Adnan Aydiner
Abdullah Igci
Atilla Soran
Editors

Breast Disease

Diagnosis and Pathology, Volume 1

Second Edition

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Editors
Adnan Aydiner
Department of Medical Oncology
Istanbul Medical Faculty, Oncology Institute
Istanbul University
Istanbul
Turkey

Abdullah Igci
Department of General Surgery
Istanbul Medical Faculty
Istanbul University
Istanbul
Turkey

Atilla Soran
Department of Surgical Oncology
Magee-Womens Hospital of the University
of Pittsburgh
Pittsburgh, PA
USA

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Preface

The goal of *Breast Disease: Diagnosis and Pathology* is to provide a comprehensive, scholarly appraisal of contemporary basic science and diagnosis. Because of advances in molecular medicine and therapeutics, this appraisal requires a more extensive understanding of the basic science of oncology than was required in the past. This book is organized into 18 chapters, and a brief summary of their content is provided below. In addition, we highlight some of the various important points in this second edition of the book.

The topic of *Benign Breast Diseases* covers a wide variety of conditions, both common and unusual, that may require additional work-up, excision, or surveillance. In addition, some benign conditions may confer an increased risk for future disease, and this risk should be explained to the patient during treatment for these entities. This section attempts to provide a basic understanding of some of the most frequently encountered benign breast conditions and various rare types, including current recommendations for work-up, management, differential diagnoses, and future surveillance. The specific conditions that are explored in this chapter include fibroadenomas, intraductal papillomas, lipomas, hamartomas, radial scars, and gynecomastia in males.

Age, family history, and both endogenous and exogenous ovarian hormone exposure have important effects on risk and have been incorporated into models that predict the individual *risk of breast cancer*. Diet, alcohol use, and other factors play smaller roles. BRCA mutation-associated breast cancer differs from sporadic breast cancer in that BRCA mutation carriers exhibit an increased risk of breast and ovarian cancer and differential sensitivity to chemotherapeutic agents. Because BRCA genetic testing is readily available, BRCA mutation status should be evaluated in high-risk women, including women who were diagnosed with breast cancer at an early age and women with a strong family history or triple-negative tumors. Given the high rate of contralateral breast cancer and ovarian cancer, mutation carriers with newly diagnosed breast cancer may choose to undergo contralateral prophylactic mastectomy or bilateral salpingo-oophorectomy. In addition, two selective estrogen receptor modulators, tamoxifen and raloxifene, and aromatase inhibitors can be used to decrease the incidence of invasive breast cancer in women who are at high risk of this condition.

Breast imaging is an essential component of breast cancer diagnosis and guides surgery and treatment options. Imaging techniques, such as mammography, ultrasound (US), and magnetic resonance imaging (MRI), enable the detection of breast cancer at earlier stages. Mammography remains the standard screening examination; however, additional imaging studies are useful in evaluating the breast. US is utilized primarily in the diagnostic setting to characterize mammographic or palpable findings and assess axillary lymph nodes. Supplemental US screening may also be useful in patients with an intermediate risk for developing breast cancer and dense breasts to increase cancer detection. In addition to mammography, high-risk patients may also have annual MRI or US screening if they are unable to undergo MRI. MRI is also performed to evaluate the extent of disease, the response to neoadjuvant chemotherapy, and the silicone implant integrity. In addition, these imaging modalities are also used to guide percutaneous biopsy, enabling minimally invasive tissue diagnosis.

In *nuclear medicine* practice, there have been many diagnostic tools developed for primary detection, staging, and evaluation of treatment response in breast cancer. In this edition, a new

chapter outlines the role of nuclear medicine both in imaging and treatment of patients with breast cancer.

Lobular carcinoma in situ (LCIS) is a high-risk indicator lesion for and a non-obligate precursor of the development of invasive breast carcinoma. The loss of E-cadherin is the hallmark pathological feature of lobular entities. Effective clinical management of LCIS requires good communication among the radiologist, surgeon, pathologist, and medical oncologist and entails surgical excision, subsequent surveillance, and systemic and surgical strategies to reduce the risk of future invasive cancer.

Ductal carcinoma in situ (DCIS) is defined as abnormally proliferating malignant cells confined to the breast milk ducts by the basement membrane. DCIS is diagnosed most commonly as a mammographic abnormality but can occasionally present as a palpable breast mass. Overall survival after breast-conserving therapy is equivalent to that observed for mastectomy. Patients undergoing mastectomy for DCIS should have sentinel lymph node biopsy (SLNB). Immediate breast reconstruction should be considered for patients undergoing mastectomy. Endocrine therapy, such as tamoxifen, is offered for 5 years to women with estrogen receptor-positive DCIS.

The topic of *Biology and Genetics of Breast Cancer* covers a wide variety of molecular studies. Understanding the mechanisms of DNA alterations leading to carcinogenesis can provide crucial insights for resolving the development of malignant processes, such as growth, invasion, and metastasis. This chapter reviews hereditary and somatic genetic alterations, epigenetic misregulations, and miRNA signatures associated with breast cancer. This chapter also emphasizes the molecular profiles of breast cancer and critical signaling pathway alterations.

Human breast cancers depend on estrogen and/or progesterone for growth, and these effects are mediated through *estrogen receptors* (ERs) and *progesterone receptors* (PRs), respectively. The *human epidermal growth factor receptor 2* (HER2) gene encodes a member of the epidermal growth factor receptor family of receptor tyrosine kinases, and its amplification with resultant overexpression plays a major role in sustaining multiple pathways in cancer growth. ERs, PRs, and HER2 status are the most important molecular markers in the standard care of all primary and recurrent/metastatic breast cancer patients and play both predictive and prognostic roles. The responsiveness of a tumor to hormone therapy is an important parameter in breast cancer management in both the adjuvant and metastatic settings. Only breast cancers with HER2 amplification or overexpression respond to HER2-directed therapies. Tumor hormonal status is prognostic for patient outcome and potential sites of metastasis. Hormonal receptor-positive disease represents an indolent and slowly growing tumor with longer time to disease recurrence. HER2 is a poor prognostic factor in the absence of HER2-directed therapies. Assessment of the ER/PR/HER2 status is an essential factor in the evaluation of every newly diagnosed breast cancer, and the standardization of assay methods is crucial.

Invasive breast carcinomas comprise a heterogeneous group of lesions that differ in their molecular and pathologic features and clinical behavior. Some patients experience long periods of disease-free survival, whereas others experience the rapid development of recurrence and metastases that are fatal within a few years of the initial diagnosis. Numerous factors in individual tumors can be evaluated to stratify patients into subsets with varying risks of recurrence and response to different therapy modalities. The *Prognostic and Predictive Factors of Invasive Breast Cancer* chapter describes the current standard prognostic and predictive factors of invasive breast carcinoma and discusses emerging data on molecular markers that can be considered in clinical practice.

Adjuvant chemotherapy and endocrine treatment decrease the mortality of early breast cancer. However, not all early breast cancer patients benefit equally from adjuvant endocrine treatment and/or chemotherapy. High-risk patients are classically identified based on clinicopathological factors, such as age, tumor size, histopathological grade, nodal status, hormone and HER2 receptor positivity, and menopausal status. However, for patients with early breast cancer, the use of these standard clinicopathological factors might not thoroughly reveal the individual risk of disease recurrence and the benefits from adjuvant systemic chemotherapy.

Many patients with early breast cancer do not derive benefit from adjuvant systemic chemotherapy. Quantitative approaches for defining prognoses and individualizing treatments are required. In recent years, *molecular signatures of gene expression* have been correlated with breast cancer recurrence risk. Several tests for genomic expression have been developed and validated on specimens from previous phase III studies to improve the prognostication of early breast cancer patients and/or the prediction of adjuvant systemic treatment.

In clinical practice, although local recurrence or distant metastasis develops in some individuals who have been assessed as low risk despite treatment, some individuals with high-risk disease do not relapse despite systemic and local therapy. Therefore, oncologists must determine objective prognostic factors to identify early recurrence and metastasis in patients with breast cancer. Based on the presumption of residual disease, clinicians have recently attempted to identify micrometastases using *disseminated tumor cells* (DTCs) in the *bone marrow* and *circulating tumor cells* (CTCs) from the peripheral blood. DTCs are known as epithelial cells in the bone marrow, and they are also considered to be micrometastases in the bone marrow. DTCs are observed in approximately 30 % of early-stage breast cancer patients. Tumor cells that circulate in the peripheral blood of patients with cancer are referred to as CTCs. CTCs are cells that have entered the peripheral blood circulation after having detached from an existing primary tumor or its metastases. DTCs and CTCs can be used to predict progression-free and overall survival as well as response to treatment.

In the *Pathology of Breast Cancer* chapter, the classification is based on the recent WHO classification of breast carcinoma, and specific gross and microscopic features of in situ and invasive breast carcinomas are explained. Morphological groups, grading of DCIS, and the necessary information that should be included in a surgical pathology report are discussed. Recent information regarding columnar cell lesions and flat epithelial atypia of the breast are discussed along with their clinical importance. Common forms of invasive carcinomas, such as invasive ductal carcinoma and invasive lobular carcinoma, special types, and rarer forms, are also discussed along with their clinical consequences.

Intraoperative pathological examination may be performed for the rapid diagnosis of breast malignancy, the assessment of the surgical margins of breast-conserving excision specimens, and the pathological analysis of sentinel lymph nodes. The most commonly used methods for intraoperative pathological examination of breast lesions are cytological and frozen section examinations in addition to gross analysis. The pathological examinations of sentinel lymph nodes necessitate careful gross examination and serial and/or step sectioning. Immunostaining using antibodies against pancytokeratin can also be performed. Sentinel lymph node metastases should be clearly defined as macro- or micrometastases or isolated tumor cells. The differential diagnosis of subtypes of metastasis and mimickers is detailed.

Fibroepithelial tumors of the breast represent a heterogeneous group of biphasic tumors composed of a proliferation of epithelial and stromal components. Fibroadenomas and phyllodes tumors constitute the major entities. These tumors are among the most challenging diagnostic lesions for pathologists. It can be difficult to make a clear microscopic distinction between fibroadenomas and benign phyllodes tumors. No reliable morphological features or immunohistochemical markers that predict phyllodes tumors are available.

