mammographic and sonographic findings are inconclusive but low suspicion and in addition biopsy would be difficult. A negative MR may offer sufficient NPV to be reassuring, and a positive MR can provide information allowing localization of the equivocal area for biopsy.

Screening High Risk Women

In women at high risk for developing breast cancer, breast MRI detects more early stage cancers than mammography or ultrasound.[33–38] Currently, the American Cancer Society recommends screening MRI for women with a 20% or greater lifetime risk of developing breast carcinoma.[39] This includes women with BRCA mutations or equivalent family history and women with a history of treatment for Hodgkin's disease. There are insufficient data to support the effectiveness of screening breast MRI in women at increased risk of breast cancer but less than 20% lifetime risk, including women with a personal history of breast cancer, history of biopsy proven atypical ductal or lobular hyperplasia, and mammographically dense breasts.

Performing Breast MRI

Equipment and Technique

1.5-Tesla magnetic field strength is the minimum required for breast MR imaging, but as there is increasing clinical availability of 3-Tesla magnets the question arises: does higher field strength improve imaging? With increasing magnetic field strength there is an increased signal to noise ratio and thus the potential for improved imaging detail.[40] Kuhl et al., evaluating a small number of women using both 1.5- and 3-T breast MR units, found overall improved image quality at 3-T, and ROC analysis demonstrated improved accuracy for 3-T units.[41] However, at higher magnetic field strengths there are physical effects that can interfere with image quality. At this time there is no evidence for definite improvement over imaging at 1.5-T.

Breast MRI requires that a dynamic contrast enhanced series be performed, using intravenous administration of a gadolinium chelate, usually at a dose of 0.1 mmol per kg of body weight. Subtraction of the pre- and post-contrast dynamic images is performed. Kinetic analysis of enhancing areas can be performed by visual assessment, or with a computer aided detection program.

There are unique considerations when a practice begins to perform breast MRI. Technologists are often unprepared for the amount of hands on patient contact that is involved in breast MR as compared to other MR examinations. For musculoskeletal, body, and neurologic exams patients generally position themselves on the MR table, with direction and perhaps a bit of hands on assistance from the technologist. In contrast, because breasts are freely mobile, a woman's breasts will not be properly positioned for the study unless the technologist physically pulls the breasts into the coil. The entirety of each breast must be gently pulled into the coil, and adjacent soft tissue (such as upper abdomen) positioned outside of the coil, for the breasts to be fully included and properly centered (Fig. 8.1). If the breasts are not properly positioned, substantial amounts of peripheral breast tissue will remain outside the coil with the edge of the coil pressing into the breast, creating both physical distortion of the tissue as well as potentially a tourniquet effect that can distort the dynamic images (Fig. 8.2). In addition to creating considerable physical discomfort leading to patient motion, this can interfere with interpretation of the dynamic images. Just as in mammography, where positioning of the breast is a skill that is so critical to the study that it comes under FDA scrutiny (in the Mammography Quality Standards Act), positioning for breast MRI is a critical component of the overall success of the study.

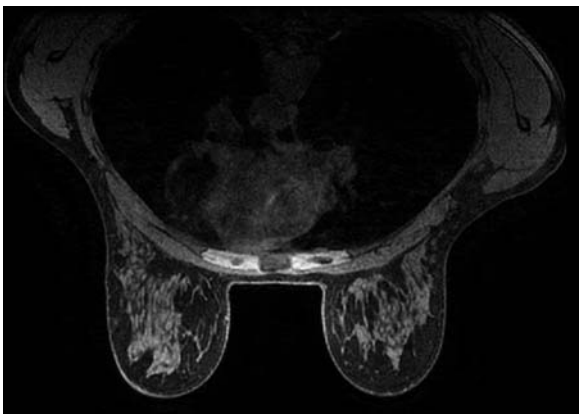

Fig. 8.1 Satisfactory positioning. The lateral and medial breast tissue have been positioned within the coil with the breasts centered

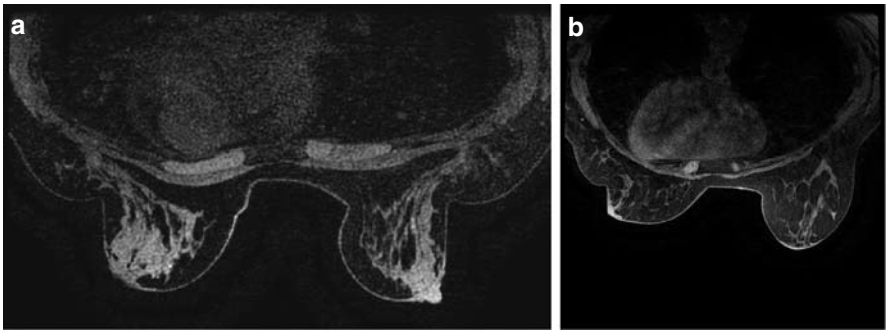

Fig. 8.2 Poor positioning: **a** (*left*) the lateral breast tissue is only partially within the breast coil and the breasts are not centered, as evidenced by the position of the nipples. Note the considerable distortion that results; **b** (*right*) the *right* breast has been positioned properly, the lateral tissue on the *left* side is only partially within the coil. The nipple is centered on the *right* but displaced to the side on the *left*. Compare the visualization of the breast tissue on the *right* side to that on the *left* side

For this reason, many practices enlist a small group of interested, usually female, MR technologists to cross train with mammography technologists and learn about breast positioning. Some practices cross train mammography technologists to perform breast MRI. In either case, the technologist needs to understand breast mobility and positioning to successfully perform the study.

Interpretation

Contrast enhancement in breast MRI evaluates patterns of vascular enhancement in breast tissue. The background enhancement patterns in normal breast tissue due to normal blood flow can present difficulties to interpretation of breast MRI, particularly in premenopausal women. To lessen the interpretive difficulties presented by normal parenchymal enhancement, breast MRI in premenopausal women is performed preferentially during the beginning of the menstrual cycle. The detection of cancer on breast MRI is based on the abnormal vascularity of tumors, including increased vascular density and increased vascular permeability. However, there is some variation in the degree of abnormal vascularity in breast cancers,[29] which creates difficulties in diagnosis. Therefore, in addition to interpretation issues due to background enhancement of normal breast tissue, interpretation can be hampered by the cancers that do not enhance (up to 11% of invasive cancers and up to 60% of DCIS) on MRI. The lack of enhancement is occasionally technical, due to faulty positioning such that the cancer is excluded from the field of view, inadequate contrast injection or motion artifact,[42] but more often is due to lesion characteristics intrinsic to the biological heterogeneity of breast cancer. Despite these limitations, high quality breast MR is highly sensitive and, as centers gain more experience with this modality, increasingly specific as well.

Breast MR evaluates both vascular and morphologic properties of breast cancer. Accordingly, breast MR sequences will be optimized for temporal resolution (dynamic sequences acquired after contrast administration, to evaluate patterns of vascular enhancement) and spatial resolution (high resolution sequences to evaluate lesion morphology). Briefly, although glandular tissue and fat have different intrinsic MR properties, normal and abnormal breast tissue have similar MR properties and therefore cancer is not detectable on non-contrast breast MR. However, normal and abnormal breast tissue respond to contrast agents differently and therefore contrast administration increases conspicuity of breast cancers.

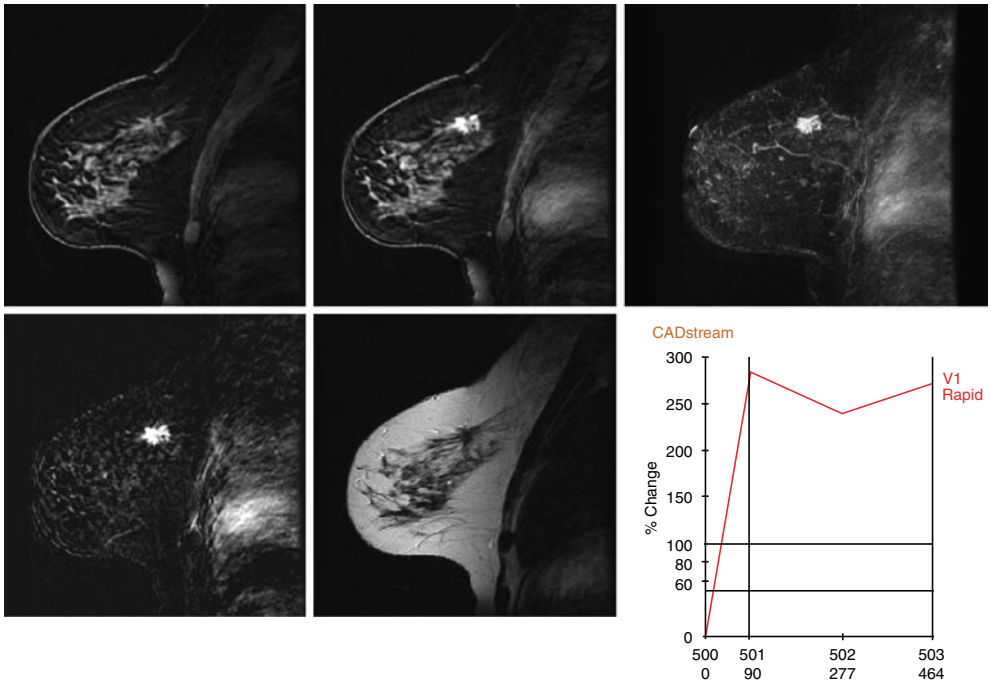

Fig. 8.3 Invasive ductal carcinoma. Contrast enhanced MR of the breast in a 52-year-old patient with biopsy proven carcinoma performed to assess for multifocal or multicentric disease demonstrates a 1.2-cm irregular heterogeneously enhancing mass with spiculated margins corresponding to the known cancer. This lesion demonstrates rapid enhancement and washout kinetics. No other areas of abnormal enhancement are seen: **a** pre-contrast T1; **b** post-contrast T1; **c** subtraction; **d** MIP (maximum intensity projection); **e** T2; **f** time intensity curve

The interpretation of lesion morphology should be performed using the Breast MR imaging lexicon[43,44] (Figs. 8.3–8.11). Using the lexicon, the lesion type, shape, margins, distribution, and internal enhancement should be assessed and described. In addition, background parenchymal enhancement requires comment. The terminology for background enhancement includes minimal, mild, moderate, and marked.

Lesion type is described as either a focus, a mass, or non-mass-like enhancement. A focus is a tiny enhancing lesion measuring less than 5 mm that is too small to characterize. A mass is a space occupying lesion measuring 5 mm or greater that has a correlate on pre-contrast T1 weighted or T2 weighted images. Non-mass-like enhancement has no correlate on pre-contrast images with normal appearing parenchyma at the site of the enhancing lesion. Differential diagnosis for a mass is between cancer and a benign solid tumor. Differential diagnosis for non-mass-like enhancement is DCIS, diffuse infiltrating lobular carcinoma, or benign entities including adenosis, fibrocystic disease, and hormonally mediated changes in normal glandular tissue.

The shape of the mass should be described as oval, round, lobulated, or irregular. Margins should be described as smooth, irregular, or spiculated. Internal enhancement descriptors are as follows: homogeneous, heterogeneous, rim enhancing, enhancing internal septations, or non enhancing septations. For all masses, location and size should be documented. Signal intensity on pre-contrast T1 and T2 weighted images should also be commented on.

Regarding non-mass-like enhancement, distribution and internal enhancement should be described as linear, linear-ductal, segmental, regional, diffuse-patchy, and/or diffuse. Internal

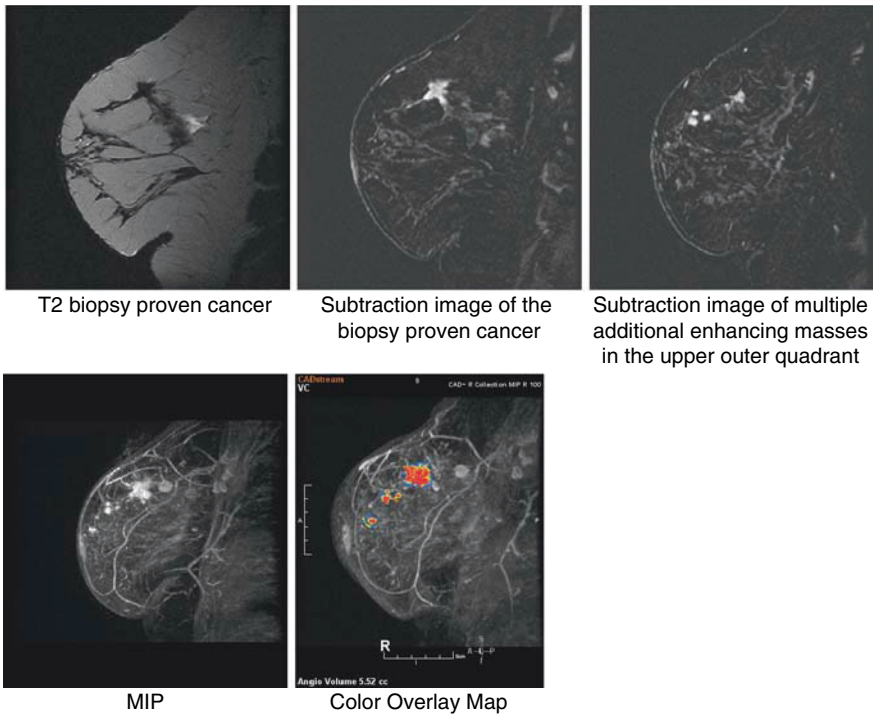

Fig. 8.4 Multifocal breast cancer. A 48-year-old patient with biopsy proven carcinoma at the 11:00 position in the right breast. MRI demonstrates an irregular heterogeneously enhancing mass with spiculated margins corresponding to the biopsy proven carcinoma in addition to multiple masses in the upper outer quadrant organized in a segmental distribution. *Color overlay map* is provided (as an alternative to time intensity curves). *Red* indicates a mass with washout kinetics. Almost all of these masses demonstrate washout kinetics and features consistent with multifocal breast cancer, confirmed on pathology. The primary cancer was detected by mammography but the multifocal disease was mammographically occult: **a** T2 biopsy proven cancer; **b** subtraction image of the biopsy proven cancer; **c** subtraction image of multiple additional enhancing masses in the upper outer quadrant; **d** MIP (maximum intensity projection); **e** color overlay map

enhancement descriptors include stippled, heterogeneous, clumped, and homogeneous. Stippled refers to punctate, similar appearing enhancing foci. Heterogeneous applies to nonuniform enhancement. Clumped implies cobblestone-like or beaded regions of enhancement and homogeneous implies confluent enhancement without internal variation. With non-mass-like enhancement, assessment for symmetry is important. Bilateral symmetric non-mass-like enhancement is usually caused by hormonal stimulation or adenosis. Asymmetric non-mass-like enhancement is a worrisome feature.

A description of initial rise and delayed enhancement kinetics should be performed for masses. Enhancement kinetics are not evaluated for non-mass-like enhancement. Initial rise describes the early phase of lesion enhancement following the injection of contrast within the first 2–3 min post-injection. Enhancement is described as fast, intermediate, or slow. Delayed phase enhancement looks at the signal intensity of the lesion 3 min after injection and relies on imaging up to 6–7 min post-injection. Delayed enhancement is described as persistent, plateau, or washout. Persistent enhancement implies that the signal intensity of the lesion continues to increase following injection. Plateau implies that the lesion signal intensity remains static following the initial rise. Washout implies that the lesion signal intensity decreases after the early phase rise.

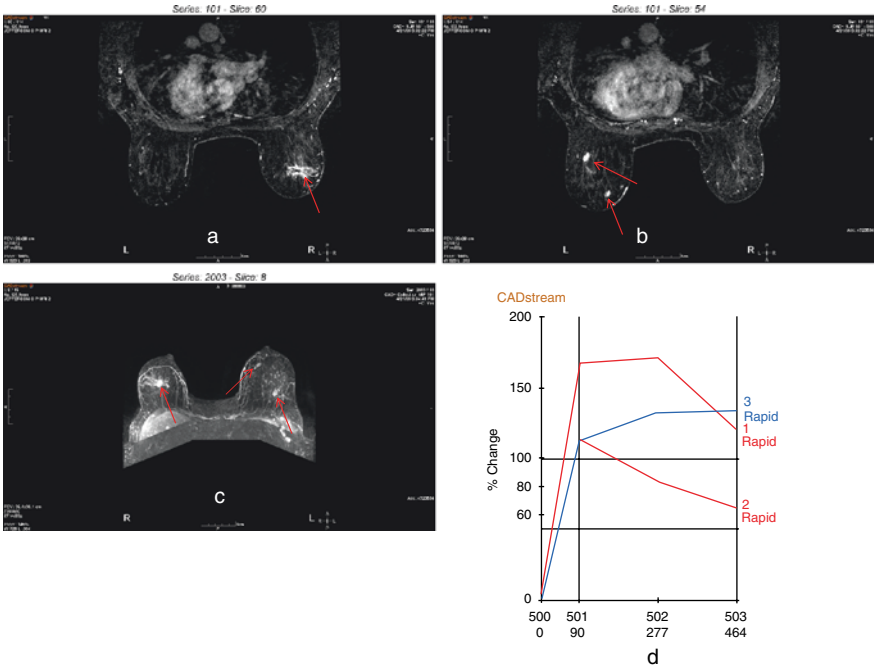

Fig. 8.5 Contralateral breast cancer. This 39-year-old woman presented with a palpable right breast mass which on biopsy demonstrated invasive ductal carcinoma. MR performed prior to surgery demonstrates an irregular heterogeneously enhancing mass in the right breast corresponding to the known cancer (**a**, subtraction image) with rapid washout kinetics (**d** *curve 1*). Two additional lesions were identified in the *left* breast (**b**, subtraction image; **c** MIP). The more posteriorly located oval mass was heterogeneously enhancing with irregular margins and had washout kinetics (**d** *curve 2*). The more anteriorly located mass in the medial breast was round, homogeneously enhancing with irregular margins and persistent enhancement kinetics (**d** *curve 3*). Both lesions in the *left* breast were biopsied. The more anterior was a papilloma but the posterior mammographically occult mass was invasive ductal carcinoma

Management of Abnormal Findings on Breast MRI

The assessment of MRI findings can be classified in the same way as abnormal findings on mammography and breast ultrasound, using BIRADS final assessment categories.[4,44] Thus, findings on MRI can be assessed as shown in Table 8.1.

These assessment categories differ from those for breast ultrasound and mammography only in the likelihood of malignancy of lesions assigned to assessment category 3. For mammography and breast ultrasound, the definition of category 3 is lesions with 2% or lower odds of malignancy, and there is research validating the imaging characteristics of lesions assigned to this category.[32] There is as yet far less research in breast MRI. There is not yet as much uniformity of criteria for characterizing lesions, and there is much less research documenting outcomes of lesions with different imaging characteristics. Therefore, the definition of what constitutes a category 3 probably benign lesion on breast MRI is not clearly defined.

Obviously, the findings of most concern are those requiring biopsy. As these findings are often non-palpable and occult on mammography and ultrasound, any facility performing breast MRI should be able to perform MR guided biopsies. An MR guided procedure, whether MR guided core needle biopsy or MR guided pre-operative needle localization, requires that the contrast enhanced MR be repeated, with the breast lightly immobilized in a grid that will allow targeting of the

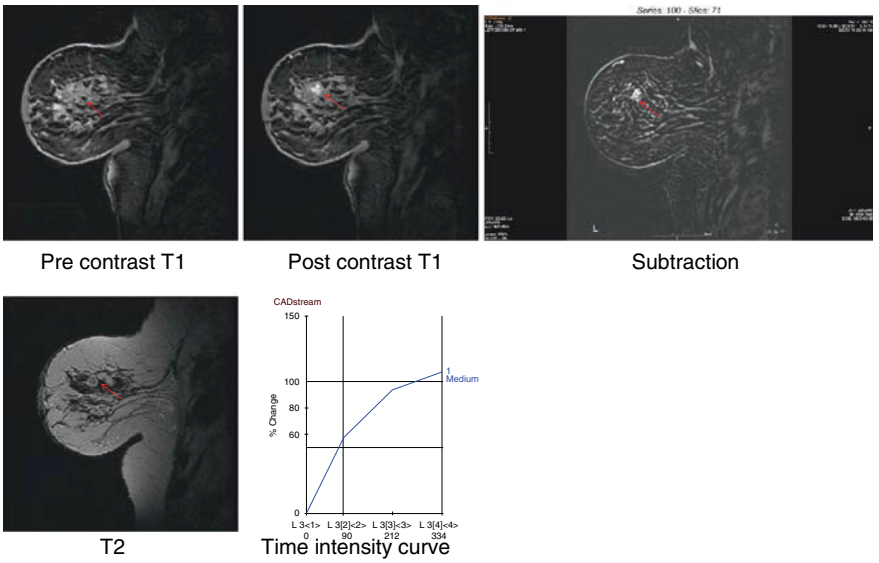

Fig. 8.6 Fibroadenoma. Screening MRI was performed in this patient with a history of breast cancer. The study demonstrates an oval enhancing mass with smooth margins, non-enhancing internal septations, and persistent enhancement kinetics. This mass is of slightly increased signal intensity on T2 weighted images. Imaging features are typical of a fibroadenoma: **a** pre-contrast T1; **b** post-contrast T1; **c** subtraction; **d** T2; **e** time intensity curve

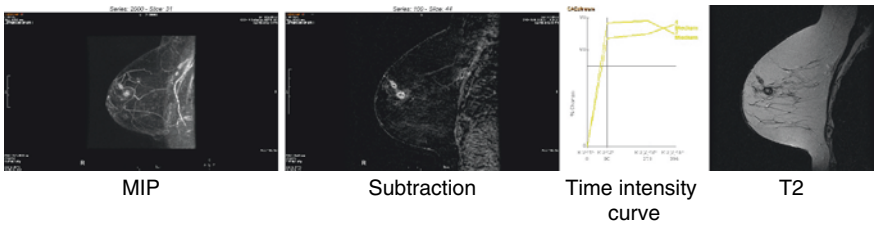

Fig. 8.7 Fat necrosis. This 48-year-old woman presented for screening MRI demonstrates two rim enhancing masses in the retroareolar breast, both with plateau enhancement kinetics. Crucial to the interpretation in this case are the pre contrast images. The T2 weighted image demonstrates fat in the center of both lesions, confirming the diagnosis of fat necrosis. The patient reported a history of an automobile accident in the past with extensive breast bruising. Fat necrosis mimics breast cancer due to its rim enhancing nature. This case highlights the importance of incorporating the pre contrast images into the overall assessment: **a** MIP; **b** subtraction; **c** time intensity curve; **d** T2

suspicious lesion.[45–49] If the lesion can be identified on the dynamic contrast enhanced study, its coordinates are located within the grid, the overlying skin and breast tissue are anesthetized, and the biopsy device or localizing needle are placed at the targeted coordinates. MR images are repeated to check the position of the device. Once the device location is confirmed at the coordinates of the lesion as determined from the initial dynamic imaging, the procedure is performed.