A variety of reactive and neoplastic lesions of the breast are characterized by spindle cell proliferation. The pathologist must be aware of the clinical, radiological, and morphological overlap between reactive and *neoplastic spindle cell lesions* of the breast. In addition, metaplastic (spindle cell) carcinoma is far more common than spindle cell sarcoma in the breast. Among the vascular lesions of the breast, angiosarcoma is more common and may appear very bland, simulating a hemangioma. Core biopsy samples must be evaluated very carefully to interpret spindle and vascular lesions. In general, excision is recommended due to morphological overlap, and clinicopathological correlation is necessary for a correct diagnosis.

In this edition, a new valuable tool, *liquid biopsy*, will be discussed. Liquid biopsy detects a group of "new-generation markers" that expand into the bloodstream from primary and metastatic tumor sites. These markers offer some advantages, such as real-time monitoring of

disease and detection of tumor heterogeneity. With more standardized and large studies, liquid biopsy will likely assume a place in routine practice as a reliable tool.

We would like to dedicate this book to postgraduate physicians in training to become breast cancer specialists. Some of the recommendations are controversial and the subject of ongoing trials. We hope this book stimulates today's young doctors to contribute to the research on which future books will be based.

Istanbul, Turkey — Adnan Aydiner, MD
Istanbul/Fatih, Turkey — Abdullah Igci, MD
Pittsburgh, PA, USA — Atilla Soran, MD

Contents

Contributors

Burak Yasin Aktas, MD Department of Medical Oncology, Hacettepe University Cancer Institute, Ankara, Turkey

Cagatay Arslan, MD Department of Internal Medicine and Medical Oncology, Bahcesehir University Faculty of Medicine, Istanbul, Turkey

Banu K. Arun, MD Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Adnan Aydiner, MD Department of Medical Oncology, Istanbul Medical Faculty, Oncology Institute, Istanbul University, Istanbul, Turkey

Soley Bayraktar, MD, MBA Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Department of Medical Oncology, Mercy Cancer Center, Ardmore, OK, USA

Zerrin Calay, MD Department of Pathology, Cerrahpasa School of Medicine, Istanbul University Cerrahpasa, Istanbul, Turkey

Ebru Cilbir, MD Division of Medical Oncology, Diskapi Yildirim Beyazit Treatment and Research Hospital, Ankara, Turkey

Emilia Josefa Borromeo Diego, MD Department of Surgery, Division of Surgical Oncology, Magee-Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

A. Lale Dogan, MD Department of Basic Oncology, Hacettepe Cancer Institute, Hacettepe University, Ankara, Turkey

Marie Ganott, MD Department of Radiology, University of Pittsburgh Medical Center, Magee-Womens Hospital, Pittsburgh, PA, USA

M. Emre Gedik, MSc Department of Basic Oncology, Hacettepe Cancer Institute, Hacettepe University, Ankara, Turkey

Brandy Griffith, DO Department of Radiology, The Ohio State University, Wexner Medical Center, Columbus, OH, USA

Abdullah Igci, MD Department of General Surgery, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Sennur Ilvan, MD Department of Pathology, Cerrahpasa School of Medicine, Istanbul University Cerrahpasa, Istanbul, Turkey

Saadettin Kilickap, MD, MSc Department of Medical Oncology, Hacettepe University Cancer Institute, Ankara, Turkey

Priscilla McAuliffe, MD, PhD, FACS Department of Surgery, Division of Surgical Oncology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Magee-Women's Surgical Associates, Pittsburgh, PA, USA

Women's Cancer Research Center of UPMC Hillman Cancer Center, Pittsburgh, PA, USA

Kandace P. McGuire, MD, FACS Department of Surgery, Division of Surgical Oncology, Magee-Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Semen Onder, MD Department of Pathology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Y. Yavuz Ozisik, MD Department of Medical Oncology, Hacettepe University Cancer Institute, Ankara, Turkey

Zeynep Gozde Ozkan, MD Department of Nuclear Medicine, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Scott M. Rudzinski, MD Windsong Radiology Group, Williamsville, NY, USA

Edward R. Sauter, MD, PhD Breast & Gynecologic Cancers Working Group, Division of Cancer Prevention, NIH/NCI, Rockville, MD, USA

Atilla Soran, MD Department of Surgical Oncology, Magee-Womens Hospital of the University of Pittsburgh, Pittsburgh, PA, USA

Zeki G. Surmeli, MD Department of Medical Oncology, Ankara Medical Park Hospital, Ankara, Turkey

Cuneyt Turkmen, MD Department of Nuclear Medicine, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Sitki Tuzlali, MD Tuzlali Private Pathology Laboratory, Istanbul, Turkey

Department of Pathology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Suayib Yalcin, MD Department of Medical Oncology, Hacettepe University Cancer Institute, Ankara, Turkey

Ekrem Yavuz, MD Department of Pathology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Breast Anatomy and Physiology

1

Kandace P. McGuire

Embryology/Development

During the 5th and/or 6th week of fetal development, the two bands of thickened ectoderm referred to as the ectodermal primitive milk streak develop between the groin and the axilla [1, 2]. This remains in the thorax to become the mammary ridge, whereas the remainder regresses in the human development [2].

The breast develops from an ingrowth of the ectoderm into the mesoderm to form a breast bud [1]. The glandular portion of the breast develops from the ectoderm. During the 12th week of development, 16–24 secondary buds will form off the primary bud [3].

Sequence of Development

The development of the breast, summarized in Table 1.1, follows a stepwise progression beginning at the 5th week post-conception and continuing until birth. In weeks 5 and 6, the primitive milk streak develops from a thickened band of ectoderm. Following development of the primitive milk streak in weeks 7 and 8, the mammary anlage will thicken, and the mesoderm will invaginate. Simultaneously, the breast buds begin to grow. This process continues until weeks 12 through 16, when mesenchymal cells begin to differentiate into the smooth muscle of the nipple and areola. Secondary breast buds will further develop and branch but remain solid structures during this time period.

At week 16, the tips of the buds become the secretory alveoli. The secondary mammary anlage differentiates into hair follicles and the sebaceous and sweat gland elements. Apocrine glands develop to form the Montgomery glands.

Table 1.1 Embryonic breast development by gestational week

Gestational week	Breast development
5–6	Primitive milk streak develops from the ectoderm
7–8	Thickening of the mammary anlage
	Invagination into the mesoderm
	Growth of breast buds
12–16	Mesenchymal cells differentiate into the smooth muscle of the nipple-areola
	Secondary breast buds develop and branch
16–20	Tips of breast buds become the secretory alveoli
	Secondary mammary anlage differentiates into hair follicles and sebaceous and sweat gland elements
	Apocrine glands develop into Montgomery glands
20–32	Breast buds canalize and become lactiferous/mammary ducts
32–40	Parenchymal differentiation; lobules/alveoli develop
	Proliferation of mesenchyme forms the nipple-areola complex
	Pigmentation of the nipple-areola complex

Beginning at week 20 of development and continuing until week 32, the breast buds will canalize to form lactiferous/mammary ducts. These ducts open into a shallow mammary pit, which will become the nipple-areola complex. In the final weeks before birth, weeks 32 through 40, parenchymal differentiation occurs. The lobules and alveoli complete the development. Finally, the nipple-areola complex develops via proliferation of the mesenchyme and becomes pigmented (Fig. 1.1) [2, 3].

Developmental Anomalies

The development of a normal breast requires perfect adherence to the sequence of development described above. Should development stray from this pattern, anomalies may occur. The three most common developmental anomalies of the female breast are (1) supernumerary breasts or nipples (polymastia/polythelia), (2) underdevelopment or lack of development of the breast, and (3) nipple inversion.

K. P. McGuire (✉)
Department of Surgery, Division of Surgical Oncology, Magee-Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
e-mail: mcguirek2@upmc.edu

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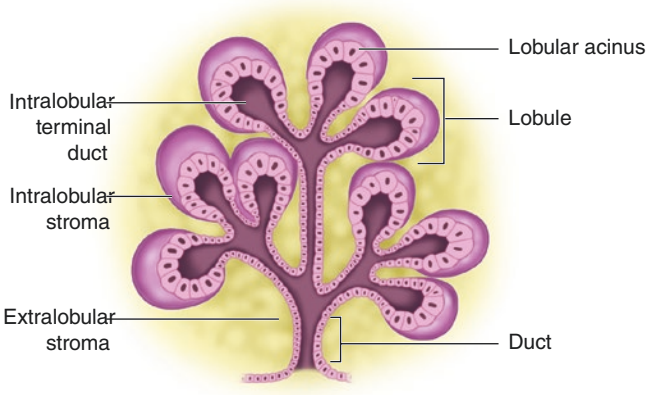

Fig. 1.1 Fully developed breast lobular unit. (From Townsend et al. [5]. Reproduced with permission from Elsevier)

Supernumerary breasts/nipples (polymastia/polythelia) can occur in both genders and are referred to as accessory if they occur along the milk line (former primitive milk streak). They are referred to as ectopic if they occur elsewhere [3].

Accessory nipples (polythelia) occur in 2.5% of the population and are much more common than accessory breasts (polymastia). Polythelia most commonly occurs in the thorax, while polymastia most commonly occurs in the axilla [2, 3].

Underdevelopment or hypoplasia of the breast can occur unilaterally or bilaterally and is usually clinically insignificant. However, severe unilateral hypoplasia of the breast can occur and is usually associated with hypoplasia of the pectoral muscle (lacking the lower third of the muscle) and deformity of the rib cage. This defect is termed *Poland's syndrome* because it was first recognized by Dr. Alfred Poland in 1841. Associated abnormalities of the hand (syndactyly and/or hypoplasia of the phalanges) may be present [2].

Amastia or lack of breast development is exceedingly rare. Athelia, a lack of development of the nipple-areola complex, can also occur, as can amazia, a lack of breast development in the presence of a nipple-areola complex [2, 3].

Failure of the mesenchyme of the nipple-areola to proliferate and elevate the mammary pit above the skin results in an inverted nipple. This failure can occur unilaterally or bilaterally and occurs in 4% of infants, both male and female [3].

Anatomy

Breast

The adult female breast lies between the second and sixth/seventh ribs. The base of the breast spans from the sternal border medially to the midaxillary line laterally and is encompassed by the superficial and deep fascia of the chest wall. Two-thirds of the breast lies anterior to the pectoralis major; the remainder lies anterior to the serratus anterior. A prolongation of the upper outer quadrant of the breast, referred to as the tail of Spence, extends into the axilla [3, 4].