There are considerations in MR guided intervention that are not encountered in stereotactic or ultrasound guided procedures. The MR detected target is usually an area of contrast enhancement which is by its nature transient. At the time of MR guided biopsy or pre-operative localization, dynamic contrast enhanced sequences are repeated and the enhancing lesion is re-identified. However, by the time the target is identified, the skin prepped and anesthetized, and the biopsy device placed, the area of suspicious contrast enhancement has often disappeared. Moreover, if the breast

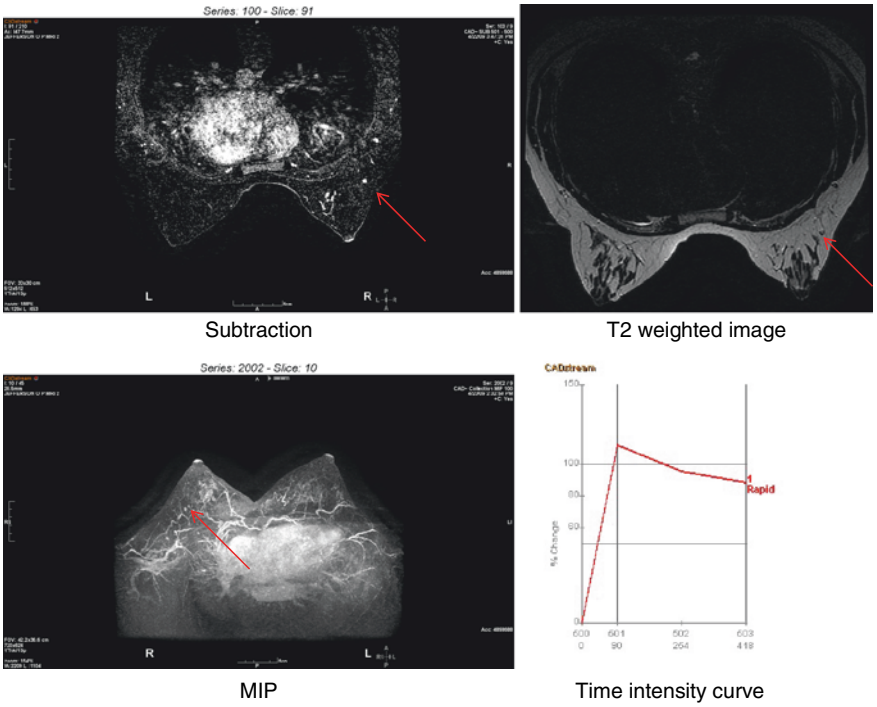

Fig. 8.8 Intramammary lymph node. Intramammary lymph nodes are usually round or oval with smooth margins, are rim enhancing and demonstrate washout kinetics. As with fat necrosis, interpretation is dependent on pre contrast images to assess for a fatty hilum, as seen on the T2 weighted image shown. In this particular case there is moderate background parenchymal enhancement with a dominant enhancing mass at the 9:00 position in the *right* breast (*arrow* on MIP). The T2 weighted image confirms that this mass represents an intramammary lymph node and no further work up is required: **a** subtraction; **b** T2 weighted image; **c** MIP; **d** time intensity curve

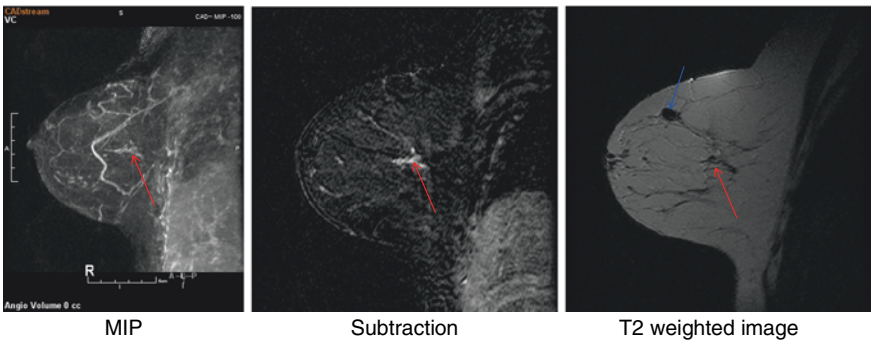

Fig. 8.9 Ductal carcinoma in situ (DCIS). This 72-year-old woman with a history of left mastectomy for cancer presented for screening MRI of the right breast. MRI demonstrates a non-mass-like enhancement in the deep central breast in a linear-ductal distribution with clumped internal enhancement. This area is low in signal intensity on T2 weighted images (*red arrow*). A non enhancing mass in the superior breast (*blue arrow* on T2 weighted image) was also seen corresponding to a hyalinized fibroadenoma visualized on mammography. Biopsy of the area of non-mass-like enhancement yielded DCIS. Kinetics are not assessed in cases of non-mass-like enhancement, as the uptake of contrast in an area of DCIS can be slow and DCIS can demonstrate persistent enhancement, limiting kinetic sensitivity: **a** MIP; **b** subtraction; **c** T2 weighted image

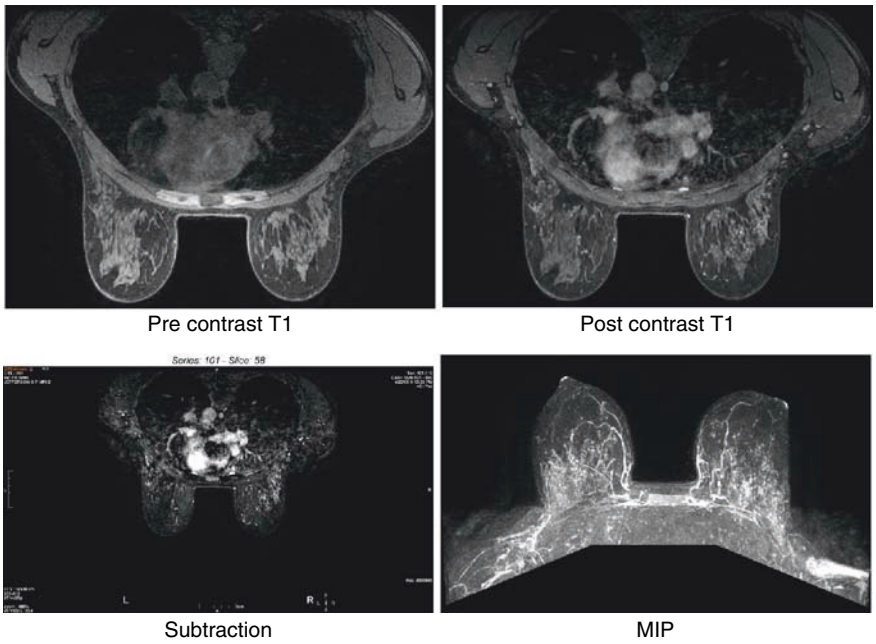

Fig. 8.10 Hormonal change. Screening MRI in this 37-year-old woman was conducted during week 4 of her menstrual cycle. There is moderate background regional enhancement in the upper outer quadrants and stippled on assessment of internal enhancement. The symmetry, distribution, and internal enhancement pattern all favor a benign etiology. In this case, enhancement is related to hormonal changes due to her imaging in the luteal phase of the menstrual cycle. Imaging, if possible, should be performed in the follicular phase during week 2 of the cycle to minimize hormone related non-mass-like enhancement that limits MR sensitivity: **a** pre-contrast T1; **b** post-contrast T1; **c** subtraction; **d** MIP

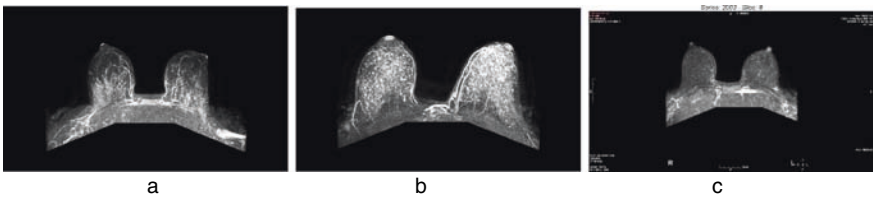

Fig. 8.11 Background parenchymal enhancement. The breast parenchyma can enhance normally following contrast administration. The degree of enhancement is variable both between patients and within a patient based on phase of the menstrual cycle. Parenchymal enhancement is minimal, mild, moderate or marked with progressive degrees of enhancement (**a–c** demonstrate increasing background enhancement). Moderate and marked background enhancement limit MR sensitivity as is evident by the illustrated images. In (**a**), there are scattered foci of enhancement, all of which measure less than 5 mm. These are too small to characterize, are bilateral, and scattered in a random distribution. No further management of the foci is required in this setting. If parenchymal enhancement is marked and the patient is not in week 2 of the menstrual cycle or is on hormonal replacement therapy, a decision can be made to repeat the MR (during week 2 or off hormones) if the patient is in a high risk category

tissue shifts during the procedure (as may happen during administration of local anesthetic or during needle placement), the lesion may be in a different location than it was on the dynamic images. We know from our experience with stereotactic and ultrasound guided procedures that lesions can shift during a procedure, sometimes from the administration of local anesthetic within the breast,

Table 8.1 BIRADS assessment categories for breast MRI images

Assessment category 1: normal
Assessment category 2: benign (a benign finding requiring no further follow up)
Assessment category 3: probably benign (a finding with very low likelihood of malignancy, short interval follow up recommended)
Assessment category 4: suspicious for malignancy
Assessment category 5: highly suggestive of malignancy.
Assessment category 6: biopsy proven malignancy

sometimes from the advancement of the needle. During these procedures, imaging is either continuous (in the case of ultrasound guidance) or repeated multiple times (in the case of stereotactic guidance) and so the shifting location of the lesion can be noted, with appropriate repositioning of the biopsy device. This is generally not possible in MR guided procedures because the target may disappear during the course of the procedure. Although tighter immobilization of the breast can minimize shifting during the procedure, it can also limit, or completely prevent, visualization of the lesion of concern[29,45] and therefore the breast is only lightly compressed. Although specimen radiography of lesions excised following MR guided localization can be performed,[50] adequacy of targeting following surgical excision or vacuum biopsy cannot generally be confirmed on specimen imaging as the target is vascular enhancement rather than a mass or calcifications. Thus, although MR guided wire localizations and percutaneous biopsies are very effective, there can be a degree of uncertainty following MR guided percutaneous biopsy or surgical excision, and follow-up MR imaging must be performed for negative biopsies.

For these reasons, suspicious lesions on MRI are often directed to second look ultrasound. If a corresponding finding is identified on ultrasound, it is biopsied with ultrasound guidance. Ultrasound guided biopsies are both more accurate and, because there is no need for a contrast injection or for MR imaging, technically simpler than MR guided biopsies. The yield of second look ultrasound will depend on the level of expertise of the sonographer performing the examination. Although this introduces an additional procedure for the patient, which may delay biopsy and will possibly increase patient anxiety, in expert hands the area of suspicion can be identified often enough using ultrasound to make the procedure feasible. Occasionally, a lesion that is indeterminate and suspicious on MR can be confirmed as benign on mammography and/or ultrasound and therefore correlation with these studies is often very important, particularly as this correlation may spare the patient an MR guided biopsy. This is particularly true for low suspicion masses such as intramammary lymph nodes and fibroadenomas, both of which are sometimes indeterminate on MR but are often clearly benign on mammography and/or ultrasound either by virtue of imaging characteristics (in the case of intramammary lymph nodes) or appearance coupled with mammographic stability.

An MR finding is more likely to be malignant if a corresponding lesion is identified on targeted ultrasound.[51,52] However, absence of a sonographic correlate is not an indicator of benignancy. In expert hands, the rate of malignancy of MR detected lesions without a sonographic correlate is still significant. Therefore, suspicious MR detected lesions require a tissue diagnosis whether or not second look ultrasound yields a sonographic correlate. For this reason, use of second look ultrasound varies in different practices. As stated previously, second look ultrasound may require an additional patient visit with an attendant increase in workup time and possibly in patient anxiety. However, the benefits of ultrasound targeting are substantial and therefore, where this level of ultrasound expertise is available, it can be used to substantial patient advantage.

Emerging Technology: Proton Spectroscopy

The variable and often low specificity of breast MRI has been cited as a disadvantage of the examination. Proton MR spectroscopy is a promising method of improving diagnostic specificity of breast MRI.

MR spectroscopy detects metabolic differences between tumors and normal tissues. Tumors commonly demonstrate elevation in compounds involved in membrane phospholipid synthesis, among them phosphocholine and glycerophosphorylcholine.[53] Proton MR spectroscopy can detect elevated levels of choline. The absence of a choline peak suggests that a lesion is benign and could potentially obviate the need for biopsy. In a small series, Bartella et al. found that the addition of proton MR spectroscopy could have raised the positive predictive value of biopsy from 35 to 82%, sparing over half of the biopsies performed.[54] A subsequent smaller series had similar findings.[55] However, these findings require confirmation in a much larger series of patients before the negative predictive value of a negative proton spectroscopy exam is fully evaluated, and therefore this study cannot be used for clinical decision making.

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Chapter 9
Genetic and Molecular Approaches to Imaging Breast Cancer

Eric Wickstrom and Mathew L. Thakur

Abstract Imaging gene expression non-invasively, with high sensitivity and specificity, would provide a more powerful diagnostic tool than any currently available. Although CT, MRI, and ultrasound have made great strides, none of the current modalities can image oncogene expression directly. No other reliable method is currently available to measure levels of specific receptors or mRNAs in vivo. In contrast to indirect approaches, noninvasive administration of SPECT or PET gene product probes allows us to image transformed cells overexpressing each specific oncogene. We have observed that radionuclide–chelator–VIP and radionuclide–chelator–AEEA–PNA–AEEA–IGF1 analogs are effective for imaging VPAC1 receptors and *CCND1* and *MYCC* mRNAs in breast cancer xenografts, with peptide mismatch and PNA mismatch specificity. Gene product imaging provides a route to the determination of malignancy in a suspicious mass, and molecular classification of a malignant mass.

Keywords Hybridization · Magnetic resonance imaging · Peptide · Peptide nucleic acid · Positron emission computed tomography · Ribonucleic acid · Single photon emission tomography

Key Issues

- Despite advances in detection and treatment, breast cancer will take the lives of more than 40,000 women in 2009. Breast cancer has already been growing for some time before a lump can be found. Mammograms and other radiological measurements suffer from high false positive and false negative rates.
- *CCND1* cancer gene mRNA elevation in ER+/PR+/Her2-xenografts can be detected specifically from outside the body by scintigraphic imaging with a [^{99m}Tc]chelator–*CCND1* PNA–AEEA–IGF1 analog hybridization agent.
- *CCND1* cancer gene mRNA elevation in ER+/PR+/Her2-xenografts can also be imaged specifically by positron emission tomography with a [^{64}Cu]chelator–*CCND1* PNA–AEEA–IGF1 analog hybridization agent.
- *CCND1* PET mRNA probes can identify sites of *CCND1* mRNA overexpression in sporadic breast lesions that arise in Her2+ transgenic mice, as opposed to normal mammary tissue, even

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when the lesions are not detected with [^{18}F]FDG. This system is the closest possible model for the clinical situation.

- Scintigraphic imaging of patients with a [^{99m}Tc]peptide specific for VPAC1/2 receptors revealed breast tumors as a function of VPAC1/2 gene expression. Importantly, an occult recurring neurofibroma not identifiable by other imaging methods was detected by SPECT with the [^{99m}Tc]peptide specific for VPAC1/2 receptors.
- Early external imaging of breast cancer gene activity with nuclear medicine mRNA probes might (1) improve the diagnosis of early breast cancer (including DCIS), (2) avoid biopsy trauma in patients with benign masses, and (3) ultimately reduce deaths from breast cancer.

Introduction

Compelling Clinical Need

Breast cancer is the most frequently diagnosed cancer in women.[1] Breast cancer has already been growing for some time before a lump can be found. Mammograms and other radiological measurements suffer from high false positive and false negative rates.[2,3] Clinical examination and mammography, the currently accepted breast cancer screening methods, miss almost 50% of breast cancers in women younger than 40 years, approximately 25% of cancers in women aged 40–49 years, and 20% of cancers in women over 50 years old.[4]

Among the ≈32 million mammograms performed every year in the US,[5] ≈7 million generate biopsies, approximately 80% of which (≈5.6 million) find benign pathology. Among the ≈1.4 million abnormal pathological results, which include ductal carcinoma in situ (DCIS), ≈250,000 are subsequently diagnosed as breast cancer.[6]

Despite advances in detection and treatment, breast cancer will take the lives of more than 40,000 women in 2009.[1] Early external imaging of breast cancer gene activity, however, might (1) improve the diagnosis of early breast cancer (including DCIS), (2) avoid biopsy trauma in patients with benign masses, and (3) ultimately reduce deaths from breast cancer.

The ability to determine noninvasively the benign or malignant status of suspected breast cancer found by mammography may minimize the number of unnecessary invasive biopsies, sparing the patient from physical and psychological trauma as well as saving health care dollars.

Available Molecular Imaging Agents

Although great strides have been made for imaging breast cancer using magnetic resonance (MRI), computerized tomography (CT), ultrasound (US), and radionuclide imaging such as positron emission tomography (PET) using 2'-[^{18}F]fluorodeoxyglucose (FDG) and single photon emission computerized tomography (SPECT) using ^{99m}Tc Sestamibi, each modality suffers from serious limitations.[2,3,7–12] The need is compelling to investigate improved imaging probes that might target specific biomarkers and thereby contribute to a greater reliability and higher sensitivity and specificity for imaging malignant lesions and to excluding benign pathology.

Aggressive tumors must take up and metabolize sugar at a high rate. [2'-^{18}F]fluorodeoxyglucose (FDG) is a well-established, commercially available agent for noninvasive determination of metabolic activity of tumors for diagnosis and for assessment following treatment.[13] FDG will serve

the purpose of determining changes in metabolic activity in breast cancer xenografts of treated animals by noninvasive PET imaging. Quantitation of uptake before and after treatment will permit assessment of the effectiveness of our agents at the metabolic level. However, FDG has limited utility for imaging breast tumors in the clinic due to their slow proliferation and metabolism, missing one-third of breast tumors.[14,15]

PET/CT fusion imaging harnesses the complementary information provided by both modalities. CT imaging provides highly detailed anatomical information and PET imaging provides functional information about the biological system. Fusion imaging is the ability to superimpose the spatially aligned images on a single display allowing the user to easily correlate features captured by the two modalities.

Progress has been made at the preclinical level in fluorescence imaging of breast cancer. For example, hydroxyapatite crystals (positive control) or calcium oxalate crystals (negative control) were implanted in rats, then detected 24 h later upon injection of a near-infrared fluorescent bisphosphonate derivative.[16] While fluorescence imaging shows promise, it is severely limited by diffraction to 2 cm penetration, and is therefore unlikely to be of use in visualizing deep-seated human tumors.

In animal studies, metastatic progression of mammary tumor cells distributed in immunocompromised mice was revealed by bioluminescent imaging after administering luciferase.[17] However, many of the constructs used for preclinical luminescent imaging, such as luciferase, have defined toxicity in humans, limiting their clinical usefulness.

Breast Cancer Genes as Diagnostic Targets

Based on the principle of hybridization of a complementary oligonucleotide with a target oncogene mRNA, radiolabeled antisense sequences have been explored for imaging applications. Design of antisense sequences for mRNA is theoretically straightforward, based on complementary base pairing rules. Noninvasive, real time imaging of oncogene expression in vivo would provide information on cellular gene expression patterns and might reveal molecular changes in diseased tissues at relatively early stages, providing opportunities for gene therapy, especially against overexpressed oncogene mRNAs.[18] In this approach of mRNA targeting, single base mismatch specificity may be achieved upon the binding of radiolabeled oligonucleotides to the target mRNA.[19] This approach is not only specific but also sensitive as it has been proposed that mRNA concentrations as low as 1 pmol/L in the tissues can probably be imaged with PET using radiolabeled probes of specific activity 1,000–10,000 Ci/mmol.[20] However, there are challenges in imaging endogenous gene expression with radiolabeled oligonucleotides such as in vivo stability, transport to the target, entry into the cell, and hybridization with target specific sequences.[21] Compared to other approaches of molecular imaging, the development of antisense imaging agents is in its infancy. However, imaging with antisense technology for early, specific, and noninvasive detection of oncogene expression is unique and warrants greater attention. Out of the 20,000–25,000 genes in the human genome, about 100 have been identified as protooncogenes.[22]

VPAC1 and *VPAC2*

VPAC1 and *VPAC2* encode G-protein coupled receptors that are overexpressed on a variety of frequently occurring human tumors including those of the breast, prostate, lung, and colon.[23] VPAC1

expression was lower on normal cells than on malignant cells,[24] on which the receptor density was high (10^4/cell).[25] VPAC1 overexpression can be imaged in breast cancer xenografts with its radiolabeled ligand vasoactive intestinal peptide (VIP).[26,27]

IGF1R

IGF1R encodes insulin-like growth factor 1 receptor, a 1,368-aa G-protein-coupled tyrosine kinase receptor for insulin like growth factor 1 (IGF1). IGF1R and IGF1 play a major regulatory role in development, cell cycle progression, and the early phase of tumorigenicity.[28] The *IGF1R* gene is amplified in ≈70% of human tumors, particularly in metastatic cells,[29] including breast cancer cells.[30] IGF1R internalizes IGF1 into endosomes that acidify, releasing cargo to the cytoplasm, followed by recycling of IGF1R back to the cell surface.[31] The S-S cyclized peptide, D(Cys-Ser-Lys-Cys), mimics a tight surface loop to direct endocytosis by IGF1R overexpressed on cancer cells.[32]

EGFR

EGFR encodes epidermal growth factor receptor (EGFR, ErbB-1, Her1), a 1,250-aa, 165-kDa transmembrane glycoprotein tyrosine kinase cell surface protein that binds epidermal growth factor (EGF), a 53-aa, 6-kDa extracellular signaling peptide, activating tyrosine autophosphorylation, leading to cell proliferation.[33] EGFR has been frequently found to be overexpressed in breast cancers, among others.[34]

HER2

HER2 encodes a 185-kDa protein, Her2, belonging to the receptor-tyrosine kinase family of cell surface proteins.[35] The Her2 protein displays strong homology with EGFR,[36] as do the closely related Her3 and Her4 receptors.[37,38] Overexpression of Her2 has been observed in 25–30% of breast cancers.[39]

CCND1

CCND1 (*BCL1, PRAD1*) encodes a 36-kDa protein, cyclin D1, which is a proto-oncogenic regulator of the G1/S checkpoint in the cell cycle that has been implicated in the pathogenesis of breast cancer.[40] The cyclin D1 protein is overexpressed in up to 80% of tumors,[41,42] indicating poor prognosis.[43]

MYCC

MYCC encodes a 65-kDa leucin zipper protein, c-Myc, that forms heterodimeric transcription factors with Max to turn on production of a broad panel of proliferative genes. *MYCC* oncogene expression is stimulated by estrogen in hormone responsive breast cancer cells in vitro. Amplification

of *MYCC* is considered to be a powerful prognostic indicator, particularly in node negative and estrogen receptor positive breast cancer.[44] *MYCC* was the first oncogene targeted by antisense oligonucleotides.[45]

BCL2

BCL2 encodes a 25-kDa cytoplasmic protein that localizes to mitochondria and increases cell survival by inhibiting apoptosis. The *BCL2* family members Bcl-XL protein and Bcl2 protein inhibit apoptosis and are upregulated frequently in breast cancer.[46]

Oligonucleotide antisense sequences specific for *IGF1R*,[47] *CCND1*,[48] *EGFR*,[49] *HER2*,[50] *MYCC*,[51] and *BCL2*[52] are reported to downregulate respective gene expression in cancers. Based on those results, mutated or overexpressed *IGF1R, EGFR, HER2, CCND1, MYCC,* and *BCL2* mRNAs are plausible targets for radiohybridization imaging (RHI).

Agents to Image Gene Expression in Animal Models and Patients

Peptide Probe Design

The 28-aa peptide VIP (Fig. 9.1), and the 27-aa peptide PACAP have high affinities for VPAC1 and VPAC2 (named after VIP and PACAP combined) oncogene receptors expressed on malignant breast cancer cell surfaces. Over the past few years we have gained extensive experience in radiolabeling VIP, PACAP, their analogs, and PNA chimeras with ^{99m}Tc and ^{64}Cu for planar and PET gene product imaging of human breast cancer, pancreas cancer, and prostate cancer in mice and have successfully used [^{99m}Tc]peptides in humans.[26,53]

Malignant breast cancer cells also overexpress IGF1R.[29] These receptors provide an opportunity to target IGF1R as a vehicle for receptor-mediated endocytosis[31] of a radiohybridization imaging probe.[48,54] Initially, we observed that synthesis of an *IGF1R* PNA dodecamer with an N-terminal cyclized D-peptide analog of IGF1, D(Cys-Ser-Lys-Cys) (Fig. 9.2), increased cellular uptake 5- to 10-fold by those cells overexpressing IGF1R.[32] A reverse sequence was synthesized with respect to the normal L-amino acid sequence to account for the reversal of chirality. We synthesized a fluorescent complementary PNA-IGF1 analog, a peptide sequence control with two D-Ala residues in the peptide in place of D(Ser-Lys), and a PNA sequence control.

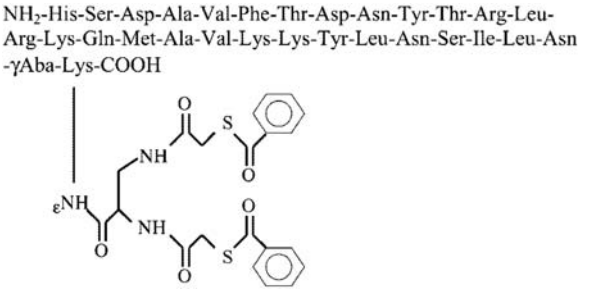


Fig. 9.1 Schematic of VIP_{28}-N_2S_2 probe TP3982 that can bind ^{64}Cu quantitatively for PET imaging of VPAC1 receptor. (Reprinted by permission of the Society of Nuclear Medicine from Thakur et al.[26,27])

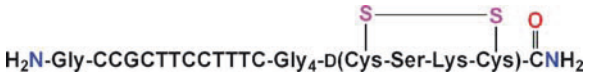


Fig. 9.2 PNA–peptide specific for *IGF1R* mRNA and IGF1 receptor. (From Tian et al.[18] Reprinted with permission of Wiley-Blackwell)

Hybridization Probe Design

Oligonucleotide potency in vivo depends upon nuclease resistance, tissue distribution, tumor cell uptake, nuclear localization, and mRNA hybridization. Naturally occurring oligonucleotides (Fig. 9.3) cannot be used directly for in vivo imaging because they are rapidly degraded in vivo by endonucleases and exonucleases.[55] Many oligonucleotide modifications are available that degrade more slowly,[55] but peptide nucleic acids (PNAs) (Fig. 9.3) are completely resistant to both nucleases and proteases.[56]

Due to their uncharged backbones, PNAs hybridize to RNA more strongly and specifically than most oligonucleotide derivatives.[56] Because PNA hybridization is so strong,[57] oligomers as short as 12 residues are active and specific enough to hybridize to oncogene mRNAs to facilitate imaging and therapy. A hybridization probe of 12 bases is also theoretically unique among transcribed sequences.[58]

Experience to date with PNA implies that the initiation codon region is the most effective region to probe.[56] The chelator–PNA–IGF1 analog probes provide hybridization arrest independent of RNase H activity.[59] As a result, PNA probes do not direct degradation of their mRNA analytes. Unconjugated PNAs are not significantly taken up by cells,[60] so that uptake of peptide–PNA–IGF1 analog chimeras will be limited to target cells expressing significant levels of receptor for the peptide analog, as we have reported.[48]

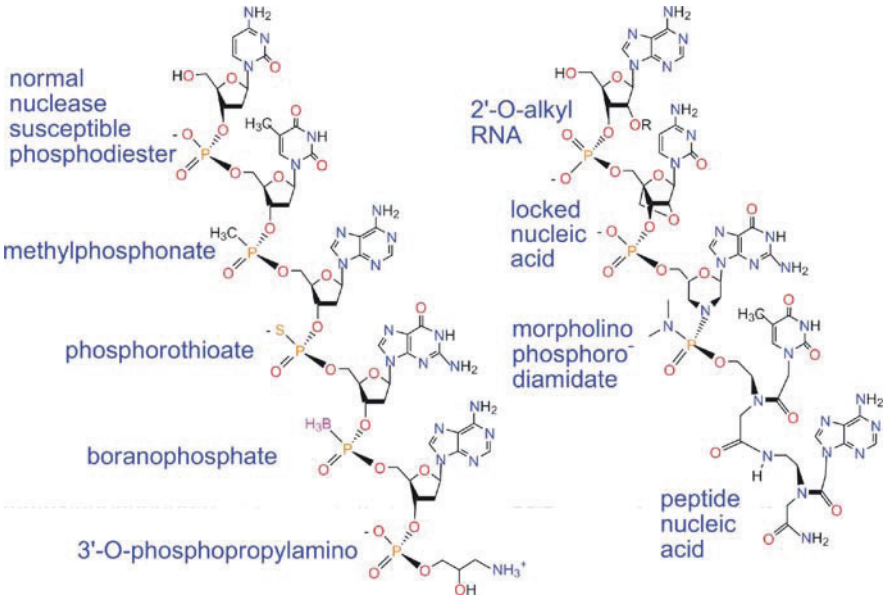


Fig. 9.3 Oligonucleotide backbone derivatives

Plasma binding proteins that carry IGF1[61] are likely to provide favorable pharmacokinetics for chelator–PNA–IGF1 analog conjugates, even though PNAs by themselves are eliminated quickly due to poor plasma protein binding.[62] PNA–peptides, at doses 10,000 times those planned for human imaging, have displayed no toxicity,[63] immunogenicity,[64] mutagenicity, or clastogenicity[65] in mice.

Solid Phase Synthesis, Purification, Radiolabeling, And Stability

We postulated that solid phase synthesis on a single resin support could be extended to continuous Fmoc coupling of all GDAGGB chelating amino acids, antisense PNA monomers, and IGF1 analog amino acids during a single, continuous solid phase synthesis on an automated synthesizer (Fig. 9.4). The chelator–PNA–IGF1 analog (Fig. 9.5)[66] and control sequences (Table 9.1) were synthesized in good yield, purified by reversed phase liquid chromatography, and characterized by mass spectroscopy and PNA–RNA melting temperatures.[48] The imaging probes were then labeled with ^{99m}Tc, analyzed by HPLC, ITLC, and SDS polyacrylamide gel electrophoresis (Fig. 9.6)[48,67] in order to determine the efficiency of labeling and the molecular mass of the final radiolabeled imaging probe.

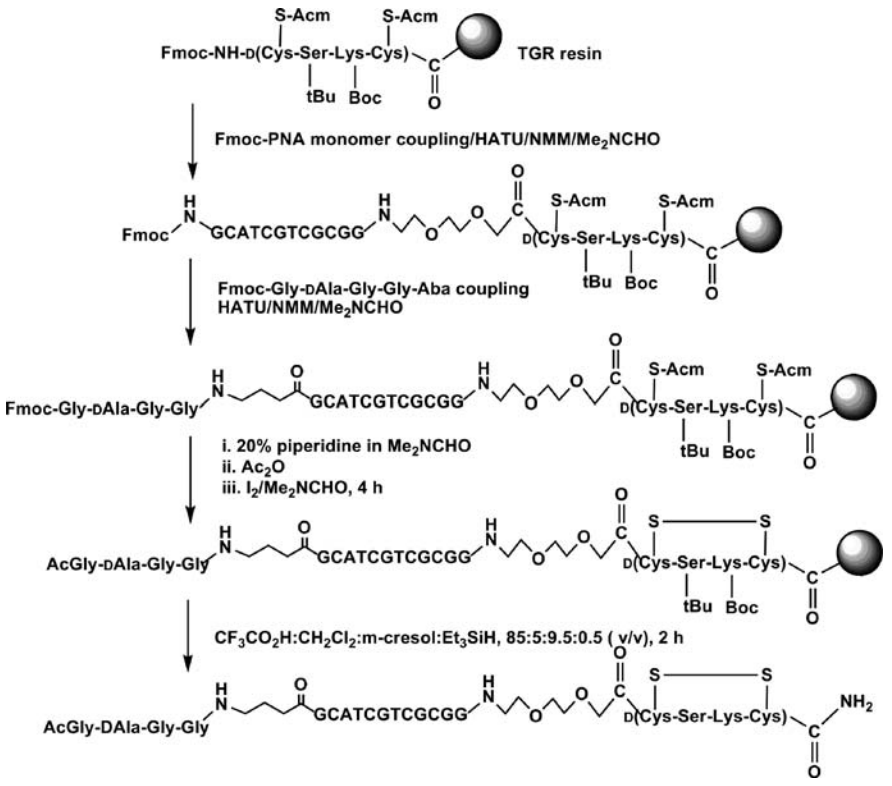


Fig. 9.4 Assembly of GDAGGB PNA–peptide. (From Tian et al.[18] Reprinted with permission of Wiley-Blackwell)

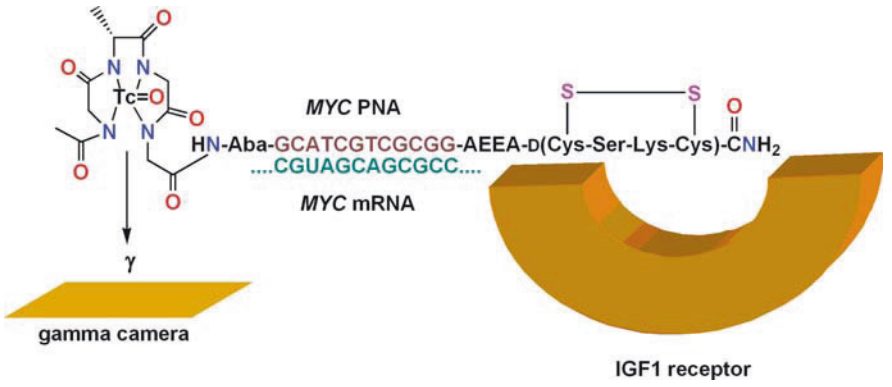


Fig. 9.5 [^{99m}Tc]GDAGGB–PNA–peptide specific for *MYC* mRNA and IGF1 receptor, designed to bind to the receptor for IGF1, internalize, and hybridize with *MYC* mRNA.[66] Scintigraphic imaging of γ-particles emitted upon decay of ^{99m}Tc. (From Tian et al.[18] Reprinted with permission of Wiley-Blackwell)

Table 9.1 GDAGGB–PNA–peptide chimera characterization

Name	Sequence	Label	Yield (%)	Calculated mass	Measured mass
PNA-free	GDAGGB-$(Gly)_4$-D(CSKC)	WT990	19.0	990.0 Da	992.0 Da
MYC PNA mismatch	AcGDAGGB-GCATGTCTGCGG-AEEA-D(CSKC)	WT4235	30.6	4235.0 Da	4234.7 Da
MYC PNA antisense	AcGDAGGB-GCATCGTCGCGG-AEEA-D(CSKC)	WT4219	27.2	4219.0 Da	4221.6 Da
CCND1 PNA mismatch	AcGDAGGB-CTGGACAACCAT-AEEA-D(CSKC)	WT4172	39.1	4172.0 Da	4174.1 Da
CCND1 peptide mismatch	AcGDAGGB-CTGGTGTTCCAT-AEEA-D(CysAlaAlaCys)	WT4113	34.0	4113.0 Da	4113.7 Da
CCND1 PNA antisense	AcGDAGGB-CTGGTGTTCCAT-AEEA-D(CSKC)	WT4185	30.6	4185.0 Da	4187.2 Da
Fl-*CCND1* peptide mismatch	SFX-AEEA-CTGGTGTTCCAT-AEEA-D(CysAlaAlaCys)	WT4361	3.0	4361.0 Da	4360.6 Da
Fl-*CCND1* PNA antisense	SFX-AEEA-CTGGTGTTCCAT-AEEA-D(CSKC)	WT4433	2.8	4433.0 Da	4433.8 Da

In order to label the hybridization probes with ^{64}Cu for PET imaging, we substituted a 1,4,7, 10-tetra(carboxymethylaza)cyclododecane (DOTA) chelator[54] for the GDAGG peptide that we used to chelate ^{99m}Tc.

These preparations were stable at 22 °C for more than 4 h, as determined by HPLC, and were stable to challenges with 100-fold molar excesses of DTPA, human serum albumin, or cysteine.

The metabolic stability of the *CCND1* probe [^{64}Cu]WT4348 was tested by administering 13 MBq (350 μCi) with a sterile 27-gauge needle into the lateral tail vein of a female NCr mouse lightly anesthetized with a mixture of ketamine (200 mg/kg), xylazine (10 mg/kg), and acetopromazine

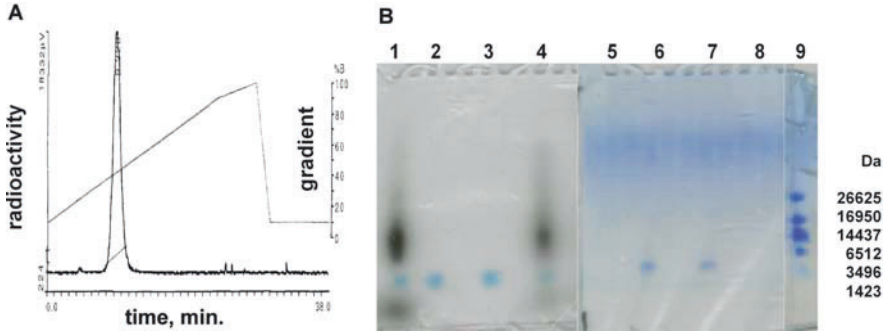


Fig. 9.6 Analysis of *CCND1* antisense probe [^{99m}Tc]AcGDAGGB-CTGGTGTTCCAT-AEEA-D(CSKC), WT 4185.[48,67] **a**, an aliquot of the labeling reaction was analyzed by C_{18} HPLC. The single labeled peak eluted at 9.3 min. **b**, denaturing gel electrophoresis on 10–20% polyacrylamide Tris-Tricine SDS gels (Bio-Rad). The *left* side is an autoradiogram, while the *right* side was stained with Coomassie blue. Lane 1,5, ^{99m}Tc labeling reaction; lane 2,6, mock reaction without ^{99m}Tc; lane 3,7, purified WT4185; lane 4,8, 9.3 min. ^{99m}Tc peak from panel A; lane 9, peptide mass standards. (Reprinted by permission of the Society of Nuclear Medicine from: Tian et al.[48])

(2 mg/kg) at a dose of 160 μL/25 g. The mouse was euthanized 3 min later and exsanguinated; 0.5 mL of blood was sedimented for 10 min. at 3,000 g in a heparinized polypropylene vial. Then 30 μL of the serum supernatant, containing 5 μCi of ^{64}Cu, was mixed with SDS-PAGE sample buffer, heated, and analyzed on an 18% polyacrylamide Tris-glycine gel (Invitrogen, San Diego CA) as previously described.[48] Duplicate gels were autoradiographed or stained with Coomassie blue.

[^{64}Cu]DO3A–PNA–peptides were determined by HPLC to be thermodynamically stable to 100-fold molar excesses of DTPA, human serum albumin, or cysteine at 22 °C for 30 min. The *CCND1*-specific [^{64}Cu]DO3A–PNA–IGF1 analog WT4348 was administered to a mouse to test for probe stability in circulating blood. Serum prepared from a blood draw 3 min after administration was analyzed by SDS–PAGE and autoradiography. Negligible ^{64}Cu radioactivity was observed over the mass range of 6–50 kDa. In particular, no ^{64}Cu radioactivity was detected at 30 kDa, the mass of Cu/Zn superoxide dismutase, which is stable under denaturing conditions on SDS-PAGE.[68]

The dissociation constant for Cu(II)-DO3A in water is 0.5×10^{-24} M.[69] Nevertheless, Cu(II) has been reported to dissociate from DOTA or TETA in vivo and bind to superoxide dismutase and metallothioneins in the liver, the principal organ for blood detoxification.[70] In addition, transchelation of ^{64}Cu to albumin in blood has also been hypothesized to enable high ^{64}Cu uptake in liver, blood and intestine, and long retention.[70] The same report, however, found that Cu(II)-DO3A was extracted intact from liver and blood proteins. Our observations, therefore, disprove the hypothesis of ^{64}Cu(II) transchelation.

Cellular Specificity and Internalization

Cellular uptake of the fluoresceinyl–PNA–IGF1 analog, the peptide control, and the PNA control, were studied in murine BALB/c3T3 cells transformed with human IGF1R,[71] and compared with two cell lines with low IGF1R expression. The transformed cells that overexpress IGF1R displayed 5- to 10-fold higher uptake of the specific PNA–AEEA–IGF1 analog after 4 h exposure at 1 μM, compared with the control PNA or the control PNA–peptide.[32] Comparable results were seen when fluoresceinyl–chelator–PNA–IGF1 analog was studied in human MCF7 breast cancer cells (Fig. 9.7).[48]

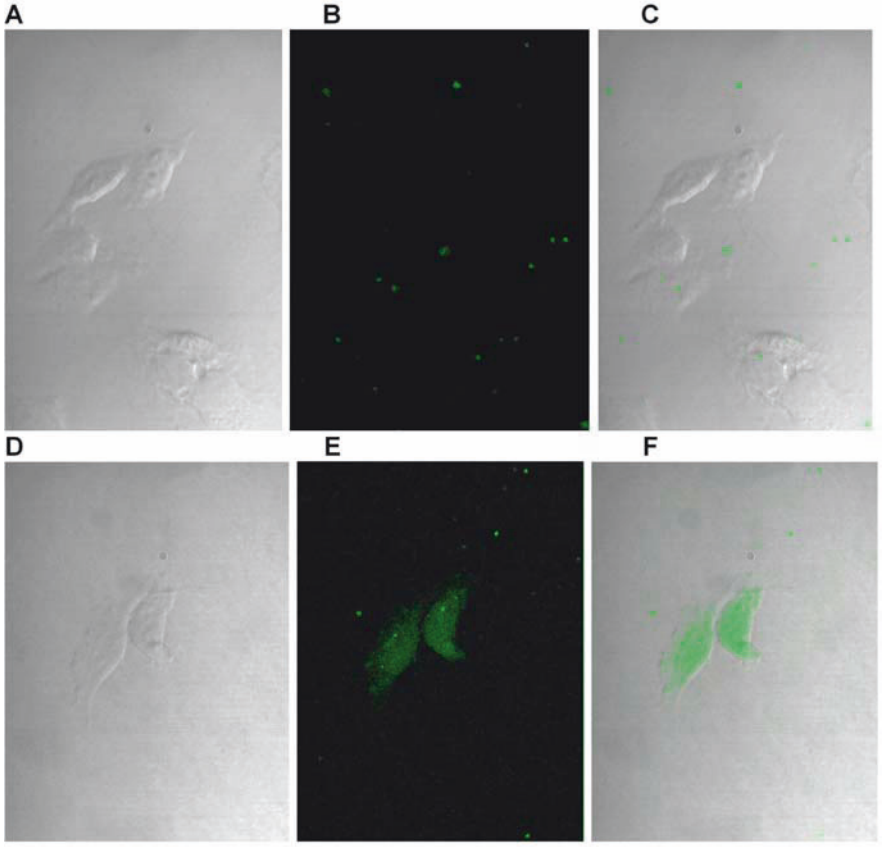


Fig. 9.7 MCF7:IGF1R cell uptake of the *CCND1* fluoresceinyl–PNA–AEEA–mismatch peptide probe, WT4361 (**a**–**c**), and the *CCND1* fluoresceinyl–PNA–IGF1 peptide probe, WT4433 (**d**–**f**). Cells were incubated in 1 μM fluoresceinyl–PNA–peptide for 8 h at 37 °C in PRF–SFM, then fixed and examined by confocal microscopy. *Left*: phase contrast; *middle*: fluorescence; *right*: overlay. (Reprinted by permission of the Society of Nuclear Medicine from: Tian et al.[48])

Administration, Pharmacokinetics, and Tissue Distribution

Human MCF7:IGF1R estrogen receptor-positive breast cancer cells, clone 17, transformed to express 1×10^6 IGF1R/cell constitutively from a cytomegalovirus promoter[72] were maintained in DMEM plus 5% calf serum, 50 U/mL penicillin, 5 μg/mL streptomycin, 2 mM glutamine, and 7.5 nM 17-μ-estradiol (Sigma) at 37 °C under 5% CO_2. For tumor induction, 5–6 $\times$ 10^6 cells in 0.2 mL of culture medium were implanted intramuscularly through a sterile 27-gauge needle into the thighs of female Ncr nude mice obtained from NIH. Tumors were allowed to grow to no more than 0.5 cm in diameter. Each injection included 10 mg of Matrigel (Becton Dickinson). A pellet that releases 4.5 mg of 17-β-estradiol over 60 days (Innovative Research of America) was implanted subdermally in each mouse. All animal studies were conducted in accordance with federal and state guidelines governing the laboratory use of animals, and under approved protocols reviewed by the Animal Care and Use Committee at Thomas Jefferson University. All animals were anesthetized by approved methods, and when required the animals were restrained using methods and

devices specifically designed to provide a minimum of discomfort to the animal. Animals were euthanized in a halothane chamber, consistent with USDA regulations and American Veterinary Medical Association recommendations.

About 500 μCi of [^{99m}Tc]probes or 200 μCi of [^{64}Cu]probes in 0.2-mL vehicle were administered into the tail vein with sterile 27-gauge needles. Radioactivity of the syringe was measured full, before administration, and after administration, to quantitate the absolute dose injected. At 4, 12, and 24 h post-injection, mice were euthanized, and tissues were dissected for measurements of pharmacokinetics and tissue distribution. These were washed free of blood, blotted dry, weighed, and radioactivity associated with each tissue was counted in an automatic Series 5,000 γ-counter (Packard), together with a standard radioactive solution of a known quantity prepared at the time of injection. Results were expressed as percent of injected dose per gram of tissue (% I.D/g).

Whole Body Imaging

We determined the sensitivity of SPECT and PET imaging of the targeted oncogene mRNAs in breast cancer xenografts relative to the nonspecific signals possible in other tissues 4, 12, and 24 h after probe administration. Full length IGF1 was used as the peptide blocking control. We combined PET imaging with CT imaging in a fusion of the two modalities to harness their complementary information. CT imaging provides highly detailed anatomical information and PET imaging provides crucial functional information about which oncogene is active, if any, in the lesion. During imaging, animals were anesthetized with a mixture of ketamine (200 mg/kg), xylazine (10 mg/kg), and acetopromazine (2 mg/kg).

Scintigraphic (SPECT) images were acquired on a Starcam (GE Medical) gamma camera equipped with a parallel hole collimator. For each image, 300,000 counts were collected. Quantitation of tumor images was provided by Digital scanning of region-of-interest intensities with the interfaced Entegra computer (GE Medical) across each scintigraphic image from the tumor-free left flank to the tumor-bearing right flank.

PET images were acquired on a Philips Mosaic PET scanner that was designed specifically for small animal imaging. Data were acquired in full 3D with an axial field of view of 120 mm allowing for high sensitivity and imaging an entire mouse at one time. Tomographic images can be reconstructed routinely into 0.5-mm^3 voxels with approximately 2.0-mm resolution.

CT images were acquired on the Imtek Inc. MicroCAT II scanner that was also designed specifically for small animal imaging. An X-ray source and X-ray detector stage (100 × 70 mm) was rotated around the animal in a step and shoot fashion. Tomographic images can routinely be reconstructed using a Feldkamp cone-beam algorithm into 100 μm^3 × 3 voxels offering approximately 200-μm resolution.

Oncogene Expression Imaging in Animal Models

MYCC

We imaged *MYCC* mRNA in ER+/PR+/Her2- breast cancer xenografts with a specific [^{99m}Tc]peptide–*MYCC* PNA–AEEA–IGF1 analog,[66] while a PNA-free control [^{99m}Tc]peptide–IGF1 analog failed to show a tumor image, as did the [^{99m}Tc]peptide–*MYCC* PNA without the

IGF1 analog.[73] In these studies, sevenfold higher intensity of *MYCC* [^{99m}Tc]chelator–PNA–D(Cys-Ser-Lys-Cys) probes was observed compared to mismatch or contralateral controls.[66]

CCND1

We hypothesized that scintigraphic detection of *CCND1* PNA gene product imaging agents with a ^{99m}Tc-chelating peptide Gly-D(Ala)-Gly-Gly-Aba on the N-terminus, and a cyclized IGF1 peptide loop, D(Cys-Ser-Lys-Cys), on the C-terminus, could detect *CCND1* mRNA in human MCF7 breast cancer xenografts in nude mice from outside the body.

IGF1R-overexpressing MCF7 xenografts in nude mice were visualized at 4, 12, and 24 h after tail vein administration of the [^{99m}Tc] chelator (Fig. 9.8).[48] At 12 and 24 h after administration of the [^{99m}Tc] *CCND1* probe WT4185, tumor image intensities were seven times greater than contralateral site intensity. The intensity ratios for the three negative controls were 1–1.5. [^{99m}Tc] chimeras distributed normally to kidneys, livers, tumors, and other tissues.

To image *CCND1* mRNA by PET, we administered approximately 0.2 mCi [^{64}Cu] DO3A–AEEA–PNA–AEEA–IGF1 analog *CCND1* probes to cohorts of five nude mice bearing MCF7:IGF1R xenografts as above to determine the sensitivity of PET imaging. Urinanalysis revealed no significant breakdown of the probe over 2–3 h.[54] Of the three probes, the antisense probe [^{64}Cu]WT4348 exhibited the highest tumor image intensity relative to the contralateral tissue at 4, 8, and 24 h (Fig. 9.9).[54] IGF1 blocking reduced the tumor image intensity to the level of the negative controls. PET image intensities in $1\times1\times1$-mm^3 voxels across the tumors revealed strong peaks of antisense probes in the heart of the tumors, compared to uniform lower levels of accumulation of mismatch probes in tumors.

In these studies, sevenfold higher intensity of [^{99m}Tc]chelator–*CCND1* PNA–D(Cys-Ser-Lys-Cys) probes was observed compared to mismatch or contralateral controls, as shown in Fig. 9.9.[65,68]

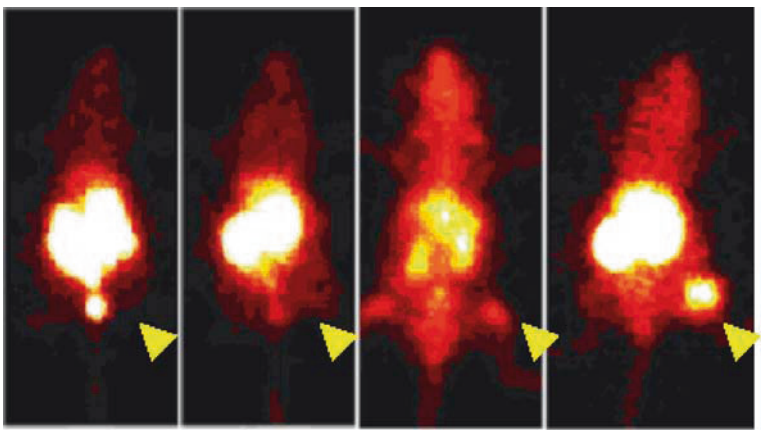
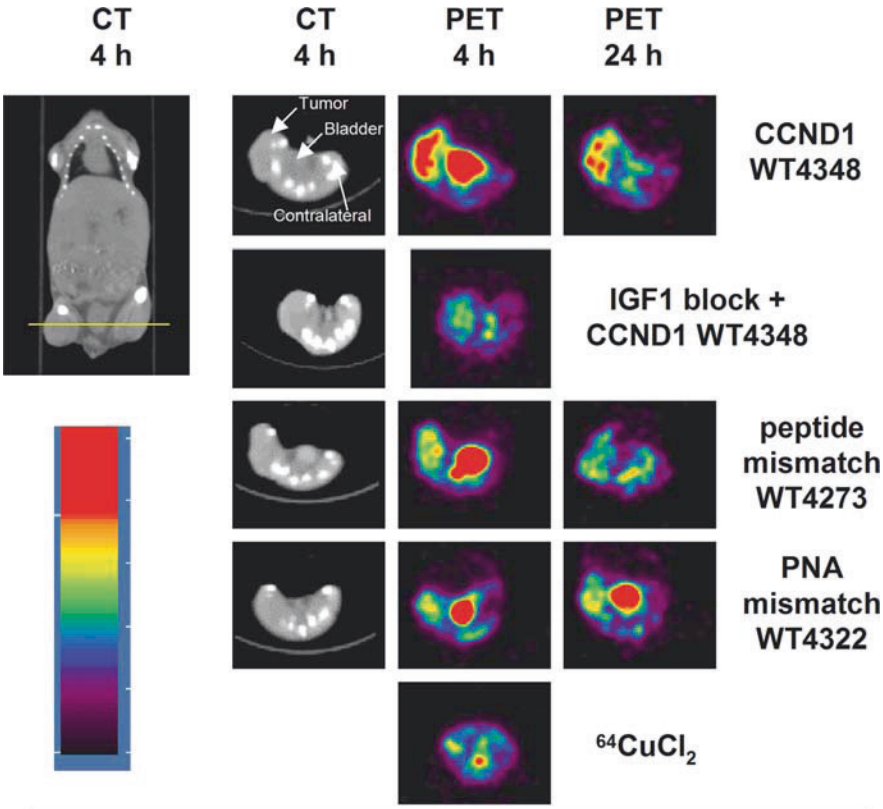


Fig. 9.8 Scintigraphic images of γ particles emitted by decaying ^{99m}Tc in immunocompromised mice carrying human MCF7:IGF1R estrogen receptor-positive breast tumor cell xenografts (*arrowhead*) at 12 h after injection of PNA–free control, [^{99m}Tc]WT990, PNA mismatch control, [^{99m}Tc]WT4172, peptide mismatch control, [^{99m}Tc]WT4113, and *CCND1* PNA antisense probe, [^{99m}Tc]WT4185. (Reprinted by permission of the Society of Nuclear Medicine from: Tian et al.[48])



Sequence	4 h	24 h
CCND1 probe [^{64}Cu]WT4348	6.83 ± 3.41 (n=5)	7.87 ± 1.99 (n=4)
IGF1+[^{64}Cu]WT4348	3.66 ± 0.17* (n=2)	not done
peptide mismatch [^{64}Cu]WT4273	3.82 ± 1.06* (n=5)	5.04 ± 0.67* (n=4)
PNA mismatch [^{64}Cu]WT4322	3.37 ± 1.43* (n=4)	4.53 ± 1.77* (n=4)
free $^{64}CuCl_2$	2.97 (n=1)	not done

*significantly different ($p<0.05$) based on Holm-Sidak all pairwise multiple comparison

Fig. 9.9 Transverse microPET images of immunocompromised NCR mice bearing MCF7 (ER+/Her2-) xenografts on their right flanks recorded at 4 and 24 h after tail vein injection of 3.7–7.4 MBq (100–200 μCi) of [^{64}Cu]*CCND1* antisense probe, [^{64}Cu]peptide mismatch probe, [^{64}Cu]PNA mismatch probe, or free $^{64}CuCl_2$ as an unchelated control. The *yellow line* on the coronal CT image shows the level of the transverse images. The *color scale* of the images was normalized to the max/min of frame to show the dynamic range of tumor uptake. Only the *CCND1* probe yielded strong tumor contrast. (Reprinted by permission of the Society of Nuclear Medicine from: Tian et al.[54])

For the mismatch controls, we postulate that excess PET intensity over contralateral background reflects nonspecific extravasation into the xenograft tissue. Therefore, ratios of peak tumor PET image intensities to contralateral muscle average intensities were calculated for equal-sized regions of interest. Of the three probes, the antisense probe [^{64}Cu]WT4348 exhibited the highest tumor intensity to contralateral muscle intensity, 7.87 ± 1.99 at 24 h post-injection.[54] This phenomenon implies that *CCND1* mRNA levels are most intense in the center of the tumors, while the periphery of the tumors shows less oncogene activity. The implication is that oxygen deficit and nutrient deficit in the core of the tumor drives more intense oncogene activity, leading to metastatic transformation.