Components of the Breast

Skin – the skin is the most superficial layer of the breast. The dermis merges with the superficial fascia [3].

Superficial fascia – this layer lies just beneath the skin. It is continuous with the superficial abdominal and cervical fascia. Along with the deep fascia, it envelopes the breast parenchyma [3].

Breast parenchyma – the parenchyma is composed of three principal tissue types: glandular epithelium, fibrous stroma, and supporting structures and fat.

Glandular epithelium comprises approximately 10–15% of the adult female breast. It is composed of 15–20 lobes, which are subsequently composed of several lobules. These lobules are referred to as terminal ductules or acini, the milk-producing glands. The major milk ducts are lined with two layers of cuboidal epithelium, while the minor ducts have a single layer. The ductal epithelium is entirely surrounded by myoepithelial cells that serve to propel milk forward through the ducts. These cells are surrounded by a continuous basement membrane. Invasion through this membrane distinguishes invasive cancer from in situ carcinoma. The ducts widen under the nipple-areola complex to form the lactiferous sinuses and then exit through 10–15 orifices in the nipple.

The fibrous stroma and supporting structures are most commonly referred to as the suspensory ligaments of Cooper. These ligaments are fibrous bands of connective tissue that travel through the breast and insert into the dermis. Tumor involvement and contraction of these bands are responsible for the puckering noted at the site of a palpable breast lump.

The remainder of the breast is composed of adipose tissue (fat). The proportion of fat to glandular tissue increases with age and is maximal in the postmenopausal breast (Fig. 1.2) [1, 4, 5].

Nipple-areola complex – As described above, each lobe of the breast leads to a ductal structure that then widens to form a large lactiferous duct (2–4 mm) that continues to form a sinus. The sinus is lined with stratified squamous epithelium. This sinus then narrows as it passes into the ampulla of the nipple (0.4–0.7 mm).

The areola comprises a combination of sebaceous, sweat, and accessory glands that form the Montgomery tubercles. Smooth muscle fibers are contained in the areola and extend into the nipple, and these fibers are responsible for nipple erection. Erection is stimulated by the sensory nerve endings and Meissner's corpuscles, which are located within the dermis of the nipple [1, 3].

Deep fascia – This layer is deep to the breast parenchyma and envelops the pectoralis major. It is continuous with the

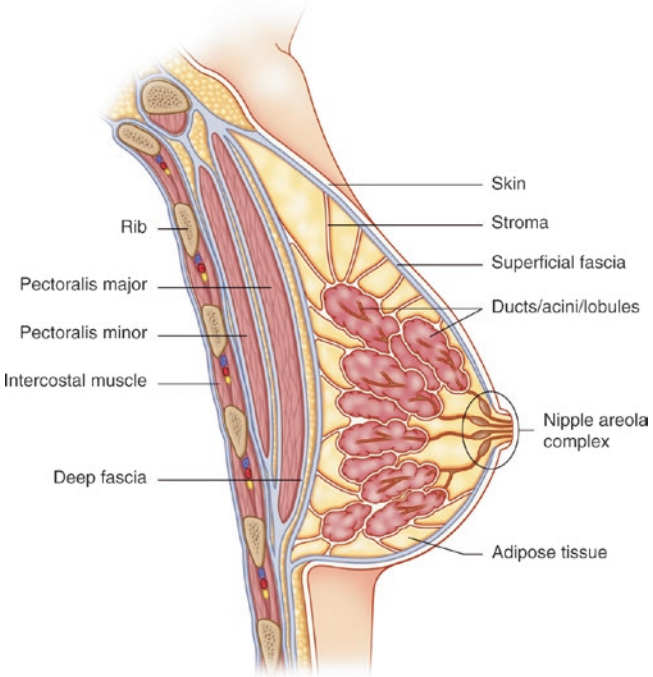

Fig. 1.2 Components of the breast

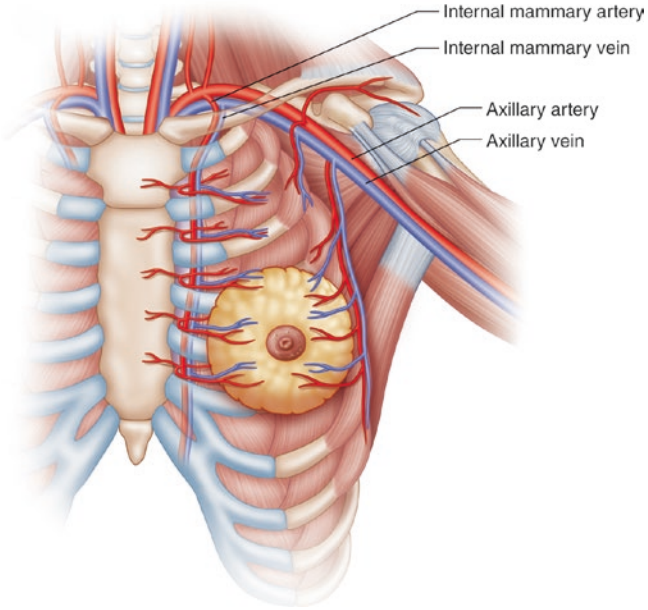

Fig. 1.3 Arterial supply and venous drainage of the breast

deep abdominal fascia caudally and spans from the sternum to the axilla laterally and to the clavicle cranially [3].

Neurovascular Structures

Arterial

The arterial blood supply to the breast comes primarily from three sources: (1) anterior perforators of the internal mammary artery (responsible for approximately 60% of the breast, mostly medial and central); (2) branches from the axillary artery, such as the highest and lateral thoracic, and the thoracoacromial artery (responsible for approximately 30% of the breast, mostly the upper outer quadrant); and (3) lateral branches of the intercostal arteries (Fig. 1.3) [1–3].

Venous

Venous drainage typically mimics the arterial supply. Thus, the primary venous drainage consists of: (1) internal mammary perforating branches, (2) tributaries of the axillary vein, and (3) branches of the intercostal veins (Fig. 1.3) [1].

Nervous

The sensory nerve supply to the breast is principally derived from the lateral cutaneous branches of the third through sixth intercostal nerves. Cranially, some sensory innervation is supplied by cutaneous branches of the cervical plexus. The nipple-areola complex is innervated by the fourth intercostal nerve [1, 3].

Lymphatic Structures

The superficial lymphatic plexus that drains the skin of the breast and the nipple-areola complex is often referred to as Sappey's plexus. Lymph flows from the skin to the subareolar plexus and then into the interconnected deep lymphatic plexus that drains the breast parenchyma via the lymphatic vessels associated with the lactiferous ducts. Approximately 97% of the lymphatics from the breast drain to the axilla; the remaining 3% drains to the internal mammary lymph nodes.

The internal mammary chain is located between the first and sixth intercostal spaces along the border of the sternum. The nodes are medial to the internal mammary vessels in the first two intercostal spaces and then become lateral to the vessels in spaces 3–6 (Fig. 1.4) [2, 4, 5]. The anatomy of the axilla and axillary lymph nodes will be discussed in the following section.

Axilla

The axilla is an important component of breast anatomy. Directly contiguous with the breast, the lymph nodes within the axilla provide a rich drainage basin for the breast. The borders of the axilla, which define the extent of axillary dissection, are as follows:

Lateral – the axillary fat pad and the bicipital groove of the humerus.

Medial – the serratus anterior and the second to sixth ribs.

Superior – the apex of the axilla bordered by the clavicle, the scapula, and the first rib.

The apex of the axilla can also be defined by the costoclavicular ligament, which is also called Halsted's ligament. The axillary vein is the superior extent of the modified

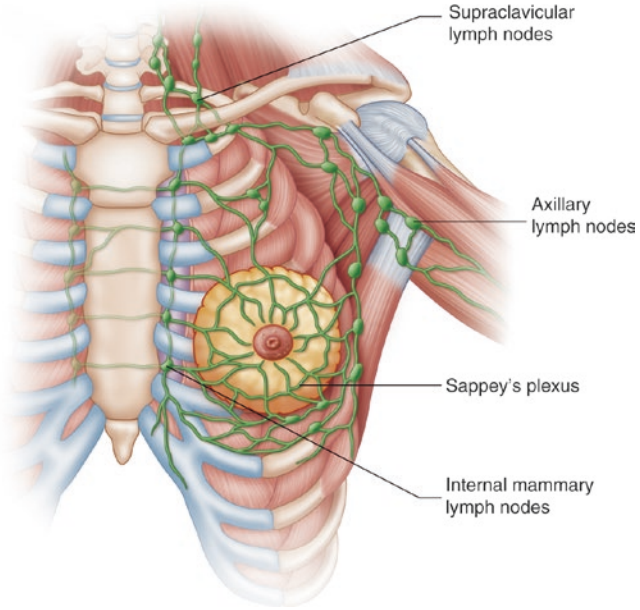

Fig. 1.4 Lymphatic drainage of the breast

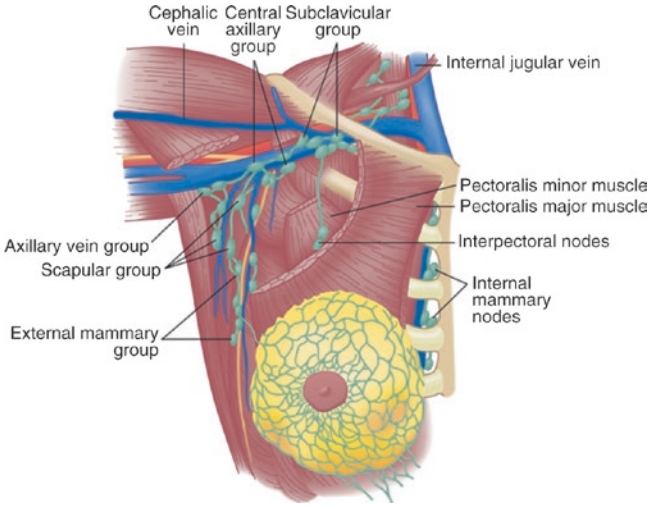

Fig. 1.5 Axillary lymph node groups. (From Townsend et al. [5]. Reproduced with permission from Elsevier)

radical axillary dissection. Medial to Halsted's ligament, the axillary vein becomes the subclavian vein.