Distribution of [^{64}Cu]WT4322, [^{64}Cu]WT4372, [^{64}Cu]WT4348, and free $^{64}CuCl_2$ in blood, tissues, and tumors were presented as % ID/g at 4 h post-injection.[54] Both [^{64}Cu]WT4322 and [^{64}Cu]WT4273 control probes exhibited high kidney, liver, lung, spleen, and intestine uptake. Within 4 h of administration, base-mismatched [^{64}Cu]WT4322 showed significantly lower uptake compared with peptide-mismatched [^{64}Cu]WT4273 and CCND1 probe [^{64}Cu]WT4348, except in the kidney, which took up more [^{64}Cu]WT4322. Both [^{64}Cu]WT4322 and [^{64}Cu]WT4273 showed slow clearance in kidney, liver, lung, spleen, and intestine. [^{64}Cu]WT4348 showed the highest tumor uptake, 2.01 $\pm$ 0.43% ID/g, and highest tumor/muscle ratio, 2.72 $\pm$ 0.67, at 4 h post-injection. [^{64}Cu]WT4348 also displayed the highest washout, compared to [^{64}Cu]WT4273 and [^{64}Cu]WT4322.[54] Free $^{64}CuCl_2$ accumulated preferentially over [^{64}Cu]DO3A–PNA–peptide in most tissues. Free $^{64}CuCl_2$ accumulated indistinguishably from [^{64}Cu]DO3A–PNA–peptide in the spleen and tumor.

BCL2

Recently an ^{111}In-labeled anti-*BCL2* sequence coupled to Tyr^3-octreotate for somatostatin receptor-mediated intracellular delivery. Although tumor uptake of ^{111}In–DOTA–*BCL2*–PNA–Tyr^3-octreotate was less than 0.2% ID/g, tumors could be imaged by 48 h p.i.[43]

Oncogene Expression Imaging in Patients

VPAC1

A [^{99m}Tc]peptide specific for VPAC1/2 receptors revealed breast tumors as a function of gene expression. Importantly, an occult recurring neurofibroma not identifiable by other imaging methods was detected by SPECT with our [^{99m}Tc]peptide. These results support our hypothesis for identifying the most active sites of oncogene expression to be sure of complete excision of transformed tissues.

A report which analyzed more than 600 tumors and their metastases using autoradiography reported that VPAC1 and VPAC2 receptors are overexpressed on a variety of frequently occurring human tumors including those of the breast, prostate, lung, and colon.[23] The authors also reported that on 100% of the human prostate tumors examined (n = 35), VPAC1 receptors were predominately overexpressed on PC tissues and VPAC2 on stroma to a lesser extent. VPAC1 expression was lower on normal cells than on malignant cells[24] on which the receptor density was high (10^4/cell).[25]

The 28 amino acid peptide, VIP, and the 27 amino acid peptide PACAP have high affinities for VPAC1 and VPAC2 (named after VIP and PACAP combined) oncogene receptors expressed on malignant cell surfaces. Over the past few years we have gained extensive experience in radiolabeling VIP, PACAP, their analogs and PNA chimeras with ^{99m}Tc and ^{64}Cu for planar and PET gene product imaging of human breast cancer, pancreas cancer, and prostate cancer in mice and have successfully used [^{99m}Tc]peptides in humans.[26,53]

Our data show that these ^{64}Cu probes are highly stable in vivo.[74] Furthermore, the high VIP affinity for receptors on malignant cells and subsequent internalization[24] minimizes its proteolysis and allows cell detection as we have demonstrated in both mice and humans.[26,53] We synthesized two more analogs of VIP_{28} that are more potent and biologically stable than VIP_{28}. The two

analogs are Lys^{12}, Nle^{17}, (3-OCH_3, 4-OH) Phe^{22}, Val^{26}, Thr^{28}-VIP, and Ac-His^1-Ala^2-Asp^3-Ala^4-Val^5-Phe^6-Thr^7-Glu^8-Asn^9-Tyr^{10}-Thr^{11}-Lys^{12}-Lue^{13}-Arg^{14}-Lys^{15}-Gin^{16}-Nle^{17}-Ala^{18}-Ala^{19}-Lys^{20}-Lys^{21}-Tyr^{22}-Leu^{23}-Asn^{24}-Asp^{25}-Leu^{26}-Lys^{27}-Lys^{28}-Ala^{29}-Ala^{30}-Ala^{31}, which is cyclized between Lys^{21} and Asp^{25} (TP3871). These analogs have the highest IC_{50} values (0.8 nM and 0.45 nM, respectively) among the many that have been synthesized and evaluated.[75]

The rationale for choosing these two VIP analogs was as follows. VIP_{28} is comprised of three aromatic moieties at Phe^6, Tyr^{10}, and Tyr^{22}, a negatively charged site at Asp^3, and a lone pair structure at His^1. Although all five sites are required for complete binding to receptors with high affinity, substitutions at position 22 of 3-OCH_3-4-OH-Phe and Lys^{12}, Nle^{17}, Val^{26}, Thr^{28}-VIP produced the best results, increasing potency by 18 times (IC50 = 0.8 nM vs 15 nM) over VIP_{28}.[76] Higher affinity may enhance tumor uptake and improve image quality. Again, our recent preliminary data in humans, obtained using Tc-99m-TP3654, a VIP analog, are consistent with this hypothesis.[26]

A VIP harboring a C-terminal diaminodithiol (N_2S_2) chelator (Fig. 9.1) was synthesized by solid phase coupling.[27] Using this technique, several peptides, such as VIP have been labeled with ^{99m}Tc in the Thakur laboratory and successfully evaluated in vitro, in experimental animals, and in humans.[26,77]

Encouraged by the pre-clinical evaluation results, we initiated a feasibility study using [^{99m}Tc]VIP for imaging tumors in humans. All tumors as identified by CT, MRI, [^{99m}Tc]SestaMIBI (methoxy isobutyl isonitrile), sonography, or mammography were known to express VIP receptors (VPAC1, VPAC2) in high density.[78] Negative controls did not display inappropriate concentration of [^{99m}Tc]VIP (Fig. 9.10).

Out of 11 patients examined thus far, there was concordance in 9. In the other two patients, only the [^{99m}Tc]VIP scan was positive for tumors known to express VIP receptors.[26] One resulted from recurrence of resected breast cancer (Fig. 9.11), and the other from a recurrence of neurofibroma in the neck (Fig. 9.12). These data demonstrate that (1) we can label peptides with ^{99m}Tc efficiently without compromising their receptor specificity and biological activity and (2) target lesions in vivo that express specific receptors. Most importantly, benign breast atypia was not delineated, suggesting an absence of false positives, while malignant recurrences not identified by current methods were clearly identified, overcoming the problem of false negatives. These positive results support our plan for radioimaging of cancer gene product expression.

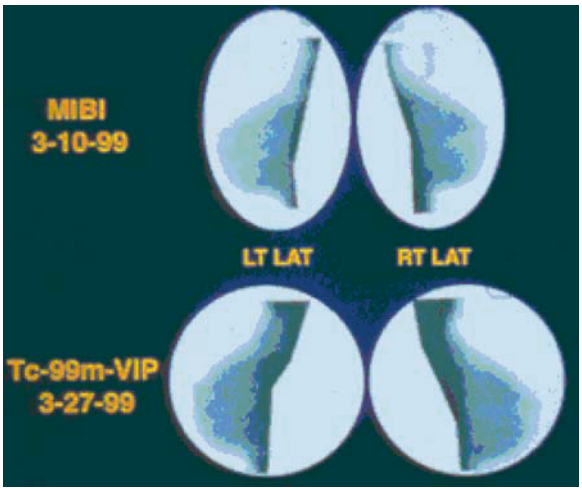


Fig. 9.10 A 47-year-old female with suspicious mammogram had normal SestaMIBI scan and normal [^{99m}Tc]VIP scan. R breast biopsy showed only calcium deposits

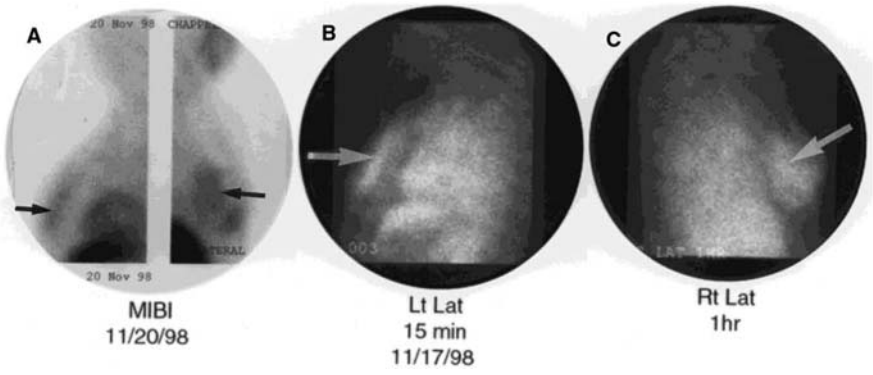


Fig. 9.11 A 42-year-old woman with prior left mastectomy presented with recurrence in right breast and left operative site. Lateral images with [^{99m}Tc]SestaMIBI (*left*) show uptake in the chest wall and right breast (*arrows*). Left-side view (*center*) obtained 15 min after injection of [^{99m}Tc]VIP and right-side view (*right*) obtained 1 h after injection of [^{99m}Tc]VIP show same lesions (*arrows*) perhaps with better intensity than on corresponding [^{99m}Tc]SestaMIBI images (*left*). (Reprinted by permission of the Society of Nuclear Medicine from: Thakur et al.[26])

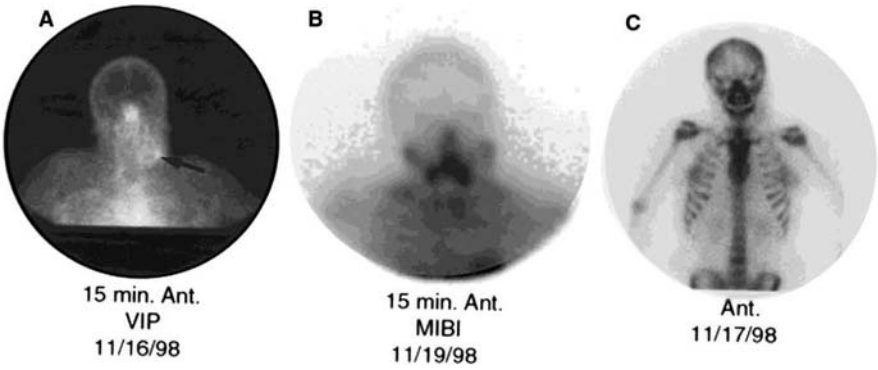


Fig. 9.12 A 20-year-old woman with a history of neurofibroma of brain in childhood presented with mass in left neck that was evident for 1 month. [^{99m}Tc]MIBI scan (*center*) was negative. Bone scan (*right*) showed faint blood pool. However, [^{99m}Tc]VIP scan (*left*) showed unequivocally positive uptake (*arrow*). Immunohistology of lesion showed that it was a high-grade spindle cell sarcoma. (Reprinted by permission of the Society of Nuclear Medicine from: Thakur et al.[26])

Future Directions

Treatment of breast cancer is hampered by a large unmet need for rapid, sensitive, specific detection, staging, and stratification of palpable and nonpalpable abnormalities. Mammography and physical examination miss many early breast cancers, yet detect many benign lesions. Genomic and proteomic molecular biology research has provided specific biomarkers, such as *CCND1, MYCC, VPAC1,* or *BCL2,* that have not yet been explored in human trials, let alone in routine clinical imaging of the breast.

Use of radiolabeled genetic probes will enable:

- Noninvasive early detection of cancer gene activation
- Informed choice of the most appropriate therapeutic intervention, based on the cancer genes that are active
- Oncogene specific therapy which could spare normal tissues and improve the quality of the patients' extended lives
- Determination of the effectiveness of therapy by monitoring downregulation of cancer gene expression noninvasively, as opposed to waiting to observe reduced standard uptake values (SUVs) with FDG

The practice of medicine will change rapidly in the next generation, driven in part by genetic and molecular targeting.

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Chapter 10
Intraductal Approaches: Nipple Aspirate Fluid to Assist in Breast Cancer Detection

Edward R. Sauter

Abstract Over 40,000 women in the United States will die this year of breast cancer. Current generally accepted techniques to detect breast cancer are limited to breast examination and imaging studies (mammography supplemented with ultrasound, and magnetic resonance imaging [MRI] for certain indications). Abnormalities found by these techniques require an invasive needle or surgical biopsy to determine if cancer is present. Our approach is to determine noninvasively whether a woman has breast cancer, and to do this before the abnormality is detectable by standard screening techniques. Herein we review the technology as it was, is, and its future potential.

Keywords Biomarkers · Cytology · DNA methylation · Image analysis · Mitochondrial DNA · Nipple aspirate fluid · Proteomics

Key Issues

- The currently accepted breast cancer screening tools for normal risk women of mammography and breast examination miss up to 40% of early breast cancers and are least effective in detecting cancer in young women, whose tumors are often more aggressive.
- Breast nipple aspiration is noninvasive, low cost, and repeatable.
- Nipple Aspirate Fluid (NAF) can be collected from the vast majority of adult women.
- The market for NAF, should it prove successful in breast cancer detection, could be as large as the entire adult female population.
- Both proteins and DNA are readily analyzed in NAF.
- It is likely that a panel of NAF markers (rather than a single marker) will be required to obtain a sensitivity and specificity sufficiently high to be accepted by the medical community.
- The population for which this technology is potentially applicable includes all adult women who have at least one intact breast nipple. Our group has collected NAF from women aged 18 to 96. Although we have attempted to collect NAF in males, we have not been successful. NAF collection is more difficult in women who have undergone subareolar breast surgery, and in women who have received breast irradiation.

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Introduction

Early detection is a major factor contributing to the 3.2% annual decline in breast cancer incidence which has been observed over the past 5 years.[1] Unfortunately, currently available breast cancer screening tools such as mammography and breast examination miss up to 40% of early breast cancers and are least effective in detecting cancer in young women, whose tumors are often more aggressive. Thus, there has long been interest in developing a noninvasive method to determine if a woman has breast cancer.

Indeed, collecting samples from the breast noninvasively has been conducted for at least 90 years. The adult nonpregnant, nonlactating breast secretes fluid into the breast ductal system.[2] This fluid normally does not escape because the nipple ducts are occluded by smooth muscle contraction, dried secretions, and keratinized epithelium. Initial studies to evaluate the breast noninvasively assessed spontaneous nipple discharge (SND), fluid which comes from the breast ducts through the nipple without compression of the breast. While bilateral spontaneous discharge is generally physiologic, unilateral single duct discharge, whether bloody or nonbloody, is generally pathologic. Although of potential use in disease diagnosis, evaluating SND does not address the evaluation of women who do not have spontaneous discharge.

Methodology

Nipple fluid is contained in the ductal and lobular lumens of the breast. The fluid is present in all women with an intact breast. Retrieving the fluid is mostly readily obtained through the nipple. The technique most commonly employed involves massage of the breast from the chest toward the nipple with the simultaneous application of suction on the nipple-areolar complex (NAC). Some but not all investigators warm the breasts prior to massage.

A device to apply suction to the NAC can be created by the investigator or, if the investigator prefers, can be purchased by a commercial vendor. Petrakis developed his own device from readily available materials in a hospital,[3] but later used a machine made device designed by Sartorius.[4] Sauter created a nipple aspiration device from materials available in a hospital, and has one of the highest reported yields to date.[5] Many investigators use a device sold by Hologic (formerly Cytyc, Bedford, MA). In short, there is no convincing evidence that the purchased devices lead to a greater NAF yield than investigator-made devices.

A reliable technique for the collection of NAF follows. After informed consent is obtained, the subject is seated in a comfortable position and the breast nipple cleansed with alcohol or similar agent.[5] After the alcohol has evaporated, a warm, moist cloth is placed on each breast. After 1–2 min, the cloths are removed, the patient massages her breast with both hands toward the nipple, and suction is applied to the NAC with the aspiration device for 10–15 s, or until the subject experiences discomfort. Fluid in the form of droplets is collected in 50-μm capillary tubes, or a similar collection device. The procedure may be repeated. Aspiration is then performed on the opposite breast, if present, in the same fashion.

Occasionally, keratin plugs rather than NAF are obtained after suction to the NAC is applied. The plugs can be removed with an alcohol swab and suctioning resumed. This may be repeated as necessary to remove all plugs. Fluid will then frequently be obtained. In order to obtain additional fluid, the nipple can be gently compressed by the participant between her fingers.

NAF, if Proven Clinically Useful, Would Likely Be Cost Effective

One of the best aspects of this technology is its low cost. The device used to collect the material can be created for little or no expense by the investigator, or purchased for a relatively modest price from a vendor. Due to its low volume, storing the material collected (generally <200 μL per collection) is generally easy, as it takes up little space, and disposal is in a standard human waste container, along with blood or other bodily fluid waste. Nurses and/or physicians who care for women with breast disease would need to be trained in the procedure. There may be personnel costs, though the amount of time required to collect a sample from most women is less than 10 min. Indeed, many women can be taught to massage their breasts, express and collect the NAF sample themselves.

Clinical Usefulness of the Technology

Sensitivity and Specificity

Initial Studies Focus on Feasibility

In 1958 George Papanicolaou reported results of the first large study evaluating fluid aspirated from the nipple rather than collecting fluid which came forth spontaneously.[6] He cleansed the nipple and applied gentle massage toward the areola. If NAF did not come forth, he used a breast pump to create mild suction. He reported a series of 917 women without breast complaints in whom he attempted to collect NAF from one or both breasts.[6] He was able to obtain a sample in 18.5% of subjects.

In order for NAF to be a useful breast cancer screening tool, it is essential to collect a sample in the vast majority of women. As a result, increasing the success rate continued to be an important area of investigation for the next 30 years. Early studies indicated that the ease of collecting NAF was related to the ethnicity of the individual, with NAF being more difficult to collect from Asians than African Americans or Caucasians.[3] This was presumed to be due to the physiology of the breast, a modified ceruminous gland, and is probably related to the secretory pattern in the breast. Ceruminous glands are known to provide less secretions in most Asians[7] and American Indians,[8] who are thought to have come from Asia, than in Caucasians and African Americans. Other variables[3] found linked to NAF collection success included age (late premenopause, 30–55 years old, had the highest yield) and menopausal status (premenopausal subjects more often provided NAF). Various nipple aspiration devices were created, notably one by Otto Sartorius,[4] which provided NAF on average in 50–60% of subjects[3,4] and in up to 80% in the highest yielding subset of subjects.[4]

The ability to collect NAF was linked not only to age, race, and menopausal status, but also to dietary habits. In a large sample of white and black women between the ages of 20 and 59 years old who did not have a history of breast cancer, the proportion of women from whom NAF was collected increased with increasing dietary fat consumption.[9] This association of NAF yield with fat consumption was especially strong among black women, and was most pronounced in women aged 30–44 years.

In the 1990s the aspiration technique was modified to emphasize warming the breast, breast massage, and multiple aspiration attempts after clearing the nipple of dried secretions.[10] Each of these techniques had been heretofore practiced, but the emphasis on persistence seemed to increase successful NAF collection, as did having the subject return for a second or third visit, if necessary, to collect NAF. This increased yield to 96% of subjects who had not undergone prior breast surgery in the subareolar region, and who had not had received breast irradiation.[10] Others have reported

success rates near 90% without repeat visits,[11] and investigators with a yield after one visit of 66% increased their yield to 78% with multiple visits.[12]

Studies Evaluating Cells in NAF

Early studies focused on morphologic changes in the shed duct epithelial cells to diagnose cancer, NAF volume, and color as predictors of breast cancer risk, and assessment of chemicals in NAF in different subject populations.

Evaluation of Cell Morphology

As previously mentioned, Papanicolaou was the first to report the presence of breast epithelial cells in NAF, and found malignant cells in 1 of 438 asymptomatic women.[6] NAF was found to contain not only epithelial cells, but also foam cells, a term used to describe the "foamy" appearance of the cytoplasm. He speculated that *It thus appears possible that under the term foam cell we are dealing with a variety of cell types that, although morphologically indistinguishable. . ., may vary in origin. . ..* Almost 50 years later, after numerous studies using panels of epithelial and macrophage markers, the origin of foam cells remains an area of debate.[13–15] In the report, Papanicolaou also evaluated breast cyst fluid collected from 100 subjects and contrasted cytologic findings in NAF with those in breast cyst fluid. He noted a relative scarcity of foam cells in breast cyst fluid, which are generally the most frequent cellular component of NAF. Leukocytes and macrophages were also scarce in cyst fluid but relatively common in NAF.

The number of epithelial and foam cells, and ratio of epithelial to foam cells, have been assessed in different breast cancer risk populations.[5,6,13] It was found that as breast cancer risk increased, the number of epithelial cells, as well as the ratio of epithelial to foam cells, increased.

Increased breast density suggests more proliferative activity. Increased breast density as seen on mammography has been linked to increased breast cancer risk.[16] Among a population of women in whom NAF cytology was collected, those with the greatest mammographic density were found to have a fourfold increased risk of cells demonstrating atypical hyperplasia.[17]

Longitudinal studies have demonstrated the usefulness of abnormal NAF cytology in predicting future breast cancer risk. A prospective study which enrolled 2,071 Caucasian women found that, after an average of 12.7 years of follow-up, the relative risk (RR) for women who yielded various cytologic categories of NAF vs women who yielded no NAF (RR = 1) were as follows: unsatisfactory specimen, 1.4; normal cytology, 1.8; epithelial hyperplasia, 2.5; and atypical hyperplasia, 4.9.[18] A follow-up study involving 4,046 women who were followed for a median of 21 years found that, compared with women from whom no fluid was obtained, whose incidence of breast cancer was 4.7%, the adjusted RRs for women with various NAF cytologic findings were 1.4 for those with unsatisfactory aspirate specimens, 1.6 for those with normal cytology in the aspirates, 2.4 for epithelial hyperplasia, and 2.8 for atypical hyperplasia. Thus, longer follow-up demonstrated a consistent, albeit somewhat lower, increased risk related to worsening NAF cytology, and is consistent with the implications of a fine needle aspiration or excisional biopsy demonstrating atypical hyperplasia.[18]

Multiple aspiration visits have been demonstrated to increase the detection of abnormal epithelial cells in NAF.[12] Two hundred seventy six women without known breast cancer underwent nipple aspiration. Among women in whom NAF was collected, hyperplastic cells were found in 34/178 (19.1%) at visit 1, which increased to 73/209 (34.9%) by visit 5. Atypical cells were found in 6.7% at the initial visit, and in 18.2% of NAF specimens in at least one of five visits.

The presence of tumor at the margin of a surgical biopsy presents a treatment dilemma, since approximately half of the time reexcision fails to find residual tumor. On the other hand, tumor

recurrence rates are significantly higher if margins are not resected until they are tumor free.[19] NAF cytology has been used to evaluate the presence of residual breast cancer. Atypical and malignant cytology observed in NAF samples collected after excisional breast biopsy but before or concurrent with definitive surgery[19] were significantly associated with residual DCIS or invasive cancer. Pathologic factors, including tumor distance from the biopsy margin, multifocal/multicentric disease, sub-type and grade of ductal carcinoma in situ (DCIS) or invasive cancer (IC), tumor and specimen size, tumor and biopsy cavity location, presence or absence of extensive DCIS, and biopsy scar distance from the nipple were included in a model to predict the presence of residual breast cancer among women with a biopsy with an involved or close tumor margin. The model[20] which included both NAF cytology and pathologic parameters was superior in predicting residual breast cancer (94%) compared to models using NAF cytology (36%) or pathologic parameters (75%) alone.