Anterior – the pectoralis (major and minor) and subclavius muscles and the clavipectoral fascia. The clavipectoral fascia envelops the subclavius and pectoralis minor and is often referred to as the costocoracoid membrane. The lateral band of the clavipectoral fascia between the first rib and the coracoid process is called the costocoracoid ligament.

Posterior – the scapula, the subscapularis, the latissimus dorsi, and the teres major. The axillary fascia lying across the base of the axillary pyramid will envelop the pectoralis major and then the latissimus dorsi. It forms the dome of the axilla. Occasionally, there can be a muscular connection between this fascia and the clavipectoral fascia, which is referred to as the suspensory ligament of the axilla [3, 5, 6].

Axillary Lymph Nodes

There are several groups of lymph nodes within the axilla. These nodes can be grouped as the apical or subclavicular nodes, which are located medial to the pectoralis minor muscle, or the axillary vein lymph nodes, which run along the axillary vein between the pectoralis minor and the humerus. The interpectoral or Rotter's nodes lie between the pectoralis major and minor muscles. The central axillary nodes are found beneath the border of the pectoralis major muscle and below the pectoralis minor. The external mammary nodes lie over the axillary tail of Spence. Intramammary lymph nodes and paramammary lymph nodes can also be found in the fat layer over the upper, outer quadrant of the breast (Fig. 1.5).

For surgical dissection purposes, there are three lymph node levels of the axilla, which are all defined by their relationship to the pectoralis minor muscle. Level I nodes are found lateral to the edge of the pectoralis minor. This level includes external mammary, subscapular, and lateral axillary lymph nodes. Level II nodes are located posterior to the pectoral minor. This level includes the central axillary lymph nodes. Level III nodes are medial and superior to the pectoralis minor. This level includes the subclavicular or apical lymph nodes [2, 6].

Structures Within the Axilla

The *axillary lymph nodes* are divided into several different groups and levels as described above and are variable in number. The maximum number identified and removed during a radical mastectomy is approximately 50, including the level I, II, and III axillary lymph nodes.

The *axillary vein* defines the superior border of the axilla during axillary dissection. It lies posterior and caudal to the brachial plexus. The axillary vein is often paired or branches during its course through the axilla.

The *thoracodorsal nerve/neurovascular bundle* innervates the latissimus dorsi and should be preserved during axillary dissection. It runs posterior to the axillary vein and medial to the subscapular vein.

The *long thoracic nerve/neurovascular bundle* innervates the serratus anterior and should be preserved during axillary dissection. If sacrificed, it will lead to "winging" of the scapula. It runs longitudinally over the serratus anterior and can be found during dissection in the axillary fat pad approximately 7 or 8 cm deep to the lateral edge of the pectoralis minor. As the long thoracic nerve/neurovascular bundle continues caudally, it will become more anterior.

The *intercostobrachial nerves* provide sensory innervation to the medial portion of the upper arm. These nerves run parallel to the axillary vein between the chest wall and the

arm. One or more of these nerves run through the axillary fat pad and may be difficult to dissect away from lymph nodes. If sacrificed, either hypo- or hyperesthesia of the posterior axillary web and the medial/upper arm can result [4, 6].

Physiology

Physiological Breast Development

Breast development is stimulated by a variety of hormones that are upregulated during the beginning stages of puberty. Estrogen and progesterone are the main hormones responsible for breast growth and development during this time. Estrogen stimulates ductal development; progesterone stimulates lobular development and epithelial differentiation.

At the onset of puberty, the hypothalamic-pituitary axis becomes less sensitive to the negative feedback of estrogen. This desensitization leads to an increase in gonadotropin-releasing hormone (GnRH) from the hypothalamus. This increase in GnRH stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary, which in turn leads to an increase in estrogen and progesterone release, thus stimulating breast development, among other developmental changes.

During pregnancy and lactation, prolactin is primarily responsible for upregulating hormone receptors and stimulating epithelial development and lactogenesis in the breast [1].

Abnormal Breast Development/Gynecomastia

Gynecomastia refers to male breast hypertrophy and can be caused by numerous factors.

1. Physiological gynecomastia: This can occur in the neonatal, pubertal, and senescent periods. Neonatal hypertrophy occurs in response to maternal estrogen. Pubertal hypertrophy occurs due to a relative excess of estradiol to testosterone. Senescent hypertrophy occurs in response to falling testosterone levels associated with aging. The enlargement is usually unilateral in puberty but bilateral in senescence. This usually does not require surgery unless the enlargement is associated with a mass by physical exam or mammogram, fails to regress, or is cosmetically unacceptable.
2. Pathologic gynecomastia: There are a number of pathological causes of gynecomastia, including true hermaphroditism, testicular tumors, adrenal cortical neoplasms, lung or hepatocellular carcinoma, endocrine disorders, cirrhosis, and nutritional deficiencies (estrogen excess states). Hypogonadism, as observed in congenital syndromes such as Klinefelter (XXY) syndrome or ACTH deficiency, can also cause gynecomastia. Secondary testicular failure from trauma, radiation, or untreated cryptorchidism can also cause hypertrophy (androgen deficiency states). Renal failure and other systemic diseases can lead to gynecomastia, as can drugs that provide exogenous estrogen or stimulate estrogen synthesis (e.g., digoxin, estrogens, anabolic steroids, marijuana, and HCG) or that inhibit the activity or production of testosterone (e.g., cimetidine, ketoconazole, phenytoin, spironolactone, antineoplastic drugs, and diazepam). Some drugs, such as reserpine, theophylline, verapamil, tricyclic antidepressants, and furosemide, lead to gynecomastia through idiopathic mechanisms [3, 5].

Physiology of Puberty

As described above, pubertal development of the breast (thelarche) begins with the stimulation of estrogen and progesterone production via the hypothalamic-pituitary axis. At this time, the breast is composed primarily of dense fibrous stroma and scattered ducts lined with epithelium. Estrogen stimulates growth of the ductal epithelium. Buds form off the terminal ductules and will eventually become breast lobules. Periductal connective tissue grows and becomes more elastic. Some studies suggest that while estrogen promotes growth of ducts, estrogen and progesterone synergistically promote full ductular-lobular-alveolar development in the breast.

There are three distinct types of breast lobules in the human breast, and the proportion of each is related to a woman's parity and hormonal status. During puberty, the breast develops mostly type I (virginal) lobules, which consist of a cluster of 11 alveolar buds around a terminal duct. These lobules have a much higher rate of proliferation than type 2 or 3 lobules.

Physiology of the Menstrual Cycle

The postpubertal breast contains fat, stroma, lactiferous ducts, and lobular units. The menstrual cycle affects not only the uterus and uterine lining but also the breast. During the follicular phase (days 4–14), levels of estrogen increase, stimulating epithelial proliferation/sprouting and an increased mitotic rate. During the luteal phase (days 15–28), progesterone increases, while estrogen abates. At this time, mammary ducts dilate, and alveolar epithelial cells differentiate into secretory cells. Often, lipid droplets accumulate, and some intraluminal secretion occurs. During this time, estrogen also exerts a histamine-like effect on the breast parenchyma, resulting in increased blood flow and breast edema just prior to the onset of menses.

During this time, type I lobules continue to predominate. The increased rate of cellular proliferation in these lobules may partly explain the differences in breast cancer rates based on parity and age at first live birth.

Physiology of Pregnancy

During pregnancy, there is a decrease in fibrous stroma along with an increase in new acini/lobules. In the first trimester, ducts sprout and branch, and lobules develop as estrogen increases. Breast enlargement is significant, with dilatation of superficial veins and breast edema. The nipple-areola complex darkens and begins to enlarge. Type 3 lobules (with an average of 80 acini) begin to develop during this time and are referred to as alveoli.

In the second trimester, levels of progesterone increase, as does lobular formation. The alveoli begin to form colostrum, which is composed of desquamated eosinophilic cells, plasma cells, leukocytes, and epithelial cells.

In the third trimester, the alveoli continue to produce colostrum. At this time, epithelial differentiation is completed, resulting in the development of secretory cells that produce and secrete milk proteins. Oxytocin increases over the last trimester, resulting in the proliferation of myoepithelial cells surrounding the ductal structures, which propel the milk forward toward the nipple-areola complex.

Physiology of Lactation

After birth, there is a sudden decrease in the levels of estrogen, progesterone, and placental lactogen, coupled with an increase in prolactin, which induces the production and secretion of milk. Hormonal levels reach their lowest levels at about the fifth postpartum day, with a concomitant decrease in prolactin-inhibiting factor (PIF). This decrease results in the secretion of prolactin. Along with additional growth factors, prolactin secretion results in the accumulation of colostrum and, subsequently, milk in the alveoli and ducts. Stimulation of the nipple-areola complex stimulates the release of oxytocin and the contraction of the myoepithelial cells surrounding the ductal system. Upon cessation of breast-feeding (weaning), levels of prolactin and oxytocin fall. Retained secretions are removed via phagocytosis. Atrophy of the glandular, ductal, and stromal elements is observed. The secretory cells responsible for milk production undergo apoptosis. However, the type 3 lobules persist.

Physiology of Menopause

After menopause, the breast parenchyma regresses and is replaced by adipose tissue. This replacement occurs by involution of the ductal, glandular, and stromal elements/connective tissue of the breast. The ductal system remains but undergoes atresia, with collapse of the lobular units. Type 1 lobules again predominate, as in the nulliparous breast. The number of lymphatic channels through the breast parenchyma also decreases [2, 4, 5].

Surgical/Oncological Considerations

Tumor Location Within the Breast

The adult breast develops in a conical form, with epithelial/ductal tissue in each quadrant of the breast. The axillary tail of Spence, as discussed previously, is an extension of the upper outer quadrant of the breast over the axilla. Because of this extension, the upper outer quadrant contains significantly more epithelial tissue than the other quadrants. Thus, this quadrant is the most frequent site of breast neoplasms and harbors more than half of both benign and malignant tumors [3, 4, 7].