While numerous studies point to the high specificity of NAF cytology in breast cancer diagnosis,[5,6,21] cytologic evaluation is occasionally difficult to interpret. Perhaps the chief difficulty is in the differentiation of benign from malignant papillary growths. This dilemma is found primarily in the cytologic evaluation of SND, which is often the result of a benign papilloma on histopathologic review but can appear suspicious for carcinoma to the cytopathologist not highly familiar with NAF and SND cytologic evaluation.[6,22]

Image Analysis (IA)

Whereas NAF cytologic evaluation is very specific in the diagnosis of breast cancer, it is not very sensitive.[5,6,23] One approach that has been used to increase the sensitivity of NAF is to evaluate the DNA content of the cells. Normal cells contain 46 chromosomes, are diploid, and have a DNA index (DI) of 1.0. An abnormal amount of cellular DNA is called aneuploidy and is associated with a high nuclear grade. Hypertetraploidy is used to describe a cell which contains more than twice the normal DNA content, and has a DI > 2.0.

Since NAF samples have limited and mixed cellularity (epithelial, foam and occasionally white or red blood cells), evaluating DNA content requires image analysis, where the cells of interest (epithelial cells) but not other cells can be evaluated for their DNA content and the percentage of cells in various stages of the cell cycle. Aneuploidy in NAF is associated with atypical and malignant NAF cytology and is associated with the presence of breast cancer.[5] Abnormal DNA ploidy is highly predictive of the presence of residual breast cancer after diagnostic biopsy.[19]

Nuclear DNA Alterations

Both deletions in DNA, evidenced by loss of heterozygosity (LOH), and changes (either gains or losses) in the number of repeat units of DNA,[24] termed microsatellite instability (MSI), had been identified in a variety of human physiological fluids from subjects with cancer, including sputum,[25] urine,[26] stool,[27] blood,[28] and SND.[29] To determine whether LOH and/or MSI could be identified in NAF from subjects with breast cancer, DNA from matched NAF and breast tissue samples was extracted and 11 microsatellite markers evaluated.[30] An identical LOH/MSI alteration was detected in NAF from 33% of proliferative and 43% of cancerous breasts which harbored the change in matched tissue.

DNA Methylation

In cancer cells, several tumor suppressor genes such as $p16^{INK4a}$, *VHL*, *hMLH1*, and *BRCA1* have been found to have hypermethylation of normally unmethylated CpG islands within the promoter regions. The hypermethylation is associated with transcriptional silencing of the gene.[31]

Hypermethylation can be analyzed by the sensitive methylation specific-PCR (MSP) technique, which can identify up to one methylated allele in 1000 unmethylated alleles, appropriate for the detection of neoplastic cells in a background of normal cells.[32] MSP has been used in recent studies for the successful detection of cancer cell DNA in bodily fluids such as liver,[33] lung,[34] and head and neck cancer in serum,[35] lung cancer in both sputum,[36] and bronchial lavage,[37] and prostate cancer in urine.[38] Using a panel of six normally unmethylated genes: *glutathione S-transferase 1* (*GSTP1*); *retinoic acid receptor-ß2* (*RARß2*); $p16^{INk4a}$; $p14^{ARF}$; *RAS association domain family protein 1A* (*RASSF1A*); and *death-associated protein kinase* (*DAP-kinase*) in 22 matched specimens of breast cancer tissue, normal tissue, and nipple aspirate fluid collected from breast cancer patients, hypermethylation of one or more genes was found in all 22 malignant tissues and identical gene hypermethylation detected in DNA from 18 of 22 (82%) matched NAF samples.[39] In contrast, hypermethylation was absent in benign and normal breast tissue and nipple aspirate DNA from healthy women.

Mutations in Mitochondrial DNA (mtDNA)

While each cell contains one matched pair of nuclear DNA (nDNA), the same cell contains several hundred to thousands of mitochondria and each mitochondrion contains 1–10 mitochondrial genomes.[40] Both because of the sheer abundance of mtDNA per cell and the tendency for mtDNA mutations within a cell to have the same nucleotide alteration (homoplasmy), mtDNA may provide a distinct advantage in terms of feasibility and sensitivity over nDNA-based methods for cancer detection, especially when one is dealing with samples of low cellularity such as NAF. A recent report documents the feasibility of detecting mtDNA mutations in NAF.[41] The authors collected six NAF samples from four women, two BRCA1 carriers and two noncarriers. mtDNA analysis was successful in four out of six samples, and one mtDNA mutation was found in a carrier.

Studies Evaluating Extracellular Fluid in NAF

NAF contains a variety of chemical substances either secreted from or which passively diffuse through the epithelial cells into the ductal lumen. These include substances of endogenous origin, such as α-lactalbumin, immunoglobulins, lipids, fatty acids, proteins, cholesterol and cholesterol oxidation products, and hormones,[42] as well as exogenous substances including nicotine and cotinine from cigarette smoking[43] and mutagenic agents of undetermined origin.[44] Many of these substances are concentrated in NAF relative to corresponding serum.

Endogenous Substances: Single Protein Analysis

Hormones and growth factors. A variety of hormones have been measured in NAF, including estrogens, androgens, progesterone, dehydroepiandrosterone sulfate, prolactin, growth hormone and the growth factors epidermal growth factor, transforming growth factor-α, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).[45–48] Elevated levels of estrogens, cholesterol, and cholesterol epoxides have been suggested to have etiologic significance in breast disease.[49]

Levels of a number of these factors have been assessed for their ability to predict disease risk. With the exception of recent parity, no relation was found between levels of estrogen in NAF and breast cancer risk. Higher levels of estradiol and estrone were found in the NAF of women with benign breast disease than in controls.[50] There is a decrease in estradiol and estrone levels in NAF following pregnancy or lactation which persists for several years before returning to prepregnancy

levels.[51] This period of decreased estrogen exposure of the breast epithelium in postpartum women has been suggested to explain partially the protective effect of early pregnancy.

Basic fibroblast growth factor and vascular endothelial growth factor are two of the most important angiogenic factors which stimulate tumor growth.[52,53] A preliminary report which analyzed 10 breast cancer patients and 10 controls found that bFGF levels in NAF were higher in women with breast cancer than in normal subjects.[54] A larger study which evaluated 143 NAF specimens[45] also found that mean NAF bFGF levels were significantly higher in women with breast cancer than in those without. VEGF levels in NAF were not associated with breast cancer. A logistic regression model including NAF levels of bFGF and clinical variables was 90% sensitive and 69% specific in predicting which women had breast cancer. Adding another biomarker linked to breast cancer, prostate-specific antigen (PSA), increased the sensitivity to 91% and the specificity to 83%.

Leptin is a hormone which plays a central role in food intake and energy expenditure.[55] Systemic levels of leptin are increased in obese individuals, and have been found to stimulate the growth of breast cancer cells in vitro. Leptin levels in NAF were more readily measured in post- than in premenopausal women, and were significantly higher in postmenopausal women with a BMI < 25.[56] While NAF leptin levels were not associated with pre- or postmenopausal breast cancer, they were associated with premenopausal BMI.

Tumor antigens. A number of proteins present in NAF have previously been associated with cancer when measured in the blood. Two of these are PSA and carcinoembryonic antigen (CEA). PSA, a chymotrypsin-like protease first found in seminal fluid and associated with prostate cancer,[57] is also found in breast tissue[48,58] and in NAF. PSA levels in cancerous breast tissue are lower than in benign breast tissue.[48] PSA is thought to cleave insulin-like growth factor binding protein-3 (IGFBP-3), the major binding protein of IGF-I. Most[10,48,59] but not all[60] studies indicate that low NAF PSA levels are associated with the presence and progression[61] of breast cancer, whereas high levels of NAF IGFBP-3 have been linked to breast cancer.[59] One explanation for the discrepancy in PSA results may be the difference in NAF yield. NAF was obtained in 97% of subjects in the studies finding an association, and in 34% of the subjects in studies where an association between NAF PSA and breast cancer was not found.[62]

Another protein which is concentrated in NAF is CEA. CEA was identified in 1965 as the first human cancer-associated antigen.[63] Serum CEA levels have been used clinically to assess and monitor tumor burden in patients with breast cancer.[64] CEA titers in NAF samples from normal breasts are typically more than 100-fold higher than in corresponding serum.[65] CEA levels in NAF from 388 women, including 44 women with newly diagnosed invasive breast cancer, were analyzed. CEA levels were significantly higher in breasts with cancer, but the sensitivity of CEA for cancer detection was only 32%.[60]

Endogenous Substances: Proteomic Analysis

Recent advances in comprehensive molecular technologies have allowed the analysis of global gene expression or protein profiles in cancerous vs normal tissues with the goal of identifying protein markers that are differentially expressed between benign and malignant tissue. One such study[66] used serial analysis of gene expression to identify molecular alterations involved in breast cancer progression. The authors concluded that many of the highly expressed genes encoded secreted proteins, which in theory would be present in NAF.

Breast tissue contains thousands of intracellular proteins. NAF contains a limited number of cells and extracellular fluid, the composition of which includes a relatively small set of secreted breast specific proteins. The few cells in NAF can be separated from the extracellular fluid. The remaining

proteins are secreted and therefore represent their final processed form, which makes proteomic analyses less ambiguous and can provide clues to changes in protein translational rates, post-translational modification, sequestration, and degradation which lead to disease.

Two Dimensional Polyacrylamide Gel Electrophoresis (2-D PAGE)

The traditional method of proteomic analysis is one or two dimensional PAGE. Using two rather than one dimensional PAGE allows better separation of proteins of equal molecular weight based on charge. Once a protein of interest is found, it can be cut from the gel and identified. Two dimensional PAGE has been used to screen NAF because it provides a convenient and rapid method for protein identification based on Matrix Assisted Laser Desorption-Time of Flight mass spectrometry (MALDI-TOF MS). At least two studies have analyzed the NAF proteome. One[67] used liquid chromatography, while the second used 2-D PAGE.[68] Over 60 proteins were identified in the first and 41 in the second study. Many of the proteins were the same, but a significant subset of proteins (35 in the first, 21 in the second) were unique to each study. Both studies should be considered when assessing the NAF proteome.

Two dimensional PAGE may serve as a screening platform to identify proteins in NAF which are differentially expressed in cancerous and benign breasts. These proteins can then be validated using one or more high-throughput proteomic approaches.[68] Three protein spots detected using 2-D PAGE were upregulated in three or more NAF samples from breasts with cancer. These spots were identified to be gross cystic disease fluid protein (GCDFP)-15, apolipoprotein (apo)D, and alpha-1 acid glycoprotein (AAG). To validate these three potential biomarkers, 105 samples (53 from benign breasts and 52 from breasts with cancer) were analyzed using enzyme-linked immunosorbent assay (ELISA), a high-throughput method of evaluating protein concentration. Considering all subjects, GCDFP-15 levels were significantly lower and AAG levels significantly higher in breasts with cancer. This was also true in pre- but not postmenopausal women. GCDFP-15 levels were lowest and AAG levels highest in women with DCIS. Menopausal status influenced GCDFP-15 and AAG more in women without than with breast cancer. ApoD levels did not correlate significantly with breast cancer.

Surface Enhanced Laser Desorption Ionization-Time of Flight Mass Spectrometry (SELDI-TOF-MS)

Although 2-D PAGE is quite powerful, it has limitations in protein separation and sensitivity. Recent advances in comprehensive molecular technologies allow the simultaneous analysis of multiple protein expression targets. The SELDI-TOF technique can be performed with 1 μL of NAF, can detect components in the high femtomole range, and the chip surface, which allows the rapid evaluation of 8–24 samples, has high throughput potential. Candidate breast cancer biomarkers can be identified using mass spectrometric techniques or an immunoassay to the suspected protein can be used to confirm its identity.

A wide array of proteins are secreted into and highly concentrated in NAF and have been associated with breast cancer. Three pilot studies[69–71] demonstrate the feasibility of SELDI-TOF analysis of NAF in a limited number of subject samples, and identified one or more protein mass peaks which were associated with breast cancer. A potential limitation of all three studies is that specific protein identification of the protein mass peak was not obtained. Although it has been proposed[72] that this is not necessary, validation studies to confirm that these protein masses are linked to breast cancer are easiest after the identification of the specific proteins, eliminating the confounder of multiple proteins of similar mass.

NAF as a Tool to Investigate the Presence of Mutagens in the Breast

It is thought that environmental mutagens stored in the adipose tissue of the breast could potentiate carcinogenesis through direct exposure to the adjacent ductal epithelial cells, and that evaluating NAF would provide information on carcinogen exposure.[73] A standard assay for the presence of mutagens is the Ames test using one of a variety of *Salmonella* strains to detect the mutagen. A number of studies using different *Salmonella* strains have been conducted.[44,73,74] One limitation of the assays performed to date is the need for approximately 10 μL of NAF, which is more than is obtained from some subjects. No association was found in the studies between mutagenic activity in NAF and breast cancer.

Alternative Intraductal Evaluation Tools

The focus of this discussion will be a brief overview on alternative tools which allow intraductal evaluation of the breast. Alternative intraductal tools are discussed in greater detail in Chapter 11. In addition to nipple aspiration, two other intraductal techniques have been investigated, one (ductal lavage, DL) which uses a catheter to irrigate one or more ducts of the breast nipple, and the other (mammary ductoscopy, MD) which enters the ducts under direct visualization and then allows irrigation ± biopsy of the ductal epithelium. Both DL and MD are usually preceded by nipple aspiration, which identifies ducts containing breast fluid which can be aspirated. NAF distends the duct, making both procedures technically easier to perform. Additionally, it has been demonstrated that breasts which provide NAF are at greater breast cancer risk than breasts which do not,[18] implying that NAF producing ducts are more likely to contain disease.

Ductal Lavage

While nipple aspiration has many strengths, a weakness is the relatively low number of epithelial cells in the specimens. DL, which cannulates one or more of the ducts through the nipple orifice, provides a sample with more cells than NAF but the location within the duct from which the cells are collected is unknown. Since the ductal fluid is diluted with saline irrigation, the initial concentration of extracellular proteins cannot be accurately estimated, limiting the analysis of extracellular proteins using DL.

Mammary Ductoscopy (MD)

Breast ductoscopy has been used as a tool to evaluate the breast for cancer for over 10 years. MD allows the direct visualization of the duct lumen, providing a more targeted approach to the diagnosis of disease arising in the ductal system, since the lesion can be visualized and samples collected in the area of interest. Initial studies of MD evaluated women with pathologic spontaneous nipple discharge (PND), while more recent reports are also using MD to assess women without PND for the presence of breast cancer. Cytologic assessment of PND has been associated with false positive readings.[75] Cytologic assessment of MD from women without PND is highly specific but less sensitive in the detection of breast cancer. Additional sample evaluation using image or molecular analysis may improve the sensitivity and specificity of MD in breast cancer detection.

Are Imaging and Intraductal Results Complementary in Breast Cancer Detection?

There is very limited information addressing this question. In different chapters in this book the strengths and weaknesses of breast imaging modalities are discussed in detail. Notably, currently available imaging technologies, including mammography, ultrasonography, and MRI, detect an abnormality which must be confirmed through diagnostic needle, core, or surgical biopsy. These procedures are painful, the needle and core biopsies are subject to sampling error, and only approximately 15–25% of the procedures demonstrate pathologic evidence of malignancy[1,2]. The noninvasive methodology of nipple aspiration is desirable in cancer detection, but thus far is not sufficiently sensitive and specific to have entered standard clinical practice.

We determined if protein profiling of NAF using SELDI-TOF analysis would be predictive of breast cancer, and if clinical variables which are available prior to surgery, including Breast Imaging Reporting and Data System (BIRADs) and NAF cytology information, would improve our ability to predict which women had breast cancer. The optimal model was 90% specific and 63% sensitive in determining if a breast requiring surgery had cancer. The model was correct 85% of the time. By comparison, magnetic resonance imaging, the most sensitive imaging tool available to detect breast cancer, was recently reported to be 77–100% sensitive and 81–97% specific in detecting breast cancer in high risk women, with lower accuracy in detecting ductal carcinoma in situ.[20] Because the population studied by SELDI included primarily women who were not at increased risk, whereas the MRI studies were performed only in high risk women, comparisons of accuracy may not be appropriate. Nonetheless, our findings suggest that SELDI-TOF analysis of NAF, in combination with clinical information known prior to surgery, is quite specific in determining whether a breast contains cancer. NAF analysis by SELDI[3] and cytology appears to be best in the detection of DCIS, whereas MRI is better in the detection of more advanced disease. These modalities, therefore, may be complementary in breast cancer screening, since they target different populations of women, and are best at detecting different stages of breast cancer.

What if the NAF is Abnormal and Standard Screening Studies Are Not?

It is important to remember that for women with PND, cytology can be atypical or rarely appear malignant when the etiology is an intraductal papilloma. Therefore, abnormal NAF cytology should be viewed with caution in women with PND. For women with NAF mild atypia, no PND, and normal or benign screening studies, it is reasonable to repeat the NAF and screening evaluation in 3–6 months. It has been our experience and that of others that mild atypia is often transient [76] and unrelated to a carcinogenic process. For women with NAF severe atypia or frankly malignant cells who do not have PND, a normal breast exam and mammography, the first step is to insure that the NAF sample was properly labeled, i.e., that it came from a subject without known breast cancer. Once this has been confirmed, the mammogram of the breast from which the abnormal NAF came should be reviewed again. If something mildly suspicious is seen, then a spot magnification view and/or breast ultrasound is reasonable, with directed biopsy if indicated. A breast MRI should be considered if the mammogram is entirely negative.

Conclusion

Cytologic evaluation of NAF is highly specific but less sensitive in the detection of cancer. DL[76] and probably MD are more likely than NAF to demonstrate atypia in women at increased breast cancer

risk since they provide a more cellular sample. Whether these more expensive and somewhat more invasive techniques will also lead to the detection of more cancers than NAF based on cytologic review of the specimens is likely. Nonetheless, cytologic review of all three intraductal samples is limited by the relatively low sensitivity of this approach. A great strength of nipple aspiration is that the device used is inexpensive, it does not require local anesthesia as does DL and MD, and it does not require the introduction of a foreign body into the breast which can rarely lead to an infection.

Nipple aspiration provides concentrated secreted proteins from the ductal epithelium. These proteins are generally readily measurable despite limited sample volume due to their high concentration relative to serum. The relative simplicity of the NAF proteome is also attractive, since the determination of candidate markers is not hindered by overlapping proteins of similar weight and charge. Both nuclear and mitochondrial DNA abnormalities can be detected in NAF which are present in matched breast cancer tissue. While changes in LOH/MSI and mitochondrial DNA are highly specific, thus far they have been somewhat less sensitive. Methylation changes in NAF DNA appear to be both sensitive and specific, and therefore are quite promising. The analysis of RNA in NAF samples is more challenging, primarily because it is less stable than DNA and protein.

Five-Year View

Optimizing the sensitivity and specificity of breast cancer detection through the combination of individually promising markers is likely to receive increasing attention. Multicenter trails will need to be conducted to validate these markers prior to their general acceptance by the medical community. Simultaneous with these translational research efforts will be increasing interest by industry to patent these marker panels for profit. Once a panel of markers with high breast cancer predictive ability is validated, training health care providers to learn breast nipple aspiration will be required. Additional large scale studies will be required to determine the optimal population of women to study. For example, studies will need to determine if nipple aspiration will prove useful in breast cancer prediction in the entire adult female population, only in high risk women, or only in women with an abnormality found on imaging and/or on breast physical examination in attempt to avoid diagnostic biopsy.

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Chapter 11
Intraductal Approaches: Mammary Ductoscopy and Ductal Lavage to Assist in the Diagnosis of Breast Cancer

William C. Dooley

Abstract Ductoscopy and ductal lavage have evolved over several decades of interest by researchers in the early identification of breast cancer and its precursor lesions. Lavage has a history that spans back to George Papanicolau but has been limited dramatically in its clinical usefulness because of the great overlap of bland malignant cytology with true benign cytology. When sub-millimeter endoscopes first became available, their use to find the source of bloody nipple discharge was a natural for surgical investigators. These technologies continue to evolve, offering new insights into breast carcinogenesis and breast cancer progression when married to new biopsy and molecular techniques.

Keywords Ductoscopy · Nipple discharge · Breast cancer

Key Issues

- The majority of non-obstructing malignant and pre-malignant breast diseases are associated with fluid production.
- Ductal lavage is a very successful method of accessing thousands of cells associated with intraductal proliferative lesions.
- Cytologic analysis of fluid produced in high-risk women can identify sub-groups at high short-term risk of breast cancer development. Molecular analysis offers opportunities to extend this to pre-cancerous detection as these technologies evolve.
- Direct ductal endoscopy with sub-millimeter endoscopes is feasible.
- Intra-luminal defects are associated with most proliferative ductal processes although there is clear overlap in the appearance of benign and malignant lesions.
- Technical developments are rapidly changing the field of sub-millimeter endoscopy – for both diagnostics and therapy.

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Historical Development of Ductal Lavage and Ductoscopy

The intraductal approach to breast cancer diagnosis was recognized for its potential early by cytologists such as George Papanicolau.[1] Unfortunately very few breast cancers could be diagnosed from the few microliters of cell-poor fluid which could be elicited from most women's breasts.[2] In the 1960s, Wrench, Petrakis, and King from UCSF began a series of studies on women in the San Francisco region to determine whether the presence of nipple fluid or its cytologic characteristics could predict future breast cancer risk.[3] We now have published data with >30 years follow-up on more than 7000 women which shows that the relative risk of cancer in women who express nipple fluid was 1.88 in the following decade. Further, if there was cytologic atypia in that fluid the relative risk jumped to 4.9 for the development of breast cancer within a decade. Interestingly, as in other series of cytologic or histologic breast epithelial atypia, the risk for subsequent cancer fell rapidly in the second and third decade after its initial detection. Fine needle aspiration studies from Fabian and Kimler showed similar risk levels associated with cellular atypia.[4] Dupont and Page defined the current histologic criteria for Atypical Ductal Hyperplasia and its natural history with a series of papers in the 1980s.[5,6] Each of these series confirm the increased risk for cancer associated with epithelial atypia in breast ducts independent of sampling method and confirm the time dependent nature of that elevated risk.

Japanese surgeons such as Okazaki began investigations of ductoscopy in the early 1990s.[7–9] Asian patients with breast cancer seem more likely to present with bloody nipple discharge from lesions that are non-palpable. This is in part due to the difficulties of mammography in a group of small dense breasted women but other biologic differences which might also contribute have not yet been well defined. Endoscope technologies in the 1990s had greatly improved optics and dropped the diameter needed for both illumination and image capture compared to the late 1970s and thus made "micro-endoscopy" feasible. Early studies on Japanese patients with bloody nipple discharge using a rigid scope <2 mm in diameter showed its usefulness in identifying the cause for the discharge, but the scopes were fragile, expensive, and rarely advanced more than 3 cm into the breast. To distend the ducts in an effort to navigate further, air was insufflated into the ducts. Unfortunately the combination of marginal optics and glare artifacts from reflection off moist surfaces led to poor quality images. Persistence with this technology and improved optics and the addition of a working channel finally allowed greater success in Asia in the mid-1990s.[10–13] The technique of ductal endoscopy for the evaluation and management of symptomatic nipple discharge using endoscopy has now spread to Korea and Hong Kong.

The Asian approach still has several drawbacks. First, the scopes are usually rigid, with the camera mounted as a heavy object at the end of a delicate optical fiber. The torque caused by such a long lever arm makes manipulating the scope around tight turns in the breast difficult. Second, the working channel has been used primarily to instill air into the ductal system. This does not relax the smooth muscle of the ductal walls and affords little distension for the investigation of more distal branches. It also causes sharp bright boundaries between fluid and air interfaces causing optical distortion and degraded images secondary to light reflection. In spite of these difficulties, Asian investigators have move beyond nipple discharge to other uses including cyst endoscopy.[14–16]

Ductal Lavage

Early U.S. attempts with sub-millimeter scopes were unsuccessful in patients with central tumors.[17] Cells recovered from the distending saline showed promise in improving cytology over those from

simple nipple aspiration or nipple fluid expression. Combining this experience with the data from Wrench and Petrakis, tools were developed to cannulate fluid producing ducts and to maximize the recovery of shed intra-luminal ductal cells – a technique we now know as ductal lavage. Data from the NSABP P-01 chemoprevention study suggested that high-risk patients with ductal atypia had the greatest reduction in future breast cancer incidence after tamoxifen treatment. Increasing the cell yield over that obtained from nipple aspiration was necessary to have a viable test to screen for ADH or other proliferative risk lesions.[18] The initial ductal lavage study enrolled women who were high risk either due to prior contra-lateral breast cancer or by Gail model with normal mammograms and physical exams.[19] The technique involved cannulation of all fluid producing ducts with a micro-catheter. The ducts were then distended with a few milliliters of local anesthetic to relax the smooth muscle and washed using saline. The study design was to compare this "lavage" technique with traditional nipple aspiration. The majority (85%) had fluid collected from at least one duct per breast. Atypia was present in 24% of the patients who successfully had a fluid-producing duct cannulated and lavaged with saline. At least 7% of this atypia was severe and bordering on malignancy using the criteria developed by Eileen King in the UCSF studies of Wrench and Petrakis.[14] Subsequent series of severe atypia detected by lavage suggest that 50–70% of the atypia is from a mammographically occult malignancy either concurrently or clinically/radiographically detectable in less than 36 months. Subsequent long-term follow-up studies will determine the natural history of lavage-detected atypia, the correlation of chemoprevention agents with atypia clearance and future risk reduction of breast cancer, and the rates and causes of false positive atypia such as papillomas.