The location of the tumor within the breast can also affect the ability to perform breast conservation (segmental mastectomy). In general, segmental mastectomy can be performed with good cosmetic outcome when the tumor volume is less than 20% of the volume of the breast [8–10]. However, this percentage can vary with tumor location. Tumors in the upper outer quadrant are much easier to resect with good cosmetic outcome because there is a great amount of surrounding tissue in the region. Tumors that lie in the lower quadrants, particularly the lower inner quadrant, have little surrounding parenchyma, and excisions in these regions can lead to significant retraction and poor cosmetic outcome after surgery and radiation are performed. Partial breast reconstruction techniques, such as small latissimus dorsi flaps and local advancement flaps, can replace volume, particularly in the outer quadrants. However, these techniques require more extensive surgery, and the patient may be better served by mastectomy and whole breast reconstruction in this situation [11–13]. Depending on tumor location, volume loss can also be addressed by oncoplastic surgical techniques ranging from simple local advancement flaps to concurrent reduction mammoplasty [14–16].

Borders of Mastectomy

There are three different types of mastectomy, all with different extents of dissection:

1. Simple or total mastectomy – several skin incisions can be made, including peri-areolar (skin-sparing) or elliptical. Dissection is performed along the superficial fascial plane superiorly to the clavicle; medially to the sternal

edge; inferiorly to the inframammary fold, just cranial to the insertion of the rectus sheath; and laterally to the edge of pectoralis major muscle. The deep border of dissection is the deep fascial plane, just superficial to the pectoralis major muscle. This method of mastectomy removes nearly 100% of the breast epithelial/stromal tissue while preserving the axillary fat pad and axillary lymph nodes.

This approach is used most often in modern practice and is often combined with immediate reconstruction. When plastic surgery is involved, it is imperative that a multidisciplinary approach to surgery be used and that both oncological and plastic surgeons are involved in incision planning. This is particularly true in the case of nipple-sparing mastectomy, in which several incisions can be used [17–23]. An important anatomic consideration is the blood supply to the nipple and areola, which can vary greatly from patient to patient. Nipples that derive most of their blood supply from the underlying parenchyma are likely to suffer partial or complete necrosis after nipple-sparing mastectomy, whereas the viability of those that derive blood supply from the surrounding skin will be largely unaffected. Blood flow can be assessed either preoperatively or intraoperatively with imaging systems that detect fluorescent dye (usually indocyanine green) injected intravenously. The resulting perfusion patterns can help guide incision planning and also identify candidates for nipple-sparing surgery [24] (Fig. 1.6).

2. Modified radical mastectomy – this operation can be performed through the same incisions as a total or simple mastectomy. The elliptical incision can be extended superolaterally toward the axilla to facilitate axillary dissection. The superior, medial, inferior, and deep borders of the dissection are the same as in a total mastectomy. However, the modified radical mastectomy involves the removal of levels I and II axillary lymph nodes; thus, the

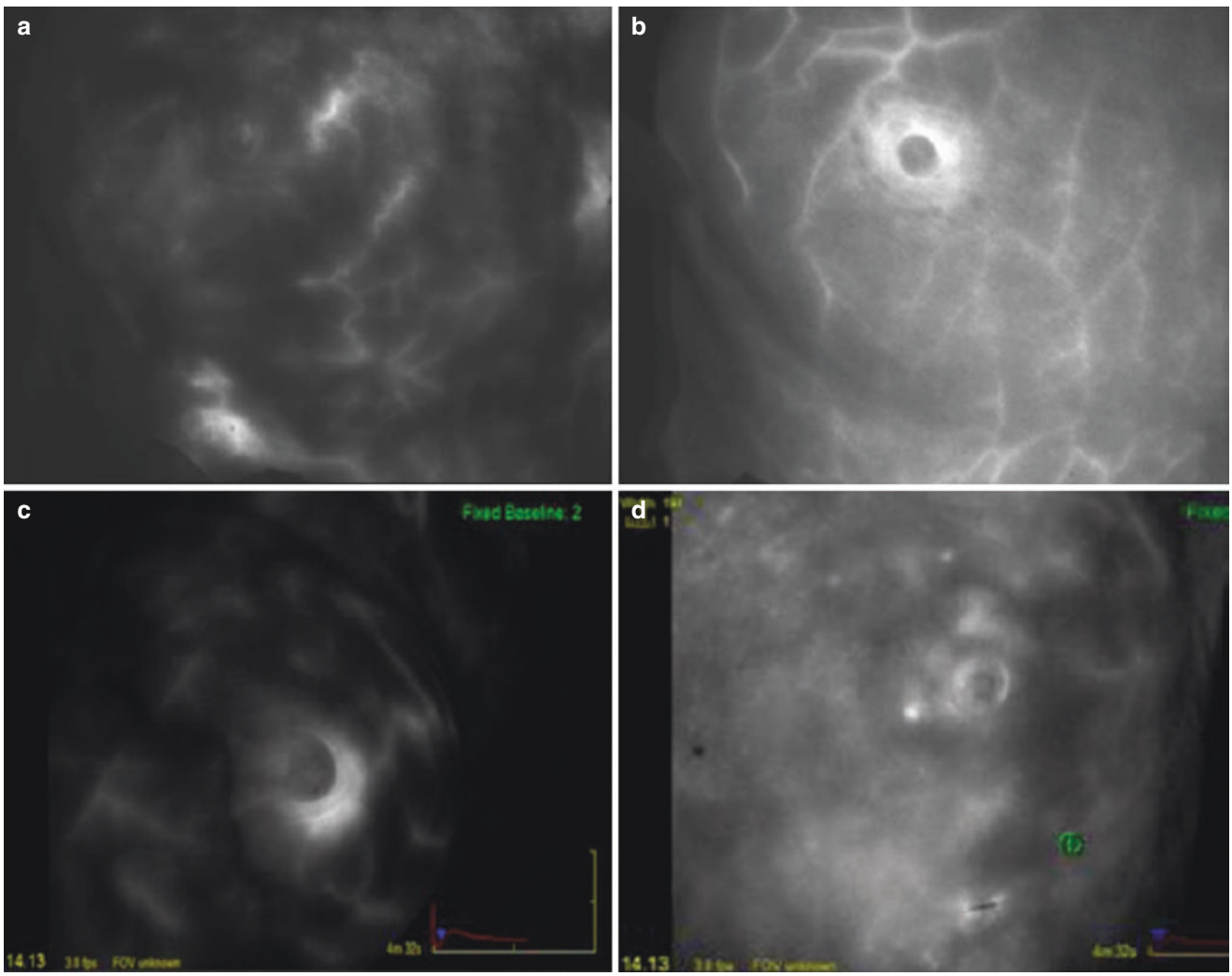

Fig. 1.6 Nipple perfusion patterns

lateral border of dissection is the latissimus dorsi extending superiorly to the axillary vein.

3. Halsted radical mastectomy – this operation is rarely performed and very rarely described in current surgical texts and atlases. The superior, medial, inferior, and lateral borders are the same as in a modified radical mastectomy. However, the deep dissection includes the pectoralis major and minor muscles. The axillary dissection includes levels I, II, and III of the axillary lymph nodes and is thus extended superior and medial to the axillary vein. This operation is performed only in the presence of locally advanced cancers that involve one or both pectoralis muscles [6].

Sentinel Node Biopsy

Sentinel lymph node biopsy was originally described as a method for detecting the lymphatic drainage of melanoma. It has been modified for use in breast cancer using the following method.

1. Isosulfan or methylene blue dye and/or technetium-99 are injected preoperatively into the superficial lymphatic plexus, either into the subareolar plexus or around the tumor.
2. This injection *can* be followed by lymphoscintigraphy to identify the area into which the radioactive dye has drained. Lymphoscintigraphy requires allowing the technetium 1 h or more to travel through the breast lymphatics into the axillary and/or internal mammary lymph nodes. As noted above, 97% of the breast drains to the axillary region; thus, this step is not necessary. It can be helpful in inner quadrant tumors, which more commonly drain to the internal mammary chain, and in patients with previous breast surgery, which might interfere with the normal lymphatic drainage of the breast.
3. Once in the operating room, the radioactive-sensitive probe can be used to localize the area in the axilla with the highest concentration of technetium colloid.
4. An incision is made in this area through the skin, the subcutaneous tissue, and the clavipectoral fascia. Once the axillary fat pad is identified, the probe can be used to localize the lymph node(s) with the highest concentration of radioactive dye. Those lymph node(s) that are both "hot" (radioactive) and "blue" (have taken up the blue dye) should be removed and sent for pathologic analysis (frozen, touch prep, or permanent). If these "sentinel" lymph nodes show evidence of malignancy, then a full axillary dissection as described for modified radical mastectomy is performed at that time or during a separate operation.

This method is based upon the anatomy of the breast lymphatic system. As described previously in this chapter, the lymph flows from the skin to the subareolar plexus and then into the interconnected deep lymphatic plexus that drains the breast parenchyma via the lymphatic vessels associated with the lactiferous ducts. Therefore, any lymphatic drainage from the breast must travel through both the superficial and deep lymphatic plexuses before leaving the breast, and an injection into the superficial lymphatic plexus will identify the main route of drainage for the breast. This drainage is standard and reproducible. Once the channels reach the axilla, they drain first to the "sentinel" lymph node(s) in either levels I or II of the axilla before draining to the remainder of the axilla. If no cancer is found in the sentinel node, there is a >95% likelihood that no other cancer exists in the axilla [2, 4, 5, 25].

Sentinel lymph node biopsy has several advantages over axillary dissection in appropriately selected patients, and in fact, axillary dissection has become increasingly rare. Several landmark trials have established the efficacy of sentinel lymph node biopsy in the setting of breast cancer. The great advantage of sentinel lymph node biopsy is the reduction in risk of postoperative arm (and to some extent breast) lymphedema. The rich lymphatic network experiences less disruption. In early studies, most notably NSABP B-32 and ACOSOG Z0010, the lymphedema rates after sentinel lymph node biopsy varied from 8% to 12%, whereas axillary dissection resulted in lymphedema rates of 14–42% [26, 27]. However, for patients who require axillary dissection, techniques can identify the lymphatics that primarily drain the arm (outside of the level III lymph nodes, which are excluded in modern axillary dissection if they are not clinically involved due to the low incidence of involved nodes in this region and the high incidence of arm lymphedema after radical mastectomy) [28, 29]. The best-described technique is axillary reverse lymphatic mapping (ARM). This technique involves injecting a small amount of blue dye in the subcutaneous tissue of the volar surface of the upper arm prior to lymphatic surgery. The axillary lymph nodes that drain the arm can be identified and frequently preserved. This can result in a lymphedema rate of 2.4% after ALND in which the identified arm lymphatics are preserved, much lower than previous reports [30–33].

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Benign Diseases of the Breast

2

Edward R. Sauter

Physical Examination of the Breast

Physical examination of the breast can be performed within the context of a clinical examination or by the individual. The former setting is often termed a clinical breast examination (CBE) and the latter a breast self-examination (BSE). Before the era of routine standardized breast imaging, physical examination was generally the primary method of diagnosing breast cancers. Breast imaging lacked standardization until the creation of the BI-RADS system in 1993 [1].

CBE is currently used as a screening test that can identify areas with breast cancer. While CBE is less sensitive than mammography, it is nonetheless the primary mode of detection for the 15% of breast cancers that are missed by mammography [2].

BSE was promoted to allow women to identify their own cancers at an early stage. Although BSE was anticipated to work for many reasons, randomized trials of BSE with increasingly sophisticated procedures for retraining and sustaining BSE practice have demonstrated 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 [2].

Examination of the Breast

The purpose of a CBE is to detect changes in the consistency of the breast tissue. Other tests are needed if an area of asymmetry is found [2]. CBE includes visual inspection of the breast first with the patient sitting and then supine. With the patient sitting, the position and contour of the breast are observed with changes in posture and arm position. Unexpected changes in breast contour should be further evaluated by breast palpation. Supine is the optimal patient position for breast palpation. Alterations in breast consistency should be further evaluated with imaging studies and/or biopsy. For women with pathological nipple discharge (PND) (discussed in more detail below), CBE can be used to localize the source of the discharge. In the absence of PND, CBE seeks 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.

Although a patient's personal and family history influences the probability that cancer will be found, history is not relevant to the CBE or when interpreting the CBE results because the majority of breast cancers occur in women without known risk factors. Models composed of silicone or other materials are widely used to teach both CBE and BSE skills, although their benefits in early cancer detection are largely unproven.

Signs and Symptoms of Breast Disease

Many of the features that students are taught to identify on CBE, such as skin dimpling, peau d'orange, hardness, and fixed mass, were first described before mammography and applied to advanced breast cancers, but these features are generally not applicable to the earlier stage cancers that are typically encountered in current practice [2]. Other findings can occur with early stage disease, such as PND, breast asymmetry, and masses. Breast inflammation is most often benign but can be an indicator of cancer. The most important consideration in any breast abnormality is to exclude carcinoma. Inflammatory carcinoma can present as erythema with or without pain in the breast. Biopsy of the skin and any associated underlying mass should be performed. The breast is also often

E. R. Sauter (✉)
Breast & Gynecologic Cancers Working Group, Division of Cancer Prevention, NIH/NCI, Rockville, MD, USA
e-mail: edward.sauter@nih.gov

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inflamed in response to radiation therapy to treat breast cancer. In this chapter, we will focus on breast symptoms that are often, although not always, related to benign disease, including mastalgia, nipple discharge, breast inflammation, and breast masses.

Pathological Nipple Discharge

CBE does not seek to determine if nipple discharge can be elicited. Nipple discharge is of interest only if it is spontaneous. Fluid can be elicited by many women from their breast with massage. This fluid is not spontaneous nipple discharge (SND), which is of concern because of its potential implications related to disease, but rather intraductal fluid that is present in the breasts of all women and is often termed nipple aspirate fluid (NAF) because a modified breast pump is sometimes used to collect it [3]. However, if a woman reports noticing fluid on her bra that she did not elicit, particularly from one but not the other breast, this symptom should be further investigated, and pressure applied to the breast sequentially in a circular pattern can help localize the source of the spontaneous discharge.

SND is common, accounting for nearly 7% of all breast symptoms. SND is most often physiological, particularly if bilateral and from multiple breast ducts. Carcinoma prevalence in women with SND varies primarily according to the criteria used to assess whether the discharge is physiological or pathological. The criteria usually include whether the discharge is from one or both breasts and from one or multiple nipple ducts and the characteristics of the discharge (bloody vs. nonbloody; clear or serous vs. white, yellow, or green). Additional criteria include the presence of an associated mass or an imaging abnormality. Because the criteria distinguishing pathological from physiological discharge vary from publication to publication, the published incidence of carcinoma among women with SND also varies [4].

PND is more likely when the discharge is unilateral and from one milk duct. The most common diagnosis in women with PND is papilloma. Among breast lesions, papilloma is unique in its frequent presentation as PND. NAF originates in the breast ducts similarly to SND but is less voluminous and can be obtained from essentially all nonlactating women after a learning period [4]. In contrast to NAF cytology, in which false positives are rare, PND cytology in patients with papillomas is occasionally falsely interpreted as containing malignant cells [5] because exfoliated cells from papillomas can appear quite abnormal when not viewed in the context of histological architecture. Although older studies reported that bloody nipple discharge was more commonly associated with cancer than nonbloody discharge, more recent studies challenge this belief [4].

Breast Inflammation

The breast is most fundamentally an appendage of the skin. Many systemic inflammatory conditions are present on the skin of the breast, including sarcoidosis, vasculitides, diabetes, and infections [6]. Sarcoidosis of the breast occurs in less than 1% of individuals with sarcoid and usually presents as a breast mass, less often with skin dimpling and peau d'orange changes [7]. Primary sarcoidosis of the breast without systemic manifestations can occur but is uncommon. Giant cell (temporal) arteritis can manifest in the breast, typically presenting as painful breast masses. Systemic symptoms related to giant cell arteritis are usually present. Other vasculitides such as polyarteritis nodosa and Wegener's granulomatosis involving the breast have also been reported [7]. Diabetic mastopathy is most often observed in premenopausal women with type I diabetes and classically presents as a hard painless mass in one or both breasts [8]. Diagnosis requires biopsy, and treatment is mass excision. IgG4-related autoimmune syndrome can present in the breast, commonly as a tender breast mass [6].

Mastalgia (breast pain) accounts for two thirds of all physician visits for breast symptoms but is not a risk factor for breast cancer [9]. The pain may be cyclical. Cyclic pain is most often related to the menstrual cycle, is bilateral and diffuse, and occurs during the luteal phase as rising progesterone levels increase the water content in the breast [9]. Noncyclic pain may be in one or both breasts and has been associated with a variety of medications, including oral contraceptives, female hormones, psychotropics, and cardiovascular medications. Larger breasted women may develop ligamentous pain if an adequate support bra is not worn. If the clinical workup, including history of diffuse pain, CBE, and mammography (in women over 40), suggests a benign etiology, treatment is generally supportive, most often starting with confirming that the woman is wearing a supportive, well fitted brassiere. Acetaminophen or a nonsteroidal anti-inflammatory medication is often effective [9].

Fat necrosis generally presents as a painful mass in the breast. There may be multiple masses, with or without skin retraction. Fibrosis and calcification are common. Women with large breasts are at highest risk [7], and necrosis usually occurs following trauma. The trauma can include cyst aspiration, breast massage, mammography, radiation therapy, biopsy, implant removal, and reduction mammoplasty. A biopsy is often needed to exclude malignancy.

Mastitis occurs most commonly in women of childbearing age. It can occur during pregnancy and lactation and in women who are not pregnant or lactating [6], although in the latter group, most women report having given birth within 5 years of mastitis onset. The normal bacterial flora of breast tissue resembles that of the skin,

and coagulase negative *Staphlococcus* and *Propionibacterium* species are the predominant organisms [6]. Mastitis is associated with pain, breast swelling, mass(es), and inflammation that resemble an abscess. Ipsilateral axillary lymph nodes are enlarged in approximately one sixth of patients [7]. Mastitis frequently leads to surgical interventions to biopsy the lesion(s) to exclude malignancy and drainage procedures to treat the inflammatory process. The interventions can lead to breast scarring and/or shrinkage. Resolution occurs in only approximately half of benign conditions. The various forms of benign inflammatory processes (periductal mastitis, Zuska's disease, comedomastitis, duct ectasia, mastitis obliterans, lactiferous fistula, and idiopathic granulomatous lobular mastitis) may be part of a common disease process termed mammary duct associated inflammatory disease sequence (MDAIDS) [7]. Smoking is linked to MDAIDS, and severe disease occurs almost exclusively in heavy smokers. Therefore, smoking cessation is a very important part of the treatment process [7]. These conditions may result from lactiferous duct obstruction, resulting in duct distention, inflammation, and ultimately rupture. SND may be observed. Treatment requires excision of the affected ductal system. Simple incision and drainage are associated with a high rate of mastitis recurrence and breast scarring.

Breast Masses and Breast Imaging

Approximately 70–80% of the breast lesions detected by physical examination or imaging and biopsied [10] are benign. Most solid or complex cystic breast lesions should undergo biopsy. Possible exceptions are lesions in young women that are highly consistent with a fibroadenoma, as long as the lesion does not enlarge over time, and lesions that have been present for years and remain unchanged. Microcalcifications are biopsied based on whether they are considered suspicious by imaging criteria.

False positive imaging can occur not only with breast cancer screening but also in the workup of other malignancies. Patients who undergo 18F-fluorodeoxyglucose (FDG) PET or PET CT for staging of cancers other than breast are occasionally found to have 18F-FDG-avid breast lesions. When biopsied, these lesions are most often benign. Among the reasons for this increased uptake include acute and chronic inflammation, physiological lactation, and benign breast masses, including silicone granuloma, fat necrosis, fibroadenoma, and postsurgical changes [11]. To decrease the number of false positive biopsies, Adejolu et al. [11] recommend correlative imaging, including mammography, sonography, or MRI.

Changes During Pregnancy or Lactation

Normal pregnancy related breast changes include growth and enlargement, tenderness and hypersensitivity, darkening of the skin of the nipple and areola, and enlargement of superficial veins near the skin surface. The nipple and areola enlarge. The breasts are more prone to leaking during pregnancy.

Most pregnancy induced conditions of the breast that are not considered normal are nonetheless benign. These benign conditions include lactating adenoma, galactocele, gigantomastia, and benign nipple discharge [12]. Cancer must be excluded by a thorough workup, including breast biopsy if indicated. During lactation, the most common problems are inflammation and infection. Organisms from the infant are the usual source of breast infections during lactation. Continuing breastfeeding with an infection in the breast is recommended because it is not known to harm the infant, and keeping the breast empty of milk promotes infection resolution by draining the material that is facilitating bacterial growth.