Until we have large follow-up series of ductal lavage patients, we can only speculate as to the clinical relevance of cytologic atypia obtained from ductal lavage. It would seem reasonable to speculate that the source of the ductal cell atypia should be no different from NAF or random FNA and therefore the relative risk increase for breast cancer about the same. Certainly ductal lavage allows access to greater numbers of proliferative ductal cells for detection, prevention or translational research in most patients. The role of ductal lavage beyond being a powerful research tool will be defined by the few but important long term follow-up studies.

Ductoscopy

Until recently, there were few tools available to identify malignant cells in women with negative clinical and radiographic findings. After a number of imaging techniques such as galactograms, magnetic resonance imaging (MRI), ultrasound (US), etc. failed to identify routinely the occult source of the atypia, I applied the Asian approach of duct endoscopy. The lavage experience had taught us how to relax the smooth muscle of the ducts and get maximal distension of the ductal system using local anesthetic topically prior to saline distension. Using that major technical variation, I began to scope first those patients with concerning atypia where imaging had failed to identify the source. The technique was successful at identifying the source of the atypia by surgical excision of intraluminal defects and in identifying multiple tumors and extensive intraductal component in early stage breast cancer.[20] Several American investigators now show the benefit of ductoscopy in the investigation of symptomatic or pathologic nipple discharge and elicitable fluid from a cancerous breast.[21–25] New technologies to direct biopsy either through the scope or in conjunction with other devices originally developed for radiographic image guided biopsy are being developed and tried in pilot settings.

Ductoscopy appears to be a viable option for the identification of intra-luminal defects which either cause symptomatic nipple discharge or give rise to severe cytologic atypia on nipple aspiration or ductal lavage. Much is said of the power of MRI to screen for multi-focal breast cancer.

Unfortunately it primarily detects invasive cancer with increased vascular flow and grossly underestimates low grade DCIS which is more often a problem in breast conservation in early stage breast cancer. My personal series shows that ductoscopy of breast cancer patients at lumpectomy yields far more evidence of wide spread proliferative disease than any MRI protocol. Further, the assumption of most MRI studies is that multiple lesions makes mastectomy necessary. In all cases so far of multifocality in my ductoscopy series, all tumors seem connected to the same duct orifice at the nipple. The operative surgeon can work out the anatomy of the ductal system and in the vast majority of cases can perform with one operation a cosmetically satisfactory lumpectomy with clear margins.[23]

Technical Considerations

The difficulty of all intraductal technologies is achieving access to the lactiferous sinus without puncturing the ductal system. The key, whether using a lacrimal duct probe, ductal lavage catheter, or a prolene suture as a soft guide wire is timing. The sphincter of each duct in the papilla has a sort of "anal-wink" when expressing fluid. It is usually open for only a fraction of a second when expressing fluid. Successful cannulation of a duct occurs when the probe is introduced during this brief relaxation of the sphincter. In women with symptomatic spontaneous discharge the sphincter is usually dilated and stays open for longer intervals. This makes the duct much easier to cannulate. Cannulating the minimally fluid producing duct associated with a peripheral sub-centimeter breast cancer is much more difficult. First the nipple should be thoroughly cleaned with a facial exfoliant until all keratin and sebaceous plugs are removed from its surface. Next the breast should be lubricated with hand lotion and massaged deeply from periphery to center to move any fluid into the lactiferous sinuses from the periphery. The kneading is similar to kneading bread and should always be done in this centripetal fashion. These first two steps are the most important and often the most neglected in achieving success with any of the intraductal technologies. Lastly radial compression of each lactiferous sinus will identify the fluid-producing orifice. As soon as an orifice is identified, stop expressing fluid until you are ready to cannulate. With cannulating tool in hand, re-express fluid while slowly distracting the nipple upward. Cannulation should occur at the time fluid first appears on the nipple surface. If you fail then do not empty the sinus of fluid before re-trying. I prefer using a tapered 2-0 prolene since it is much too soft to penetrate most duct walls and will not advance unless you meet minimal or no resistance. All harder objects must be approached with greater caution.

Once in the duct, you can use a 26 or 24G angiocath and Seldinger technique with a 2-0 prolene as a guide wire. Progressive dilation will allow up to 1.2 mm external diameter objects to be placed into the ductal system with ease. Injection of 3–5 cc of buffered local anesthetic into the ducts comes next. It is important then to wait for 2–5 min to get relaxation of the ductal walls before starting saline distension. Success in your cannulation technique can readily be measured by following the cellularity of ductal lavage samples. If the lactiferous sinus is perforated then the samples will have few epithelial cells (<100). If the ducts are intact the cell counts from both normal and abnormal ducts will be >1000.

For the reasons given above, patients with spontaneous nipple discharge will be the easiest for the novice to scope. Further, because of chronic fluid production the ducts are often of larger diameter and it is easier to manipulate the scope. For the first several cases I would suggest getting a preoperative galactogram. Until you learn how to manipulate through some of the lengthy papillomas, it can be very disconcerting to put in a scope and find yourself in a yellow kelp forest of papilloma without a central lumen. The best starter cases are those with 1–1.5 cm of normal duct before a lesion is found, so that you can get oriented before pathologic findings distort landmarks. After

success with spontaneous discharge patients, the next patients to tackle are those with central DCIS with or without invasive cancer. The spectrum of visual findings associated with malignant and pre-malignant breast disease can then be identified and the surgeon's expertise at recognition of differing pathologic lesions can rapidly improve (see Figs. 11.1, 11.2, 11.3 and 11.4).

The last 3 years has seen rapid evolution of sub-millimeter endoscopy and its application to breast ducts. Optics have improved, allowing manufacturers the ability to devote a smaller cross sectional diameter to light and image capture and leave available larger working channels for visually directed biopsy. These new smaller scopes and new vacuum assisted biopsy techniques have begun to allow investigators to sample serially up and down the ductal tree and begin to map the pathologic changes.[26,27] The value of this is confirmed by Hunerbein and colleagues at Berlin who

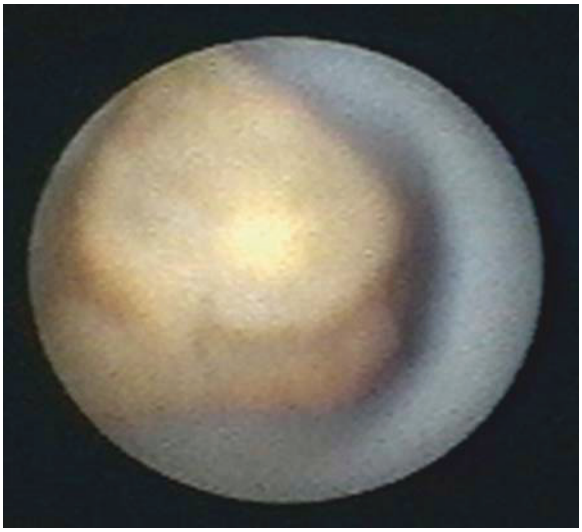

Fig 11.1 Papilloma

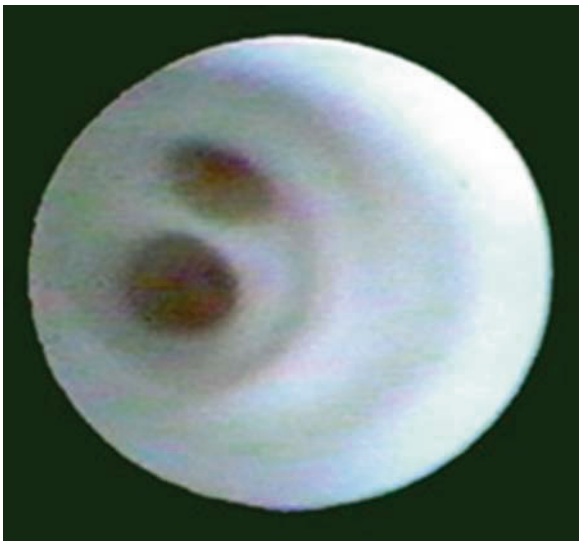

Fig 11.2 Bifurcation normal duct

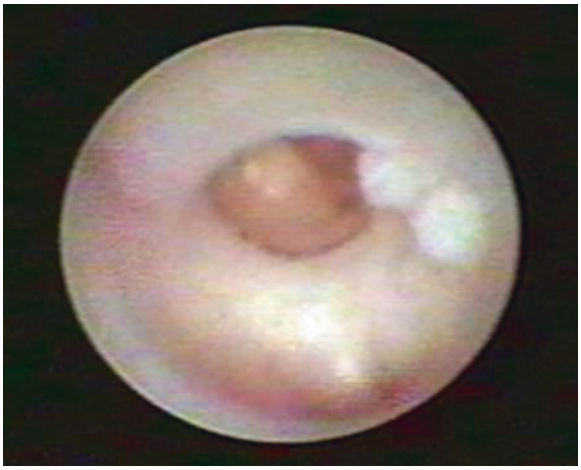

Fig 11.3 Low grade DCIS

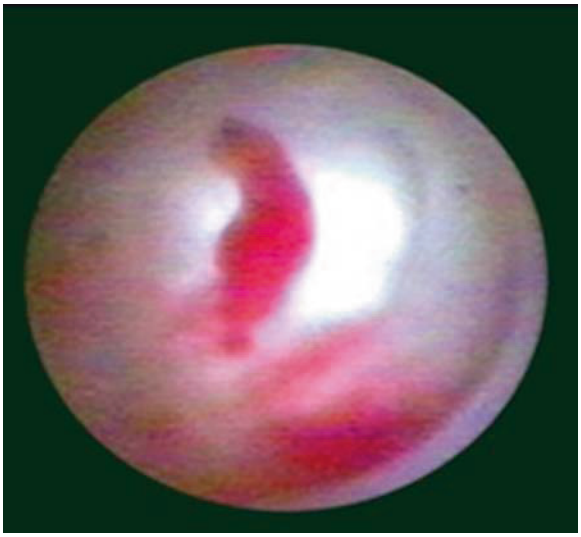

Fig 11.4 High grade DCIS

have now been able to find extensive intraductal carcinoma beyond what can be seen by traditional mammography techniques.[28] Ductoscopy and mammography were complementary in this series with a combined sensitivity of 95%. Their approach was similar to mine in that they assumed that all visible intra-luminal defects might be associated with malignant or pre-malignant disease. Other American authors have presented convincing data that not all intra-luminal defects are DCIS as assessed by routine pathology.[21,22] Unfortunately, the sampling bias of routine histology may miss relevant proliferative disease, as both my and the German series suggest.

The further development of auto-fluorescence techniques by Jacobs and colleagues from Munich opens the potential of being able to distinguish visually benign from more suspicious lesions on the basis of simple biologic differences in the ductal lining tissue.[29] The overlap in appearance of benign and malignant intraductal lesions has led some authors to question the value of ductoscopy as a clinical tool.[30–32] The application of auto-fluorescence as in bronchoscopy will further enhance

our ability to use this technique and separate confusing intra-luminal growths into more suspicious and less suspicious. Combining ductoscopic findings with results of other imaging techniques seems already to offer substantial benefits in determining the presence and extent of small cancers.[31–33] As ductoscopic techniques continue to evolve, some are using the scopes not just through the nipple but also through direct cyst puncture.[34] Refinements of both instrumentation and techniques will further expand both the clinical and research utility of ductoscopy in the near future.[35,36]

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Chapter 12
Blood Markers

Mark W. Duncan

Abstract Breast cancer is a complex and heterogeneous disease and this diversity, especially at the molecular level, makes it challenging to develop blood-based tests to detect the disease, in its early stages. Although several biochemical markers aid in diagnosis, no existing test is sufficiently sensitive and specific for early detection.

Currently there is justifiable optimism that the "omics" technologies will deliver the additional tools that will fill the clinical gap. These methods can determine hundreds to thousands of analytes in a sample simultaneously and, if thoughtfully employed in well-designed clinical studies, they should be able to identify multiple independent components (markers) that characterize the full spectrum of a heterogeneous disease such as breast cancer. The tests might ultimately be based on one or more molecular types (e.g., microRNA, methylation status, gene expression (mRNA), proteins, and/or metabolites) monitored in any of several possible tissues or biological fluids.

Most investigators have targeted their focus at circulating protein biomarkers. A set of analytes in blood potentially offers a non-invasive approach to early disease diagnosis and sub-classification based on biochemistry, and could provide the clinician with both prognostic and predictive information. A comprehensive panel could also be useful in monitoring symptom regression, the onset of adverse reactions, patient compliance and disease recurrence.

While omics methods can expedite the discovery process, biomarker validation remains the rate-limiting step. Extensive, well-designed clinical studies are essential and attempts to streamline this process ultimately cut corners that delay clinical implementation, increase costs and generate false hope. This chapter reviews biomarker discovery and validation with special emphasis on the practice and potential offered by evolving analytical methods, most notably those that allow simultaneous quantification of hundreds to thousands of components.

Keywords Proteins · Proteomics · Biomarkers · Lipids · Metabolites · Autoantibodies

Key Issues

- Currently available breast cancer screening tools such as mammography and breast examination fail to identify up to 40% of early breast cancers and are least effective in detecting cancer in

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young women whose tumors are often more aggressive. Alternative diagnostic approaches are required.
- A screening test is the Holy Grail, but this is a demanding challenge given the genetic and biochemical heterogeneity of breast cancer. This diversity indicates that the only feasible strategy to minimize false positive and false negative results is to develop tests based on a panel rather than a single analyte.
- Blood markers offer promise as non-invasive measures of breast cancer and could deliver greater sensitivity and specificity than mammography.
- Representative members of several molecular classes are potentially useful markers, including DNA, RNA, peptides, proteins, and small molecules.
- While emphasis has been on generating the "perfect" biomarker, a test with sub-optimal performance can be used in conjunction with existing strategies, such as mammography.

Introduction

Heterogeneity is a feature of breast cancer and is evident both in the malignant cells and the host background. This leads to a complex continuum of molecularly distinct tumor types that, although similar in clinicopathologic parameters, are different phenotypically in their responsiveness to therapy, and with respect to clinical outcome. This molecular diversity is a challenge when the objective is to diagnose or screen for early disease by biochemical measures.

Over the last few decades several biochemical markers have evolved as useful aids in diagnosis and patient management but, as yet, no test is sufficiently sensitive and/or specific enough for early detection. It is becoming increasingly apparent that a single analyte test will not fill the existing void. The molecular diversity of breast cancer demands a multi-analyte panel that represents the full biochemical spectrum of the disease. Unfortunately, its creation and validation is no simple task and requires considerable time, money, meticulous study design, the analysis of comprehensive patient populations, and relentless verification and validation in a multi-institutional setting.

The development of such a test panel would put the breast oncologist in an enviable position. Biomarkers already afford the clinician with objective criteria for the optimal treatment of each patient given the characteristics of their cancer, but the missing pieces of the clinical puzzle are tools for breast cancer diagnosis. There is justifiable optimism that the omics technologies will deliver panels of diagnostic biomarkers and these might be based on one or more molecular types (e.g., microRNA, methylation status, gene expression (mRNA), proteins, metabolites).

Definitions

The NIH Biomarkers Definitions Working Group defines a biological marker (or biomarker) as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[1]

The use of biomarkers in delivering appropriate treatment is not a new concept: HbA_{1c} is used as a predictor/marker of diabetes, oxidized LDL is a marker for sub-clinical development of atherosclerosis, and a range of pituitary and target gland hormones are routinely used to direct endocrine therapy.

Biomarkers can be genes, proteins, or other molecular types that broadcast a biomedical phenotype before it is clinically apparent and they can be applied in many settings (Table 12.1). This

Table 12.1 Some settings for the application of biomarkers

Risk assessment	Enables preventive intervention in high-risk individuals
Diagnosis, screening and early detection	Allows appropriate intervention at an earlier stage
Staging of disease	Allows objective clustering of a heterogeneous patient population into homogeneous groups that require similar treatment and will have similar outcomes
Prognosis of disease	Enables more aggressive therapy for patients with poorer prognosis, e.g., those with metastases
Toxicity and safety assessment	Provides objective measure of the onset of adverse outcomes
Monitoring efficacy of a therapy	Allows identification of responders/non-responders, or provides objective markers of response

chapter focuses on a discussion of the essential continuum of tasks from biomarker discovery through to routine clinical application with a focus on disease diagnosis.

Discovery and Development of Biomarkers: The Problems

Biomarkers have to be discovered, methods developed to measure them, and then they must be rigorously validated in a clinical setting. It is in the discovery phase where omics methods are having their greatest impact because they allow many hundreds of analytes to be quantified simultaneously, but comprehensive coverage comes at a high price: there is a significant compromise in analytical throughput, sensitivity, and quantitative precision. In other words, discovery is performed with a blunt tool.

Once candidate biomarkers are identified, the next challenge is the development of methods appropriate for their routine measurement. Key analytical parameters such as analyte stability, analytical precision, trueness, limit of detection, limit of quantification, and linearity must defined and optimized. These principles are addressed in an extensive range of documents released by the Clinical Laboratory Standards Institute (CLSI) (http://www.nccls.org/).

Clinical validation studies follow assay validation and aim to establish whether the biomarker offers clinical utility. This process begins by defining the frequency distribution of the candidate biomarker(s) in healthy individuals and establishment of reference interval(s) so that later in the process, patient results can be compared to these values.[2,3]

Studies follow which include the analysis of appropriately collected, well-defined clinical samples. Because most diseases are multifactorial by nature, and several different disorders can lead to the same apparent phenotype, patients in each test group must have the same disease. Moreover, for the test to offer high sensitivity and utility, the full spectrum of related disorders must be represented in additional test populations. The effects of age, ethnicity, gender, environment, concomitant diseases, nutrition, and medications are additional variables that could potentially confound test development and it may be necessary to address a subset of these specifically.

Sample size is a critical consideration: It must be adequate to allow sufficient statistical power to draw valid conclusions. Several sources including " Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests" (http://www.fda.gov/cdrh/osb/guidance/1620.html) and "The Early Detection Research Network (EDRN)" (http://edrn.nci.nih.gov/) cover these and additional issues.

In summary, it is apparent that bringing a biomarker(s) to the clinic is a complex and collaborative venture involving clinicians, scientists, pathologists, bioinformaticists, diagnostic companies,

regulatory authorities, and instrument manufacturers. All too often, however, the process is compromised because (1) studies are under-powered in the discovery phase, (2) the full spectrum of clinical disease is not represented, (3) related disorders that allow assessment of specificity are excluded, (4) independent validation is not undertaken, and/or (5) inaccurate or imprecise assays are used in the validation phase. There is no substitute for careful design, rigorous control, and the analysis of many clinical samples and, contrary to common belief, the analytic methods are rarely, if ever, rate-limiting in the process.

Perhaps the best way to demonstrate the slow evolution of the biomarker process is by way of an example. Carcinoembryonic antigen (CEA) is the most widely used tumor marker and its measurement can be useful in the management of breast cancer patients. The following section illustrates some of the work that brought us to our current understanding of the role of CEA in the management of breast cancer.

The Slow Evolution of a Biomarker: CEA in Breast Cancer

In 1935, Greenough wrote of the importance of the early detection of breast cancer and discussed the enormous diversity of the condition,[4] but it took decades before biochemical markers and methods for their determination became available.

CEA, a heavily glycosylated protein with a molecular mass of ca. 200,000, was first described as a biomarker for colorectal cancer in 1965.[5] CEA is a normal cellular product that is over-expressed in several different types of carcinomas, including colon, breast, and lung, but elevations are also seen in various non-neoplastic diseases, such as liver cirrhosis.

In the late 1970s, Tormey and Waalkes summarized the state of cancer biomarker research[6] and described CEA as a relatively nonspecific test capable of detecting most patients with metastatic disease and post-operative patients at high risk of relapse. They also suggested that CEA is a predictive marker of response to combination chemotherapy in metastatic disease.[6] Shortly thereafter, Coombes and colleagues reported on the measurement of 19 biochemical parameters in 51 patients with breast disease[7] and they concluded "All the parameters studied here are relatively nonspecific and much more fundamental work will be needed to obtain a sensitive and specific tumor-index-substance for breast cancer."[8]

In 1978, Barna and Deodhar suggested that CEA has potential as a prognostic indicator.[9] The same year, Falkson and colleagues reported on analysis of CEA in 234 patients with pathologically proven breast cancer: 181 with advanced metastatic disease and 53 without distant metastases but with nodal involvement at the time of mastectomy.[10] Normal CEA values were recorded in 109 patients considered to be in complete remission. Furthermore, of 63 patients with progressive disease, 22 had normal values and, in 6 of these, clinical relapse preceded CEA elevation by several months. Tumor burden and abnormal serial CEA values positively correlated in 38 patients and in 30 patients, change in clinical status and CEA values occurred simultaneously. Only two patients showed an increase in CEA values in advance of clinical documentation of relapse.

In a more extensive study published the same year, Myers et al. reported on CEA levels in 742 postoperative patients with breast cancer and suggested that levels are associated with an increased risk of developing recurrent disease.[11]

Follow up studies became progressively more comprehensive and addressed other issues. For example, Zangerie and colleagues measured 5 tumor markers, including CEA, in a series of 935 healthy subjects. They reported that levels of CEA (along with kappa-casein, HCG and beta-HCG) increased at the start of the clinical evolution of breast cancer pathology when compared with the

highest level observed in normal subjects. However, a "pathological concentration" of at least one of these markers was observed in 5.5% of the subjects presenting with benign mastopathy and persistently elevated levels of CEA marked disease recurrence or metastatic spread.[12]

In 1979, Cove and colleagues reported on the clinical usefulness of eight potential tumor markers, including CEA.[13] Serum CEA concentrations were elevated in 13% of patients with local and 65% of those with advanced breast cancer. In patients with clinical evidence of progression or regression of tumor, serum CEA levels changed appropriately in 83% of cases. An accompanying editorial in the same issue of the British Medical Journal concluded: "Though impressive, these cumulative abnormalities should be interpreted with caution for several reasons. Firstly, the more substances measured the more false-positive results will occur, unless the normal ranges are expanded. Secondly, even in advanced disease, many of the abnormalities are only just above the normal range, suggesting that they are unlikely to be sensitive guides to the amount of tumor. Thirdly, the *abnormalities reported with some of the markers vary greatly among authors and even among different publications from the same authors. These apparent discrepancies cannot be explained by differences in the patients studied and are in part due to assay differences*. . . . The fourth factor is that the value of studying acute-phase proteins is likely to be limited because they can be affected by treatment that alters the host's immune responsiveness, irrespective of the effects on the amount of tumor. Biochemical changes within the normal range are difficult to interpret unless the physiological variation is known."[14] Importantly, these insightful reservations are no less true of many biomarker studies that are published today.

Progressively, population sizes increased as additional emphasis was placed on validating the role of CEA and other markers. In 1980, serum CEA levels were determined in 2,095 patients following mastectomy for breast cancer.[15] Of 1,462 patients free of metastases, 91% had normal levels (i.e., less than or equal to 3 ng/mL; 98% had levels less than or equal to 5 ng/mL). By contrast, 54% of 633 patients with overt metastases had values greater than 3 ng/mL (43% had greater than 5 ng/mL) and levels were dependent on tumor burden and metastatic location. The authors concluded that the CEA test is a valuable adjunct to monitor the clinical response to chemo/hormonal/radiotherapy in metastatic breast cancer.

In 1984, plasma CEA was reported to be elevated in a small percentage of patients with early breast cancer and in about 60% of patients with distant metastases. These authors concluded that CEA was neither sufficiently sensitive nor specific to be used for mass screening and is of limited use as a diagnostic aid. Monitoring patients following mastectomy with serial estimations of plasma CEA was also reported to be of little value in detecting disease recurrence, although measurements were helpful in objectively assessing response to treatment in patients with disseminated breast cancer.

There are currently over 250 papers which report on the value of CEA determinations in breast cancer diagnosis and management. After decades of investigation the consensus is that CEA determinations are of little value in breast cancer screening, diagnosis, staging, prognosis, or surveillance, but they can prove useful for monitoring patients with metastatic disease when employed in conjunction with diagnostic imaging, history, and physical exam. Consistent with these findings, in 2007 the American Society of Clinical Oncology (ASCO) updated recommendations for the use of CEA as a tumor marker in breast cancer. CEA measurement is not recommended for breast cancer screening, diagnosis, or routine surveillance; instead, CEA could be used to monitor response to therapy as outlined in Table 12.2.

The protracted history of the role of CEA in breast cancer illustrates a critical point: no single study identifies, validates, and unambiguously establishes the utility of a marker. The evolution from discovery to routine clinical application takes years to decades. Misdirection is common and is often associated with inadequacies in the methods used to measure the analyte. Finding a biomarker that

Table 12.2 CEA uses to monitor response to therapy

(1) When monitoring patients with metastatic disease during active therapy, CEA can be used in conjunction with diagnostic imaging, history, and physical exam
(2) Based on present data, CEA alone should not be used for monitoring response to treatment
(3) When there is no readily measurable disease, an increase in CEA may be used to indicate treatment failure
(4) CEA determinations can be used with caution while monitoring the first 4–6 weeks of a new therapy

appears abnormal in a population under examination is only the first step in a complex process and, thereafter, well-designed longitudinal studies defining marker concentrations in relation to disease progression/regression are critical additional components.