Pregnancy associated masses are usually discovered during patient self examination. Ultrasound is the imaging modality of choice to further delineate the lesion and is often useful if a biopsy is indicated. Lactation should be suppressed prior to biopsy in nursing women to reduce the risk of abscess and milk fistula formation [13]. Fine needle aspiration is less reliable during pregnancy and lactation due to the hyperproliferative features in the tissue of the pregnant, lactating, or involuting breast [14].

During pregnancy and lactation, the breast can be affected by a variety of benign disorders, including inflammatory and infectious diseases, juvenile papillomatosis, and benign tumors. Fibroadenomas may manifest with growth or infarction. Galactocele is the most commonly observed breast lesion during lactation [15]. It manifests as either a cystic mass with a fat-fluid level or as a pseudohamartoma. The tumors and diseases that affect the breasts during pregnancy and lactation are also observed in nonpregnant women but may have a different appearance. The sensitivity of mammography in pregnant and lactating women is decreased due to increased parenchymal density. Instead, ultrasonography is the most appropriate radiological method for evaluating breast masses in this setting and is particularly useful in the diagnosis and treatment of abscesses.

Three percent of breast carcinomas occur in women aged 35 or younger. Breast cancer is the leading cause of cancer related death for women 15–29 years of age [16]. Studies of how reproductive factors influence the development of breast cancer are increasing our understanding of why carcinoma presents at an advanced stage among women who are pregnant or lactating. Specifically, concurrent or recent pregnancy is associated with increased tumor aggressiveness and

poorer survival. More than 15% of women younger than age 40 who develop breast cancer do so during pregnancy or lactation [17]. Pregnancy associated breast cancer (PABC) is classically defined as breast cancer diagnosed during pregnancy or within the first 12 months postpartum. The average age of women with PABC is 32–38 years [18]. The incidence of PABC may increase as more women choose to postpone childbearing until their mid to late 30s. Pregnancy related Burkitt's lymphoma characteristically manifests with bilateral and diffuse involvement of the breasts [15].

Pathology of Benign Breast Disease

Fibroadenomas and disorders related to breast growth are the most common breast diseases in adolescent women. The assessment of breast disorders in adolescents generally involves CBE and, when needed, ultrasonography. Fibroadenomas can be treated conservatively unless they continue to grow. When the diagnosis is secure and surgical removal is selected, enucleation is the procedure of choice. Breast abscess is mainly due to duct ectasia [19]. Phyllodes tumors (PTs) of the breast are biphasic neoplasms in which interactions between the epithelium and stroma are critical for tumor development and progression. Intratumoral genetic heterogeneity is common in PTs and may account for the reported lack of correlation between histological grading and clinical behavior [20].

Desmoids are benign, slow growing fibroblastic neoplasms that are characterized by an infiltrative and locally aggressive growth pattern and frequent recurrence but no metastatic potential. Breast desmoids are rare and often misdiagnosed because they can mimic other breast lesions, including carcinoma. Desmoid tumors should be considered in the differential diagnosis of patients presenting with hard breast lumps [21].

Diabetic mastopathy is a proliferation of fibrous tissue in the breast that mimics a tumor. No imaging modality is entirely reliable in differentiating diabetic mastopathy from malignancy, and core biopsy is essential for accurate diagnosis when mammography and/or ultrasonography are indicative of potential malignancy [22]. Breast calcium deposits in the media of arterioles are more frequently detected in the mammograms of diabetic subjects and must be differentiated from suspicious breast microcalcifications.

Pseudoangiomatous stromal hyperplasia (PASH) is a benign, proliferative mesenchymal lesion of the breast that typically affects women of reproductive age [23]. PASH is frequently an incidental histological finding in breast biopsies. Rarely, it can present as a firm, painless breast mass. When presenting as a mass, it is well circumscribed, firm, and rubbery. Histologically, it demonstrates dense collagenous stroma. The most important differential diagnosis is angiosarcoma. When incidentally found, no treatment is required. When PASH forms a tumor mass, it is treated by local surgical excision with clear margins.

Papillary lesions of the breast are common and morphologically varied, ranging from benign to atypical to malignant. Cytologic assessment is very challenging and often inconsistent with the histologic assessment of the same lesion [24]. Completely excised papillary lesions have an excellent prognosis, whereas incompletely excised lesions may recur or persist as carcinoma. Complete excision is therefore recommended for all papillary lesions [24].

Ectopic breast tissue in axillary lymph nodes is a benign condition that must be differentiated from primary or metastatic carcinoma. Rarely, proliferative conditions such as an intraductal papilloma can occur in ectopic breast tissue [25].

The growing use of breast image detected biopsies has led to increased diagnosis of benign breast disease (BBD). As a group, BBD is a known risk factor for breast cancer among both Caucasian and African American women [26]. When separated into individual pathological entities, BBD ranges from diagnoses with no increased cancer risk to those with a consistently documented increased cancer risk. The lesions of highest risk, which are sometimes referred to as "borderline" lesions, contain atypical changes and include atypical hyperplasia and lobular carcinoma *in situ*. Some also classify ductal carcinoma *in situ* as a borderline lesion, although others do not. These "borderline" lesions can be difficult to diagnose, particularly because biopsy sample size is often limited [27]. Borderline lesions are associated with an increased risk of neighboring malignancy, particularly when the biopsy sample is small. Some of the most challenging scenarios include the differentiation between atypical ductal hyperplasia and low grade ductal carcinoma *in situ*, lobular neoplasia versus solid low grade ductal carcinoma *in situ*, correctly classifying papillary lesions with atypia, and classifying the spectrum of columnar cell changes [27]. Consensus criteria and uniform terminology for the diagnosis of these lesions do not exist.

Management of Palpable Breast Masses

In general, palpable breast masses are evaluated by CBE and imaging. Although imaging is not initially required, it does provide a more accurate assessment of mass size and shape and the involvement of the mass with surrounding structures. Most palpable breast masses should undergo biopsy, regardless of whether the lesion appears suspicious on breast imaging, because some cancers appear benign based on imaging criteria. Exceptions include simple cysts and tumors that are consistent with fibroadenoma in a young woman and do not continue to enlarge. Palpable intramammary lymph nodes are generally benign but rarely contain tumor spread from a

nearby primary breast cancer. Treatment of simple cysts is generally therapeutic. Bloody and/or recurrent cysts should generally be excised [28]. The increasing use of screening mammography, liability risks, and volume control legislation by the federal government poses a major challenge to clinicians to safely select patients for breast biopsy. Despite a normal mammogram, a palpable breast mass often requires aspiration or excisional biopsy. Careful clinical judgment must prevail if observation is elected. A biopsy should be performed on a clinically suspicious mass regardless of whether the mammogram is suspicious. Management of the patient with a nonpalpable mammographic abnormality requires a close working relationship among the surgeon, pathologist, and radiologist. Thoughtful clinical judgment and interdisciplinary cooperation promote an acceptable benign-to-malignant ratio for breast biopsies.

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Benign Breast Tumors

3

Emilia Josefa Borromeo Diego

Fibroadenoma

Definition

Fibroadenomas are benign breast tumors that are composed of epithelial and stromal elements arising from the terminal duct lobular unit. They are frequently diagnosed in young women, predominantly in the second or third decade of life, but can occur at any age [1].

Clinical Presentation

Most fibroadenomas are likely asymptomatic; they were present in up to 8–10% of women based upon autopsy findings of "normal breasts" in 1955 [2]. More recent reports indicate that this incidence may be as high as 25% in asymptomatic women, with 13–20% having multiple fibroadenomas [3].

If the fibroadenoma causes symptoms, the most common presentation is a firm, movable mass that does not adhere to the chest wall or the skin of the breast. These masses are frequently painless but can occasionally cause discomfort, particularly when they are larger or located in areas that are pressed upon, such as the underwire of a female brassiere.

Medical attention is frequently sought in scenarios of pain, rapid growth, cosmetic deformity, and fear of malignancy.

Radiological Findings and Workup

The imaging workup for a fibroadenoma usually begins with an ultrasound because these masses are frequently detected in younger women for whom a suspicion of cancer is relatively low. For women who have a personal history or a family history of breast cancer, are over the age of 35, or have symptoms that are not clinically congruent with a fibroadenoma, a bilateral baseline mammogram should be considered as an adjunctive test.

The characteristic ultrasonographic finding for a fibroadenoma is a round, oval, or lobular well-circumscribed hypoechoic mass [4]. On mammogram, these masses are distinct and occasionally have calcifications. The ultrasound is a more specific test than the mammogram for diagnosing fibroadenomas and should be considered the first imaging modality in young women presenting with a breast mass [5]. See Figs. 3.1 and 3.2.

Core needle biopsy should be obtained when the diagnosis is uncertain due to suspicious features on imaging or there is change in clinical findings (e.g., rapid growth) that may affect surgical planning.

Pathology

The gross examination of a fibroadenoma reveals a firm, smooth, tan-whitish, lobulated mass that is well-marginated and distinct from the surrounding breast tissue. Sectioning of the specimen demonstrates a homogeneous mass that can have a "bulging" appearance. These masses range in size from sub-centimeter to >4 cm. Fibroadenomas that are larger than 5 cm are termed giant fibroadenomas and juvenile giant fibroadenomas, specifically, when found in younger women [6]. Historically, the term Brodie's disease of the breast has been used to indicate large, excessively cellular, and long-neglected fibroadenomas [7]. See Fig. 3.3.

Microscopically, fibroadenomas have epithelial and stromal elements with smooth, well-circumscribed borders that can exhibit one of the two growth patterns, pericanalicular or intracanalicular, which pertain to the architecture of the ductal elements. When there is evidence of sclerosing adenosis, metaplasia, or hyperplasia, they are termed complex fibroadenomas [8]. See Figs. 3.4, 3.5, and 3.6.