Existing Biochemical Tests and Breast Cancer

The Holy Grail in breast cancer clinical practice remains a screening test capable of cost-effective and reliable detection of early stage disease, even in asymptomatic individuals. Currently, mammography is the best tool available, but it is universally acknowledged that better methods are essential if we are to have a significant impact on morbidity and mortality. Mammography finds breast cancer earlier than breast self-examination, but screening mammography has high rates of false-negative results and many women reject routine mammograms. False positive findings are also a problem and result not only in increased costs and complexity of screening, but also bring significant psychosocial consequences. Mammography is also time and resource consuming, poor at detecting cancer in women with dense breasts, and of limited value in quantifying changes in tumor mass over time.

Biochemical tests offer the promise of improved detection and screening for breast cancer. At this time, however, they are only used to support the diagnosis. Optimal current testing includes a complete blood count, liver and renal function tests, alkaline phosphatase, calcium, CA 15–3, CEA (indicative of spread to other areas of the body), CA 27.29, and CA125. (However, testing of CA 15–3, CEA, CA 27–29, and CA 125 are not routine in many practices.) Additional prognostic tests of the tumor tissue, such as chromosome number, hormone receptor status, oncogene over-expression, HER2/neu overexpression and, increasingly, gene profiling (e.g., Onco*type* DX), are often included to assist with managing the patient.

The early hope that one marker would be diagnostic of the disease and be present in cancer and absent from no-cancer was quickly replaced by the realization that a sensitive and specific test requires the determination of multiple independent markers that define the heterogeneous breast cancer population.

Multivariant Testing and Omics Technologies: The Methods and the Molecules

General Comments

Simply put, existing breast cancer biomarkers suffer from low diagnostic sensitivity and specificity and have not made inroads on reducing cancer burden. Instead, multivariate tests that combine several uncorrelated analytes into an integrated panel is the likely way forward because breast carcinomas are heterogeneous in behavior, outcome, and response to therapy.[16]

Although there are now omics methods that allow the simultaneous measurement of most classes of analytes including DNA, RNA, proteins, and metabolites, this chapter focuses on proteins and proteomics. Any one of the molecular classes, however, is illustrative of the approach, problems, and promise of these tools in a discovery setting. In common, all these approaches interrogate thousands of variables in a single study, and are therefore well suited to unearthing a comprehensive set of multiple, independent markers related to a disease. The hope is that a set of biomarkers, when measured concurrently, will push the boundaries of detection of smaller tumors and offer improved specificity and sensitivity. The approach is a powerful alternative first-step to hypothesis-driven studies because it operates without recourse to existing knowledge and is less dependent on insight, instinct, and experience. A single omics study can lead to the formulation of dozens of testable hypotheses and, as T. C. Chamberlin suggested, when we have multiple lines of independent investigation, it limits our potential to embrace a single hypothesis with too much affection, to press our theory to fit the facts, and to press the facts to make them fit our theory.[17] Multiple independent hypotheses help us to be more objective, to distribute our effort, and divide our affections. Each hypothesis (or biomarker candidate) then suggests "... its own criteria, its own means of proof, its own method of developing the truth, and if a group of hypotheses encompass the subject on all sides, the total outcome of means and of methods is full and rich." But, as Platt suggests, each hypothesis needs to be investigated: no corners can be cut; no alternative explanations of the findings overlooked.[18] Put another way, the application of omics methods does not profoundly change the scientific process; it simply improves our odds of successful discovery.

Proteins and Proteomics

Of the multiplexed discovery tools, proteomics has attracted the most attention because it has the potential to complement and enhance the wealth of information accessible through genomics. The proteome is the full complement of gene products of a cell, organ or organism and, although derived from a relative small set of genes, remarkable phenotypic diversity is introduced through co- and post-translational modifications such as phosphorylation, glycosylation, alternative splicing, and specific cleavages. The proteome is therefore a complex, information-rich, and dynamic entity with both temporal and spacial specificity. Most important, it offers information that cannot be accessed at the level of mRNA. The plasma proteome is of special significance because blood is readily accessible, and its analysis reveals thousands of proteins that reflect the collective expression of all cellular genomes. In theory, the judicious analysis of plasma can unearth biomarkers of most (if not all) diseases, but in practice the process is complicated.

Plasma proteins are diverse in properties and distributed across an enormous dynamic range.[19] Further, only a handful of proteins make up the bulk of the mass of the proteome,[19] and these mask attempts to measure others. Investigators have therefore applied a range of techniques to enrich their samples and/or to remove abundant components selectively. However, each additional step in the work-up (e.g., protein precipitation, resolubilization, fractionation, chromatography) introduces its selectivity and reduces quantitative precision. Typically only a small fraction of all proteins is identified, modified and low abundance proteins are missed, and quantitative errors are large.

Despite these limitations, proteomics has delivered protein markers of potential interest and some of this work is reviewed below. Notably, few investigators have employed serum or plasma for their analysis and, if they did, profiling strategies were favored over 2D electrophoresis or liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). One exception is the work of Rui and colleagues in which sera from 54 unaffected women and 76 patients with breast cancer were

analyzed by two-dimensional (2D) electrophoresis and the proteins identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). Two candidate markers were identified in this study: HSP27 (up-regulated) and 14-3-3 sigma (down-regulated). In a blinded verification study these biomarkers were then used to classify an independent set of 104 serum samples. The discriminatory pattern correctly identified all 69 breast cancer cases, and of the 35 cases of non-malignant disease, 34 were recognized as non-cancer. Surprisingly, 2D gels were used for verification rather than more precise and cost effective ELISA assays but, regardless, these findings justify additional study.[20]

Most "proteomic" studies have employed MALDI-TOF profiling.[21] In this approach laser irradiation is used to ionize the components of a prepared biological sample (serum, plasma, tissue extract, or intact tissue) inside a MALDI-TOF MS. The charged particles (ions) are then accelerated down the instrument's flight tube through the application of an electric field and their time-of-flight (TOF) is determined and converted to a mass-to-charge ratio (*m/z*). The mass spectrum is a plot of abundance (y-axis) vs mass-to-charge ratio (x-axis) for all the ions recorded. Typically, the y-axis shows percent relative abundance with the most abundant species (*m/z* value) arbitrarily assigned a value of 100%.

In profiling studies, statistical data analysis is used to derive unique features within distinct groups of MALDI spectra. (A representative MALDI mass spectrum of unfractionated human serum is included as Fig. 12.1.) Although this approach is often referred to as "proteomics" it is important to note that the peaks in the spectrum are typically not identified, but are only represented by their characteristic *m/z* values. Furthermore, only a small fraction of the proteins present in any sample are evident in the spectra.

Several studies have adopted a profiling approach to serum or plasma analysis. For example, in a randomized block design, pre-operative serum samples from 78 breast cancer patients and 29 controls were analyzed by MALDI-TOF MS. Spectra were generated following C8 magnetic bead

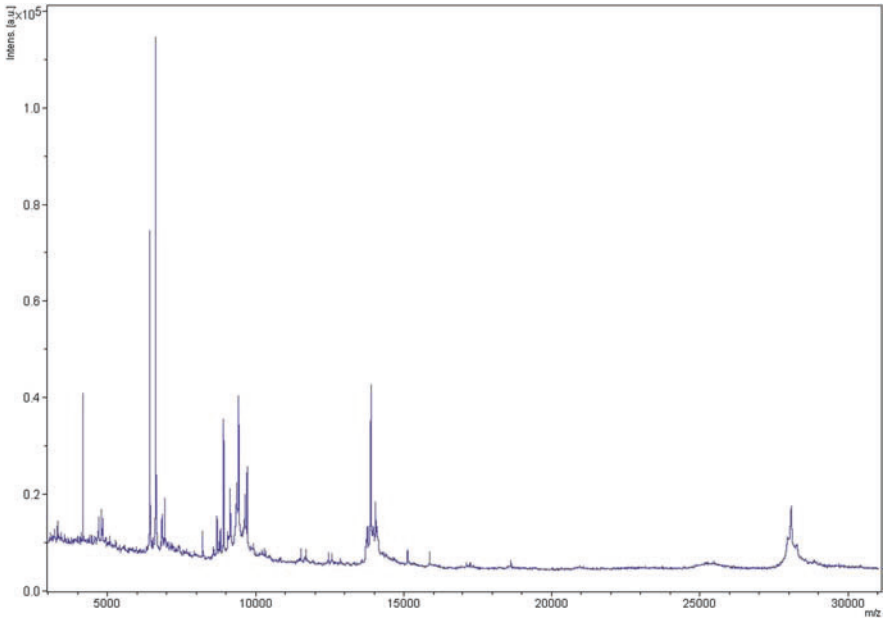

Fig. 12.1 A representative MALDI-TOF mass spectrum of unfractionated human serum

extraction, smoothed, binned, and normalized after baseline correction. Linear discriminant analysis with double cross-validation, based on principal component analysis, was used to classify the protein profiles. The authors report a total recognition rate of 99%, a sensitivity of 100%, and a specificity of 97.0% for the detection of breast cancer.[22] These exceptionally promising data warrant further attention. Although the authors claim that double cross-validation demonstrated that the classification is based on the information content of the spectra rather than chance, validation in additional control and breast cancer sample sets is essential to eliminate the possibility of overfitting. Additionally, validation studies should include other disease groups because non-specific acute phase reactants could account for these findings, rather than direct products of the tumor itself.

Callesen and colleagues examined preoperative serum samples from 48 breast cancer patients and 28 controls. They used a rigorous sample collection protocol to ensure high quality specimens and to minimize bias associated with preanalytical factors. Nine mass spectrometric protein profiles were obtained for each serum sample and a total of 533 common peaks were defined and represented a 'reference protein profile' (Of these, 72 peaks reportedly exhibited statistically significant intensity differences ($p<.01$) between cases and controls). Based on these findings a diagnostic rule was constructed that exhibited a cross-validated sensitivity and specificity of about 85% for the detection of breast cancer. Their approach reportedly distinguished early stage cancers from controls without major loss of sensitivity and specificity.[23]

The reproducibility of profiling approaches has recently been addressed and it has been suggested that, despite known problems, there is convergence toward a set of common discriminating, reproducible peaks for breast cancer when multiple studies are compared.[24] These initial findings offer hope that additional profiling studies will narrow in on a set of classifiers that can distinguish breast cancer with high sensitivity and specificity.

Glycoproteins and Carbohydrates

Aberrant glycosylation has long been recognized as a feature of proteins present on the cell surface or secreted by cancer cells. N-glycans of the total serum glycoproteins from advanced breast cancer patients and healthy individuals have been compared, and a significant increase in a trisialylated tri-antennary glycan containing alpha 1,3-linked fucose, which forms part of the sialyl Lewis x epitope, was noted. When breast cancer patients and controls were compared, a twofold difference in this glycan marker was noted. Further, when 10 patients were monitored longitudinally, a positive correlation between the glycan marker and disease progression was observed. A related pilot study indicated that acute-phase proteins α1-acid glycoprotein, α1-antichymotrypsin, and haptoglobin beta-chain were contributors to the increase in the glycan marker and the authors concluded that specific glycans and glycoforms may be improved markers of breast cancer.[25]

Recent work used immunoaffinity chromatography to isolate and identify potential cancer biomarker glycoproteins. Glycoproteins were selected from plasma of disease-free and breast cancer patients, and of the 26 proteins identified, 9 were reported to be potential breast cancer markers. Appropriately, however, the authors acknowledge that much larger, more diverse populations of breast cancer patients need to be studied.[26]

MALDI-TOF MS has been used to generate a glycomic profile of permethylated glycans in sera from 92 breast cancer patients (12 Stage I; 11 Stage II; 9 Stage III; and 50 Stage IV) along with sera from 27 disease-free women.[27] The serum glycoproteins were first enzymatically deglycosylated, then the released glycans were purified and permethylated prior to analysis. Statistical analysis of the data showed that sialylated and fucosylated N-glycan structures could serve as biomarkers of breast

cancer and that increased sialylation and fucosylation of glycans indicates cancer progression. MS-based N-glycomic profiling of serum constituents may therefore offer a sensitive approach to staging the progression of cancer.

Consistent with these reports, quantitative profiling of N-linked and O-linked oligosaccharides in different breast cancer cell lines confirms a statistically significant difference in certain neutral, sialylated and fucosylated structures.[28] Other studies have also identified glycan biomarkers of breast cancer in cell lines following chemical cleavage of the oligosaccharides (glycans) from glycosylated proteins. Kirmiz and colleagues analyzed the free glycan species by MALDI Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), generated (high resolution) glycan profiles for each cell line, and compared them. Sera from a mouse model of breast cancer and a small number of human serum samples from breast cancer and control subjects were also analyzed. The authors acknowledged that their sample size was inadequate, but report that glycosylation profiles were sufficiently different to distinguish patients with cancer from those without.[29]

Lipids

Differential alterations in plasma lipid profiles have also been reported to be associated with breast cancer.[30] Plasma lipids (namely total cholesterol [TC], high-density lipoprotein [HDL], low-density lipoprotein [LDL], very-low-density lipoprotein [VLDL], and triglycerides [TG]) were analyzed from 70 controls, 30 patients with benign breast disease (BBD), 125 untreated breast cancer patients and 93 individuals post-treatment. Plasma TC and HDL were lower and VLDL and TG were higher in breast cancer patients than in controls. Further, plasma VLDL and TG were higher in breast cancer patients than in patients with BBD. Plasma levels of TC, HDL, VLDL, and TG could significantly distinguish (p=.01, p=.002, p=.001, p=.002, respectively) between controls and breast cancer patients and plasma levels of VLDL and TG could discriminate (significance unclear) between patients with BBD and breast cancer. Odds ratio analysis indicated that higher levels of TC and HDL were significantly associated with a reduction in breast cancer risk (p=.01 and p=.0001, respectively), whereas higher levels of VLDL and TG were significantly associated with increased breast cancer risk (p=.001 and p=.002, respectively). Plasma VLDL and TG levels were lower in complete responders as compared with pretreatment levels (significance unclear), and plasma TC and LDL levels were significantly lower in non-responders as compared with pretreatment levels (p=.015, p=.009, respectively). The reported correlation between lipid levels, breast cancer risk, disease status, and treatment outcome is of considerable interest, but once again additional studies are required. It has also been suggested that lysophospholipids alone or in combination with other markers may aid in the early diagnosis of breast cancer, but the authors acknowledge that many scientific and technical challenges first need to be resolved before the potential of this approach can fairly be assessed.[31]

Metabolites

Metabolomics aims to monitor metabolic status and provide data complementary to genomics and proteomics. Increasingly, metabolomic methods are being used to develop diagnostic tests and the approach holds considerable promise because it can provide markers of cellular integrity, cell and tissue homeostasis, morphological alterations, cell damage, cell death, and more.[32] Several groups

are currently applying metabolomic methods to studies aimed at identifying diagnostic biomarkers for breast cancer.

Autoantibodies

The development of autoantibodies against tumor-associated proteins can be used to mark cancer exposure and, because serum autoantibodies are detectable when antigen expression is low, this approach may allow very early detection.[33] The antibody response to tumor antigens appears to be robust and stable, but currently we know little of the specificity of the antibody immune response to breast cancer.[34] Nevertheless, measurement of autoantibodies against one or a panel of antigens shows promise in the detection and diagnosis of early primary breast cancer.[35]

Summary

No biomarker for the early diagnosis of breast cancer is validated, but we are drowning in a sea of promising candidates that may prove to be of clinical value, especially if utilized cooperatively, or employed in conjunction with other testing modalities. At this stage, however, none has undergone thorough evaluation across large patient populations and in several independent laboratories and, without these data, discussion of the value of any candidate is premature.

As we progress towards a system of personalized medicine aimed at early diagnosis and optimal treatment at minimum cost, breast cancer continues to serve as our poster child. Disease management has already made the paradigm shift away from empirical approaches to objective individualized therapy where clinical decisions are made with the aid of specific molecular markers. With the inclusion of other markers for diagnosis, the biochemical armory will be complete. Conceivably, an optimized panel of a dozen or more circulating analytes measured simultaneously in blood will then provide diagnostic, predictive, and prognostic information to the clinician, while others will monitor the regression of symptoms, the onset of adverse reactions, and the patient's compliance.

A constant theme in this chapter is that enhancements in our analytical methods (i.e., with respect to sensitivity, precision, accuracy, throughput, cost effectiveness, and ease of operation) help us with biomarker discovery, but they don't markedly accelerate the process of biomarker validation. More importantly, enhanced analytic methods do not negate the requirement for a thoughtful study design incorporating the right samples from the right patients. Cutting corners vitiates the process and generates false hope. As was illustrated in the case of CEA, biomarker adoption is not constrained by analytic methods, but by the totality of the discovery and validation process – most notably accrual of large sets of appropriate and carefully defined clinical samples.

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Chapter 13
Circulating Tumor Cells in Breast Cancer

Michail Ignatiadis and Dimitris Mavroudis

Abstract The presence of disseminated tumor cells (DTCs) in the bone marrow has been shown to predict poor clinical outcome in early stage breast cancer. However, peripheral blood is easier to obtain and allows for real time monitoring of minimal residual disease (MRD). Towards this end, circulating tumor cells (CTCs) in the blood are detected using either direct methods, mainly antibody-based assays (immunocytochemistry, immunofluorescence, flow cytometry), or indirect methods, mainly nucleic acid-based assays (detection of mRNA transcripts by reverse transcriptase polymerase chain reaction, RT-PCR). The detection of CTCs using RT-PCR for CK19 was shown to be an independent prognostic factor in women with early breast cancer. Furthermore, there has been considerable progress in genotyping, phenotyping and profiling micrometastatic cells. The challenge now is to integrate minimal residual disease as a prognostic and predictive tool in the management of breast cancer. This requires the standardization of micrometastatic cell detection and characterization which will allow the incorporation of CTCs into prospective clinical trials testing their clinical utility.

Keywords Breast cancer · Circulating tumor cells · CK19mRNA · Disseminated tumor cells · Immunocytochemistry · Micrometastatic cells · Prediction · Prognosis · RT-PCR

Key Issues

- Disseminated tumor cells (DTCs) in women with breast cancer are defined as occult epithelial tumor cells found in the bone marrow.
- Circulating tumor cells (CTCs) in women with breast cancer are defined as occult epithelial tumor cells found in the peripheral blood.
- Micrometastatic cells are usually detected after an initial enrichment step using either direct methods, mainly antibody-based assays (immunocytochemistry, immunofluorescence, flow cytometry), or indirect methods, mainly nucleic acid-based assays (mRNA transcripts by RT-PCR)
- A meta-analysis showed that the presence of bone marrow DTCs predicted poor clinical outcome in women with early stage breast cancer.

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- A single center study showed that the detection of CTCs using RT-PCR for CK19 was an independent prognostic factor in women with early stage breast cancer.
- Detection of CTCs is not always prognostically relevant which could be explained by sampling error, the suboptimal sensitivity of the assays, the detection of apoptotic cells, or cells that are not able to self-renew and generate metastases.
- Phenotyping/profiling/genotyping CTCs may be crucial not only to identify new targets which could be used to eliminate minimal residual disease (MRD) but also could serve as a real-time monitoring system to assess the evolution of genetic changes in tumor cells with potential prognostic and therapeutic implications.
- The detection of CTCs in breast cancer should not be used in clinical practice as a "standard evaluation tool". Standardization of micrometastatic cell detection and characterization as well as the incorporation of CTCs into prospective clinical trials is urgently needed before using this exciting new tool in clinical practice.

Introduction

The TNM breast cancer staging system cannot accurately identify prognosis for some women, especially those with small, axillary node-negative tumors who relapse and die of breast cancer.[1] Therefore, many investigators have hypothesized that the detection of micrometastases in the bone marrow (Disseminated Tumor Cells, DTCs)[2–4] or peripheral blood (Circulating Tumor Cells, CTCs)[5–7] might provide prognostic information beyond the TNM system. Furthermore, micrometastatic cells that are undetectable by classical imaging and laboratory studies (Minimal Residual Disease, MRD), when present after potentially curative surgery, are thought to contribute to disease relapse and therefore are obvious targets of adjuvant treatment strategies. Consequently, the study of these cells, apart from the impact on refining prognosis, has the exciting potential of individualizing adjuvant treatment for women with breast cancer.

Bone marrow DTCs were shown to have less advanced genomic changes than primary tumor cells, suggesting that tumor cell dissemination occurs early in the disease course and therefore breast cancer should be considered a systemic illness, even when diagnosed at an early stage.[8,9] However, the American Society of Clinical Oncology (ASCO) 2007 recommendations for breast cancer tumor markers suggested that present data are insufficient to recommend assessment of bone marrow DTCs for the management of patients with breast cancer.[10] Similarly, the panel concluded that the measurement of CTCs should not be used to make the diagnosis or to influence treatment decisions in patients with breast cancer.[10] However, this is the first time that DTCs/CTCs have been included in the ASCO guidelines and the panel concluded that the data are intriguing and should be further evaluated in future studies.[10] Herein we will critically review the literature concerning the clinical implications of DTCs/CTCs and present the potential that the study of MRD might have in changing the way we manage breast cancer. Micrometastasis in axillary lymph nodes (included in the last TNM edition[11]) is beyond the scope of the present review.

Definition of Occult Tumor Cells

Disseminated tumor cells (DTCs) in women with breast cancer are defined as occult epithelial tumor cells found in the bone marrow. CTCs in women with breast cancer are defined as occult epithelial tumor cells found in the peripheral blood. Epithelial cells are only rarely found

in the bone marrow and peripheral blood of otherwise healthy women. Several investigators have provided evidence that most epithelial cells detected in the bone marrow[9,12,13] or peripheral blood[14] of women with breast cancer harbor genomic alterations characteristic of malignant cells.

Methods and Limitations for the Detection of Occult Tumor Cells

By definition, micrometastatic cells are undetectable by standard hematoxylin-eosin staining. These cells are usually detected after an initial enrichment step (e.g., density gradient centrifugation, filtration, immunomagnetic selection techniques) using either antibody-based assays (e.g., immunocytochemistry, immunofluorescence, flow cytometry) or nucleic acid-based assays (mRNA transcripts by RT-PCR).[15–20] Several investigators have compared different detection methods.[21,22] The nucleic acid-based assays have generally been considered more sensitive, while immunocytochemistry has the advantage of allowing the assessment of morphology of the stained cells.

The CellSearch™ system, a semi-automated system based on immunofluorescence and flow cytometry (Veridex, Warren, New Jersey, USA), has been approved by the FDA as an aid in the monitoring of patients with metastatic breast, colorectal and prostate cancer. However, the clinical utility of monitoring CTCs with the CellSearch system remains to be proven. The peripheral blood sample is enriched for cells expressing the epithelial-cell adhesion molecule (EpCAM) with antibody-coated magnetic beads, and cells are labeled with the fluorescent nucleic acid dye 4,2-diamidino-2-phenylindoledihydrochloride. Fluorescently labeled monoclonal antibodies specific for leukocytes (CD45-allophycocyan) and epithelial cells (cytokeratin 8,18,19-phycoerythrin) are used to distinguish epithelial cells from leukocytes.[23]

Since automated digital microscopy (ADM), the preferred method of CTCs detection, is too slow to scan large substrate areas, Krivacic et al. developed an approach that uses fiber-optic array scanning technology (FAST).[24] FAST cytometry enabled a 500-fold speed-up over ADM with comparable sensitivity and superior specificity.[24] The combination of FAST and ADM allowed the investigators to detect rare epithelial cells from whole unseparated blood after immunofluorescence staining with a pan-cytokeratin antibody.

Another method for the detection of Circulating Epithelial Tumor Cells (CETCs) from whole unseparated blood uses laser scanning cytometry after staining with anti-EpCAM and anti-CD45 fluorescent antibodies (MAINTRAC™).[25]

Recently, Nagrath et al. described the development of a microfluidic platform (the "CTC-chip") capable of efficient and selective separation of viable CTCs from peripheral blood samples, mediated by the interaction of target CTCs with antibody (EpCAM)-coated microspots under precisely controlled laminar flow conditions, without requiring pre-labelling or processing of samples (CellPoint Diagnostics, California, USA).[26] The "CTC-chip" can detect and isolate rare CTCs with high purity from small blood volumes (2–3 mL).[26]

For the detection of CTCs, different markers have been chosen based on their expression in epithelial but not mesenchymal cells (epithelial-specific markers) or based on their specific expression in breast tissue (breast tissue-specific markers). Among these markers, cytokeratins (CKs), which are intermediate filament keratins found in the cytoskeleton of epithelial cells, have been most extensively used.[27] The CKs most commonly studied in breast cancer are CK19 and CK8,18. However, false positive results have been observed using either nucleic acid-based or antibody-based assays.[16] Contaminating genomic DNA during RNA extraction, illegitimate expression, or stimulation of CTC markers in normal mononuclear cells or lymphocytes by cytokines and the presence

of CK19 pseudogenes have been considered responsible for the false positive results when using nucleic acid-based assays.[16,28–31] The use of quantitative RT-PCR, which can sometimes discriminate low illegitimate background expression from the higher levels found in breast cancer as well as the design of primers that do not amplify genomic DNA or pseudogenes might in part address and resolve the above concerns.[32] Similar limitations have been described using antibody-based techniques. Many of the antibodies directed at epithelial and breast cancer cells are also known to stain occasionally hematopoietic cells displaying illegitimate expression of cytokeratins (CK19) or MUC1. Non-specific staining of plasma cells can also occur due to alkaline phosphatase reaction against the kappa and lambda light chains on the cell surface.[16] Optimizing the antibodies and using the appropriate negative controls in staining experiments have been employed to overcome the above problems.

Diagnosis

Several studies have used CTC detection as a tool to assist breast cancer diagnosis.[33,34] Reinholz et al, reported that molecular detection of CTCs can be used in combination with mammography and physical examination for the early detection of breast cancer. They used mammaglobin-A (MGB1) and B305D-C genes to construct a diagnostic test that correctly classified women undergoing biopsy for mammographically-detected breast abnormalities as having breast cancer with a sensitivity of 70.5% and a specificity of 81%.[33] Chen et al. developed a membrane array-based method to simultaneously detect multiple peripheral blood mRNA markers for use in breast cancer diagnosis.[34] The assay achieved a sensitivity of 80.6%, and a specificity of 83.8% for breast cancer detection.[34]

Prognosis

CTCs have been studied for their impact on prognosis estimation in breast cancer (see Table 13.1).

Metastatic Breast Cancer

Most studies reporting on the prognostic value of CTCs in patients with metastatic breast cancer have used the CellSearch™ system. The presence of ≥ 5 CTCs per 7.5 mL of whole blood in 177 patients with measurable metastatic breast cancer before a new treatment was started, was an independent predictor of progression-free survival (PFS) and overall survival (OS).[35] Furthermore, CTC detection by CellSearch was suggested to be a surrogate endpoint than is superior to current radiology imaging studies for assessing response to treatment and predicting OS in metastatic breast cancer patients.[36] To provide further evidence that CTC detection can improve clinical outcome in metastatic breast cancer, the Southwest Oncology Group (SWOG) has launched a phase III trial (ClinicalTrials.gov NCT00382018) to test the strategy of changing chemotherapy compared with continuing the same chemotherapy for metastatic breast cancer patients who have elevated CTC levels at first follow-up assessment. Several other investigators have reported other less standardized or validated antibody- or nucleic acid-based assays to detect CTCs in metastatic breast cancer.[37–41]

Table 13.1 Representative studies on the prognostic value of CTCs in breast cancer

Stage	Tissue	Volume (mL)	Enrichment/ detection method	Marker	Pts (No)	Detection rate (%)	Cut-off	Independent prognostic value	Ref
IV	PB	7.5	IS	CK	177	49	≥5 CTCs/7.5 mL	PFS, OS	35
IV	PB	24	Ficoll/RT-PCR	Multi[2]	103	29.1	Based on healthy women	No	38
I–IV	PB	5	Filtration/ICC	CK8	123	I–III:25 IV:11.6	I–III: ≥4 CTCs/5 mL IV: ≥13 CTCs/5 mL	I–III:No IV:TTP	37
I–IV	PB	10	EL/RT-PCR	Multi[3]	65	69	[5]	NR	74
IV	PB	10	Ficoll/IS/PCR ELISA	Telomerase	25	84	Based on healthy women	NR	41
I–III	PB	10	Ficoll/RT-PCR	CK19	148	29.7	Nested[6]	DFI, OS	5
I–III	PB	20	Ficoll/RT-PCR	CK19	444	40.8	0.6 MCF7 Eq/5 μg RNA	DFS, OS	7
I–II[1]	PB	20	Ficoll/RT-PCR	CK19	167	21.6	0.6MCF7 Eq/5 μg RNA	DFS, OS	6
I–III	PB	20	Ficoll/RT-PCR	Multi[4]	175	44	0.6 MCF7 Eq/5 μg RNA Nested[5,6]	DFS	76
I–III	PB	20	Ficoll/RT-PCR	MGB1	101	13.9	Nested	DFI	64
I–II	PB	20	Ficoll/RT-PCR	HER2	214	21	Nested	DFI	67
I–III	PB	50	IS/ICC	CK, HER2	35	48.6	≥1 CTCs/50 mL	No	72
I–III	PB	22.5	Ficoll/IS/IF (CellSearch™)	CK 8, 18, 19	1,500	9.5	>1 CTC/22.5 mL	NR	54
I–III	PB	7.5	EL/IF	EPCAM, CD45	91	100	10-fold change in CETS during treatment	RFS	55
NED	PB	40	Ficoll, IS, ICC	CK	341	CTCs: 10	≥1 CTCs/10×10^6MNCs	NR	85
I–IV	PB	7–14	Ficoll, ICC	CK	114	CTCs: 24.5	≥1 CTCs/3×10^6MNCs	No	86
I–IV	PB	9	Ficoll, RT-PCR	CK19	148	PB:15	Based on healthy women	No	87
I–IV	PB	5	Ficoll/RT-PCR	CK19	109	44	Nested	NR	52
I–IV	PB	10	IS/RT-PCR	MUC	94	37	Ct ≤37	NR	60
NED	PB	10	EL/RT-PCR	MGB1	310	2	Nested	No	62
I–IV	PB	10	Ficoll/RT-PCR	EGFRvIII	62	43.5	Nested	NR	68

Pts: Patients, NED: Non-Evidence of Disease, PB: Peripheral Blood, NR: Not Reported, CTCs: Peripheral Blood Circulating Tumor Cells, MNCs: Mononuclear Cells, ICC: Immunocytochemistry, IF: Immunofluorescence, IS: Immunomagnetic Separation, EL: Erythrocyte Lysis, CK: Cytokeratin, Muc: Mucin, MGB1: Mammaglobin-A, EGFRvIII: Epidermal Growth Factor Receptor variant III, I–II:[1] Node-negative tumors, Multi-marker:[2] (CK19, p1B, PS2 and EGP2), Multi-marker[3] (human chorionic gonadotropin [hCG], c-Met, 134-*N*-acetylgalactosaminyl-transferase [GalNAc-T], and tumor–associated antigen [MAGE-A3]), Multi-marker:[4] (CK19, MGB1 and HER2):[5] For a patient to be considered CTC-positive, at least one mRNA marker should be positive, DFS: Disease-Free Survival, DDFS: Distant-Disease-Free Survival, BCSS: Breast-Cancer-Specific Survival, OS: Overall Survival, DMFS: Distant-Metastasis-Free Survival, RF: Recurrence-Free Survival, DFI: Disease-Free Interval, PFS: Progression-Free Survival, TTP: Time-To-Progression, RT-PCR: Reverse-Transcriptase Polymerase Chain Reaction, Ct: Cycle threshold, Nested:[6] In case of nested RT-PCR, results are expressed as detectable or non-detectable, Eq: equivalents

Early Breast Cancer

DTCs

Several studies have evaluated the prognostic value of bone marrow DTCs in early breast cancer and some of them have failed to demonstrate an independent prognostic value of DTCs when controlled for the "well-known" primary tumor and clinical characteristics.[2,3,42–48] However, a meta-analysis involving 4,703 early breast cancer patients provided adequate statistical power to address this question.[4] DTCs were detected in 30.6% of the patients at the time of primary surgery and their detection was an independent prognostic factor for poor outcome. For the subset of low risk patients with pT1N0 tumors (n = 1,036), the presence of DTCs was associated with an increased risk of distant metastasis and death during the first 5 years.[4] However, the ASCO 2007 recommendations concluded that these retrospective data do not justify differential recommendations for adjuvant therapy[10] for a patient with bone marrow micrometastases.

Apart from the above meta-analysis where DTCs were examined at the time of primary surgery, Janni et al. reported that the persistence of DTCs during recurrence-free follow-up in patients with breast cancer was an independent prognostic factor for short recurrence-free survival (RFS) and OS.[49]

CTCs

Stathopoulou et al. first reported that the detection by nested RT-PCR of CK19mRNA-positive cells in the peripheral blood of women with early stage breast cancer was an independent prognostic factor for worse disease-free survival (DFS) and OS.[5] Later on, the same investigators developed a real-time RT-PCR for the quantification of CK19mRNA transcripts.[50] Xenidis et al. used the above assay and detected CK19mRNA-positive cells in the peripheral blood of 21.6% of 167 patients with axillary lymph node-negative breast cancer before the administration of adjuvant chemotherapy. Their presence was an independent prognostic factor for worse DFS and OS.[6] In an expanded cohort of 444 women with Stage I–III breast cancer, Ignatiadis et al. detected CK19mRNA-positive cells by the same real-time RT-PCR assay in the blood of 40.8% of patients before adjuvant chemotherapy.[7] The presence of these cells was an independent prognostic factor for shorter DFS and OS. Furthermore, Xenidis et al. demonstrated that CK19mRNA-positive cells were detected post-adjuvant chemotherapy in 32.7% of 450 early breast cancer patients and their presence was an independent prognostic factor for reduced DFS and OS.[51] Several other investigators have reported on the molecular or immunocytochemical detection of CTCs using cytokeratins by taking into consideration standard clinicopathological characteristics like tumor size, lymph nodal status, and histology grade.[52,53]

In the SUCCESS trial, >1 CTC/23 mL of blood was detected by the CellSearch System in 9.5 and 8.7% of 1,500 node-positive and high risk node-negative early breast cancer patients before and after adjuvant chemotherapy, respectively.[54] After a 12-month median follow up, detection of >1 CTC/23 mL after but not before adjuvant chemotherapy was associated with shorter disease-free and overall survival.[54] Pachmann et al. used the MAINTRAC technology and identified 1–100,000 circulating epithelial cells (CETCs)/mL of peripheral blood in women with early breast cancer.[55] All 91 women had detectable CETCs and a 10-fold increase in CETCs' numbers between blood samples drawn before and after adjuvant chemotherapy was an independent predictor of relapse.

There are obvious and significant differences in CTC detection rates between the molecular techniques, the CellSearch and the MAINTRAC platform which could only be addressed through a direct comparison of these technologies in the same patient population with early stage breast cancer.

Detection of CTCs: Is it Always Prognostically Relevant?

Only 30% of patients with CK19mRNA-positive CTCs relapse whereas 15% of patients without these cells still relapse and die of breast cancer after a 5-year median follow-up.[7] For patients who relapse with no detectable CTCs, this could be explained by sampling error or could be attributed to the suboptimal sensitivity of the assays or cytokeratins as a marker of occult tumor cells. Indeed, tumor cell dissemination has been linked to the epithelial-mesenchymal transition and the loss of epithelial cell markers.[56] On the other hand, for patients with CTCs who did not relapse, this could be due to the detection of apoptotic cells or cells that are not able to self-renew and generate metastases. Moreover, microarray and comparative genomic hybridization (CGH) studies have shown that breast cancer is a genetically heterogeneous disease[57,58] and that even the micrometastatic cells of any given patient with early stage breast cancer may exhibit diverse genomic profiles.[13] Therefore, in order to improve the sensitivity/specificity of CTC detection as a prognostic tool as well as to define subpopulations of CTCs with aggressive biological behavior that could be used more precisely as surrogate markers for relapse, several approaches have been employed.

Other Markers (Apart from Cytokeratins) for the Detection of MRD

Investigators have used a variety of markers for the detection of micrometastatic cells including mucins, mammoglobin-A (MGB1), maspin, carcinoembryonic antigen (CEA), HER2, EGFRvIII, and cathepsin D.[59–68] Since HER2 oncoprotein has been associated with aggressive biological behavior in breast cancer, several groups have studied the expression of HER2 on micrometastatic cells. Apostolaki et al. used nested RT-PCR to detect peripheral blood HER2mRNA-positive cells in 21% of 214 patients with early breast cancer after the administration of adjuvant chemotherapy; their detection was an independent prognostic factor for reduced DFI.[66] Wulfing et al. used double immunocytochemical staining to identify HER2-positive CTCs that correlated with shorter DFS and OS in early breast cancer patients treated with adjuvant chemotherapy or hormonotherapy.[69] Moreover, Ignatiadis et al. have shown that women with the CK19mRNA-positive/HER2mRNA-positive molecular profile in the blood had shorter DFS compared with CK19mRNA-positive/HER2mRNA-negative patients.[70] Although the above studies have used different methodologies for the detection and characterization of CTCs' HER2 status, HER2-positive cells have been consistently detected in approximately half of early breast cancer patients presenting CTCs and these patients had worse prognosis than their counterparts with CTCs not expressing HER2.

Based on the heterogeneity of CTCs, several multi-marker RT-PCR assays have been reported[33,71–74] Taback et al. used human chorionic gonadotropin (hCG), oncogene receptor (c-Met), 134-*N*-acetlgalactosaminyl- transferase, and a tumor-associated antigen (MAGE-A3) to develop a multi-marker RT-PCR assay combined with an electrochemiluminescence automated detection system for the detection of CTCs in breast cancer.[71] Zehentner et al. chose four different markers, namely MammaglobinA, B305D, GABRP, and B726P to develop a multi-marker, real-time RT-PCR assay for the detection of CTCs in breast cancer patients.[72] Mikhitarian et al. used a panel of seven genes for the molecular detection of CTCs (mammaglobin-A, CEA, CK19, PIP, muc1, PSE, EpCAM).[74] However, no correlation between CTC detection using the above multi-marker assays and clinical outcome has been reported. Recently, Ignatiadis et al. reported for the first time that the use of a multi-marker (CK19, MammaglobinA, and HER2) RT-PCR to detect CTCs predicted poor clinical outcome in early stage breast cancer patients and this assay had increased accuracy as compared to CTC detection by real-time RT-PCR for CK19 alone.[73] The presence of two or three positive

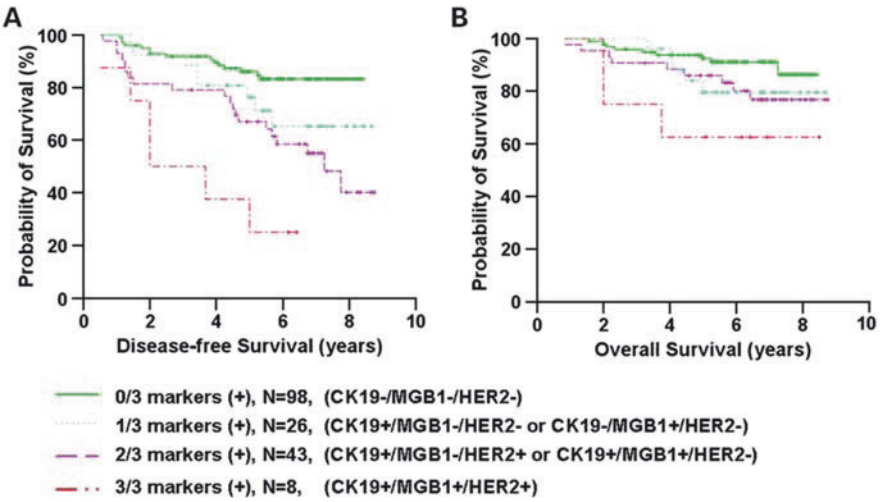

Fig. 13.1 Disease-free survival (DFS). (**a**) and Overall survival (OS) (**b**) in early breast cancer patient groups based on the molecular detection of CTCs using a panel of three markers (*CK19*, MGB1, and HER2) (From Ignatiadis et al.[73] Reprinted with permission. © 2008 American Association for Cancer Research. All rights reserved)

markers in the peripheral blood was associated with progressively worse prognosis compared with that of one positive marker (Fig. 13.1).

Assays to Distinguish Between Apoptotic and Non-apoptotic or Viable Micrometastatic Cells

In general, when using nucleic acid based-assays, the detection of an mRNA transcript in a blood sample suggests the presence of a viable cell since the viability of RNA once released from cells is poor and the presence of mRNA suggests active transcription machinery. Moreover, Alix-Panabieres et al. used epithelial immunospot (ELISPOT) to detect viable, nonapoptotic CTCs.[75] The assay allowed the detection of protein secretion at the individual cell level.

Study of Micrometastatic Cells with a "Stem Cell-like" Phenotype

Given the recent identification of breast cancer tumor-initiating cells with the CD44+CD24-/low phenotype,[76] it could be hypothesized that micrometastatic cells with this phenotype might represent a prognostically relevant subpopulation. A study that detected CTCs with the CD44+CD24-/low, "stem cell-like" phenotype has recently been reported.[77]

DTCs vs CTCs

Compared with the bone marrow, peripheral blood sampling is easier and more acceptable to patients and their treating physicians. Therefore, an important question is whether peripheral blood sampling can replace bone marrow aspiration for the evaluation of MRD. Wiedswang et al. compared the prognostic value of CTCs vs DTCs detected by immunocytochemistry in 341 breast cancer patients with sampling performed at a median follow-up 40 months after the initial operation.[78] Although both CTCs (10% of the patients) and DTCs (14% of the patients) were significantly associated with clinical outcome, DTCs were more informative than CTCs.[78] Pierga et al. compared the detection

of cytokeratin-positive CTCs vs DTCs with an automatic-assisted immunocytochemical detection system in a cohort of 114 breast cancer patients.[79] In non-metastatic patients ($n = 75$), the presence of DTCs but not CTCs was prognostic for poor DFS.[79] In another study, investigators performed real-time RT-PCR for the detection of CK19 and mammaglobin-A in 148 patients with early and metastatic breast cancer.[80] Patients with either an elevated CK19 or mammaglobin-A expression level in the bone marrow but not in the peripheral blood had worse OS.[80] Although the above studies suggest that DTCs are prognostically superior to CTCs in early breast cancer, the best validated techniques for the detection of bone marrow DTCs (i.e., immunocytochemistry[4]) and peripheral blood CTCs (i.e., real-time RT-PCR for CK19[6,7]) have never been directly compared.

MRD and Breast Cancer Molecular Subtypes

Ignatiadis et al. recently reported the first study examining the prognostic value of micrometastatic cells in relation to early breast cancer molecular subtypes.[7] After a median follow-up of 5 years, the presence of CK19mRNA-positive CTCs before the initiation of adjuvant chemotherapy predicted worse outcome in patients with estrogen receptor (ER)-negative but not ER-positive early stage breast cancer, despite the similar proportions of patients with CK19 mRNA-positive CTCs in both subgroups. Moreover, the presence of CK19mRNA-positive CTCs was associated with shorter DFS and OS in the triple-negative and HER2-positive, but not in the ER-positive/HER2-negative subgroups[7] (Fig. 13.2). Based on results of the above study, Ignatiadis et al. hypothesized that *CK-19* mRNA-positive CTCs had different biologic behavior in patients with ER-negative (basal-like CTCs) and ER-positive (luminal-like CTCs) tumors. However, molecular and immunophenotypic characterization of *CK-19* mRNA-positive CTCs in patients with ER-negative and ER-positive disease is required to validate this hypothesis further.

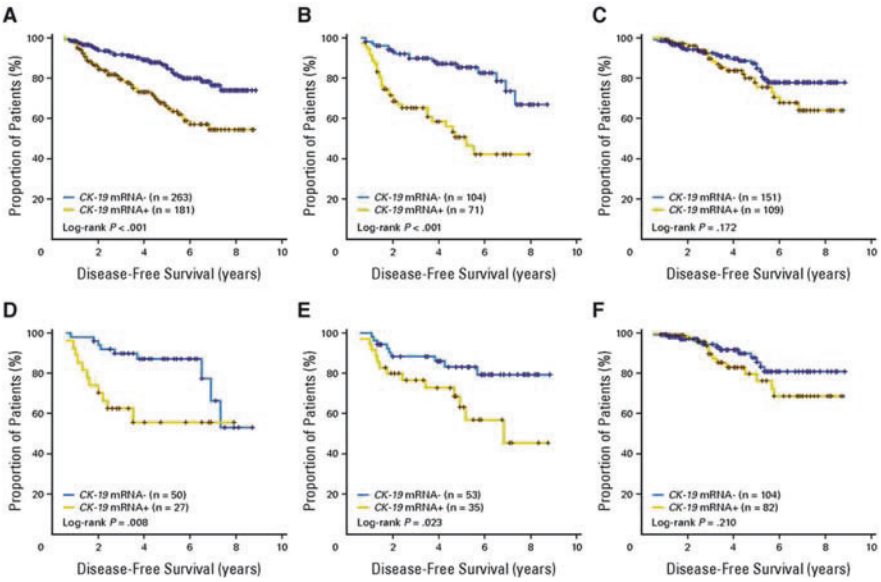

Fig. 13.2 Disease-free survival of patients with and without cytokeratin-19 (CK-19) mRNA-positive circulating tumor cells (CTCs): (**a**) entire patient population; (**b**) estrogen receptor (ER)-negative; (**c**) ER-positive; (**d**) triple-negative; (**e**) HER2-positive; (**f**) ER-positive/HER2-negative subgroups (From Ignatiadis et al.[7] Reprinted with permission. © 2008 American Society of Clinical Oncology. All rights reserved)

Interestingly, according to most primary tumor gene expression signatures, the majority of ER-negative tumors are assigned to the poor-prognosis group, whereas ER-positive tumors comprise a mixture of poor- and good-prognosis tumors.[58,81] Therefore, it would be interesting to assess prospectively the hypothesis that, by combining information from primary tumor gene expression profiling and the detection of micrometastatic cells, we could further improve prognosis in early stage breast cancer.

Prediction

Apart from refining prognosis, the most exciting field is the role of studying MRD as a predictive tool. There is experimental evidence that micrometastatic cells have less advanced genomic alterations than primary tumor cells.[9] Since micrometastatic cells are the true targets of adjuvant systemic treatment, when choosing therapy it is likely important to consider both the characteristics of the primary tumor and those of micrometastatic cells in order to improve outcome of early breast cancer patients.

CTCs Phenotyping, Profiling and Genotyping

Several investigators have tried to phenotype individual micrometastatic cells. Meng et al. used a sensitive blood test to capture CTCs and evaluate their *HER-2* gene status by fluorescence in situ hybridization.[82] They reported that 9 of 24 breast cancer patients with HER2-negative primary tumors had acquired HER2 gene amplification in their CTCs during cancer progression.[82] Meng et al. also demonstrated a marked tendency for co-amplification of HER2 and urokinase plasminogen activator receptor (uPAR) genes on individual CTCs.[83] On the other hand, Kallergi et al. provided evidence of enhanced expression of activated signaling kinases as well as HER2 on individual CTCs.[84] Moreover, Smirnov et al. using microarray technology identified global gene expression profiles from CTCs of metastatic cancer patients and created a list of CTC-specific genes.[85] Intriguing results have demonstrated that gene expression profiling of single cells is feasible with oligonucleotide arrays.[86] Finally, using the CellPoint platform, Maheswaran et al. detected EGFR mutations in CTCs from patients with lung cancer treated with gefitinib. This study provides proof of principle for the feasibility of minimally invasive (blood sample instead of tumor biopsy) serial monitoring of tumor cell genotypes during treatment.[87] Based on these data, it seems that CTC phenotyping/profiling/genotyping may be crucial not only for identifying new targets which could be used to eliminate MRD but also could serve as a less invasive and therefore more feasible and acceptable real-time monitoring system to assess evolution of genetic changes on tumor cells with potential prognostic and therapeutic implications.

Chemotherapy and Hormonal Therapy

Several studies have demonstrated that CTCs are relatively resistant to chemotherapy,[51,88] probably due to their low proliferative potential.[89] Xenidis et al. showed that the persistent detection of CK19mRNA-positive cells in 119 patients with hormone receptor-positive tumors during tamoxifen administration was an independent prognostic factor for short DFS and OS.[90] Therefore, the persistent detection of CK19mRNA-positive cells during adjuvant tamoxifen administration should be

further investigated as an indicator of tamoxifen resistance and the need to switch to an alternative adjuvant hormonal therapy such as an aromatase inhibitor.

New Targeted Agents

Since conventional chemotherapy and hormonal therapy cannot eliminate all micrometastatic cells in all patients, several investigators have targeted CTCs using monoclonal antibodies. Bozionellou et al. reported for the first time that a short course of trastuzumab could eliminate chemotherapy- and hormonotherapy-resistant CK19mRNA- and HER2mRNA-positive CTCs in 20 (67%) of 30 patients with early and metastatic breast cancer.[91] Similar data have been reported in a xenograft SCID mice model.[92] Furthermore, HER2-positive CTCs have been reported in patients with HER2-negative primary tumors.[69,70] Therefore, it would be interesting to test prospectively the hypothesis that this subpopulation of women could also benefit from adjuvant trastuzumab administration.

CTCS as Prognostic and Predictive Tool: Is it Ready for Prime Time?

CTCs can serve as a real-time "biopsy" to evaluate and monitor treatment response since their detection can be easily repeated at different time intervals, whereas this is not the case for bone marrow DTCs. Therefore, most ongoing and planned clinical studies use peripheral blood CTCs instead of bone marrow DTCs in order to study MRD as a prognostic and predictive tool in breast cancer. Although there are emerging data that CTC assessment might in the near future provide an exciting new prognostic and predictive tool to individualize breast cancer treatment, several problems have to be addressed before they could be used in clinical practice.

a. *Detection of CTCs is not yet standardized.* Several efforts have been made toward CTC detection standardization during the last year.[93,94] However, since investigators have reported CTC detection in breast cancer patients using different peripheral blood volumes per patient, different enrichment procedures, and different methods or epithelial tumor markers for their detection, comparisons across studies is extremely difficult. CTC detection has also mainly relied on CKs. However, even studies using the same method and marker did not use common standardized procedures (e.g., different primers, amplification conditions, and platforms for the RT-PCR, or different antibodies, protocols and detection systems for the immunocytochemistry or immunofluorescence). Therefore, the assays used have in many cases been suboptimally standardized with low reproducibility, whereas the cut-off values chosen for defining CTC positivity have not been adequately validated.
b. *Published studies on CTCs are flawed with many problems.* Many CTC studies had poor statistical design, suffering from small sample sizes, were retrospective and were not reported in a rigorous fashion.[95] Therefore, it is important that authors publishing in the field of CTCs should adhere to reporting recommendations for tumor marker studies.[95]
c. *No published studies have as yet shown that by using CTCs as a prognostic and/or predictive biomarker we can improve clinical outcome of patients with breast cancer.* Henry et al. published a tumor marker development flow chart in which they described how a tumor marker can reach clinical practice. First, an accurate method to measure the tumor marker has to be developed. Then, a preliminary, preclinical hypothesis for this marker should be validated, ideally in archived specimens from prospective trials.[96] Finally, a prospective clinical trial should be designed so

that treatment decisions in the experimental arm are based, at least in part, on CTCs. In this way, definitive proof will be provided that CTCs can be used to improve clinical outcome in breast cancer.

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Note: The letters 'f' and 't' following locators denote figures and tables respectively.

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