E. J. B. Diego (✉)
Department of Surgery, Division of Surgical Oncology, Magee-Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

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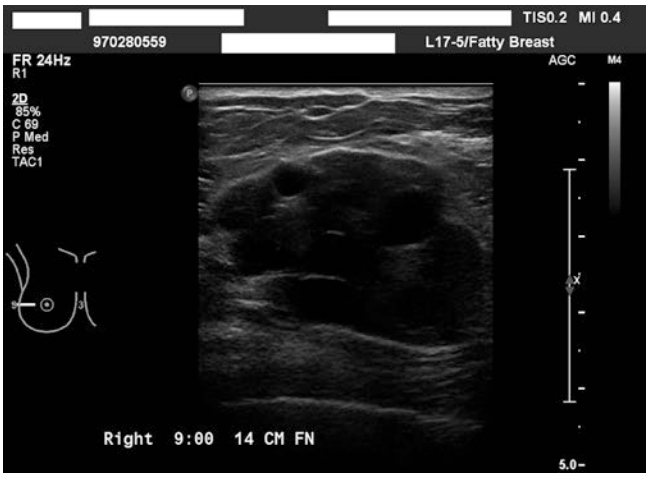

Fig. 3.1 Ultrasound appearance of a fibroadenoma

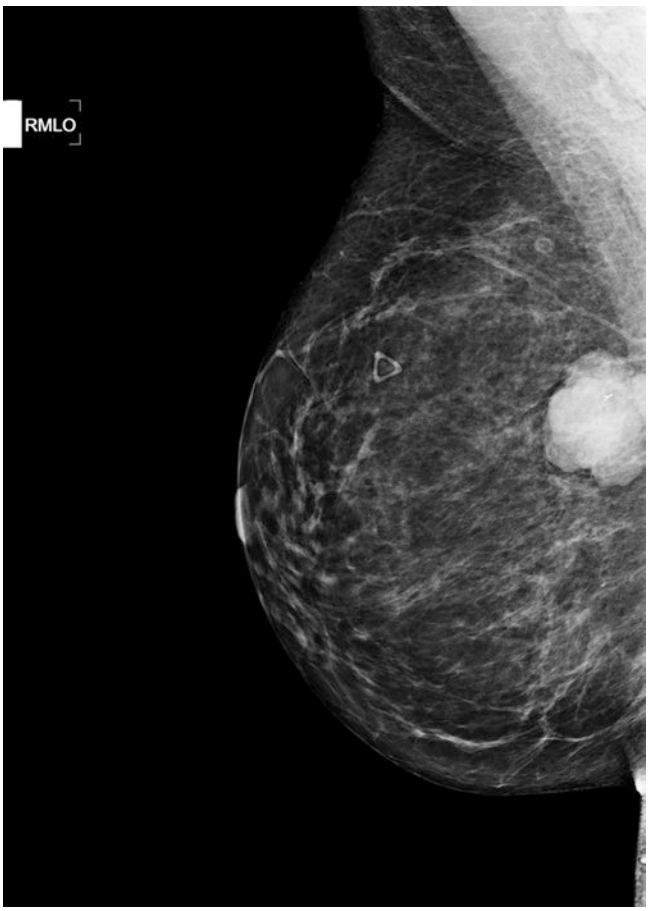

Fig. 3.2 Mammogram of a fibroadenoma

Special types of rare fibroadenomas are classified based upon histological features [1]. Tubular adenomas or pure adenomas have prominent adenosis and very little stromal elements [2]. See Figs. 3.7 and 3.8. Lactational adenomas exhibit

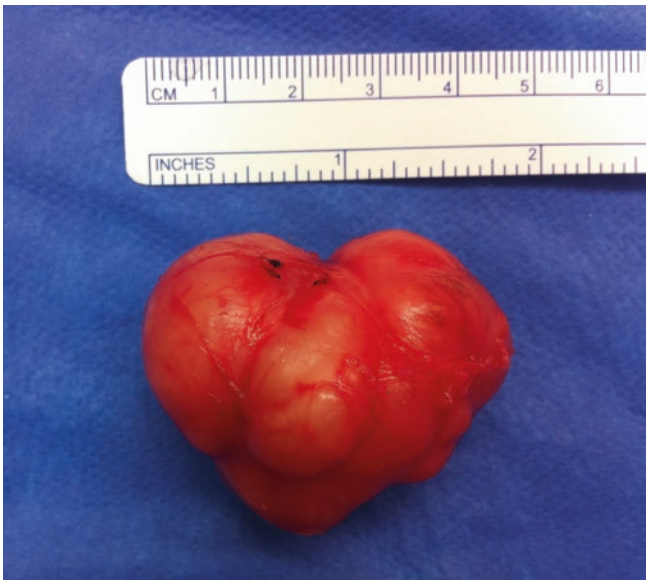

Fig. 3.3 Gross appearance of a fibroadenoma

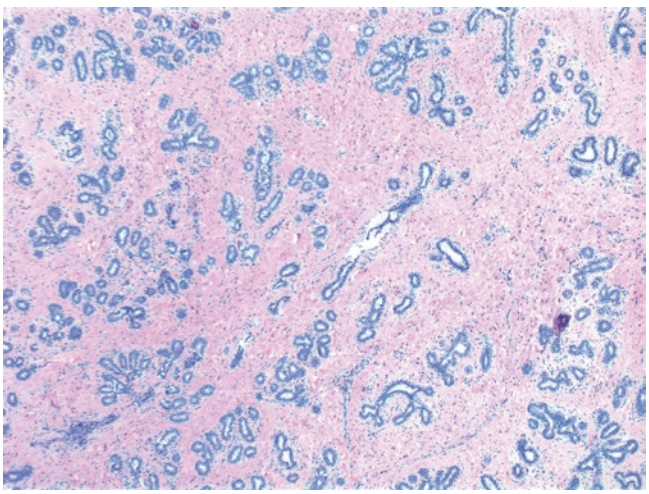

Fig. 3.4 Low-power magnification view of an intracanalicular fibroadenoma

lactational changes in secretory glands in fibroadenomas of pregnant or breastfeeding women [9]. See Figs. 3.9 and 3.10.

Management

Simple fibroadenomas that are asymptomatic and <3 cm, without evidence of growth, in a patient who has no personal or family risk factors for breast cancer can be safely observed. The incidence of malignant transformation is extremely low, and there is no increased lifetime risk of breast cancer [10].

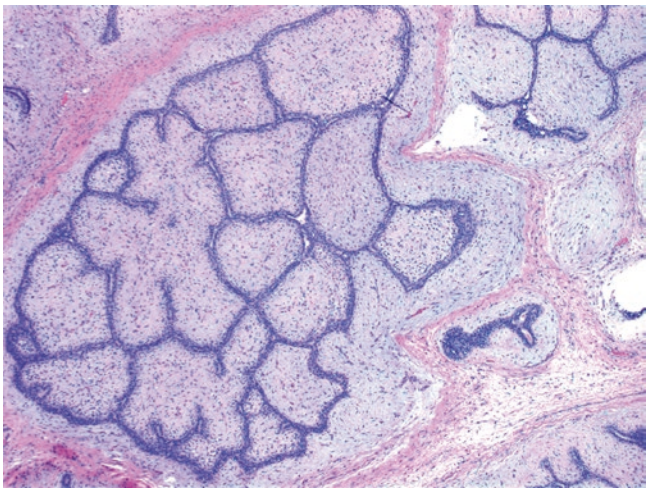

Fig. 3.5 Low-power magnification view of a pericanalicular fibroadenoma

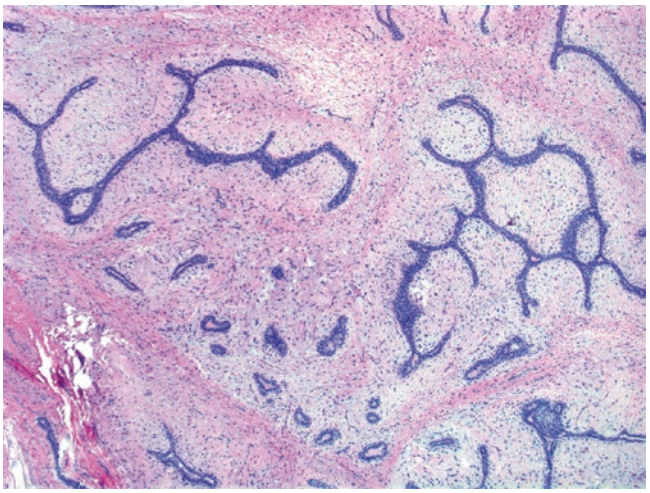

Fig. 3.6 Low-power magnification view of a mixed intracanalicular/pericanalicular fibroadenoma

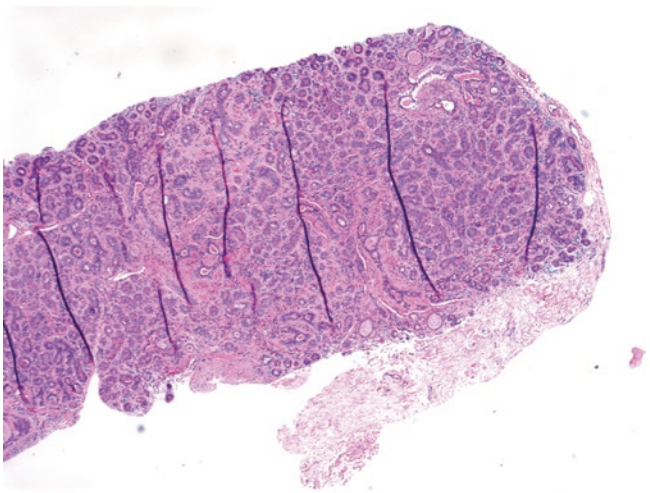

Fig. 3.7 Low-power magnification view of a tubular adenoma

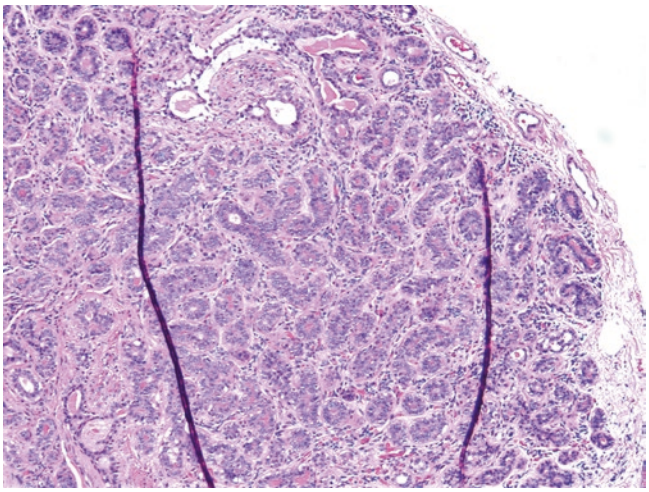

Fig. 3.8 High-power magnification view of a tubular adenoma with prominent adenosis and few stromal elements

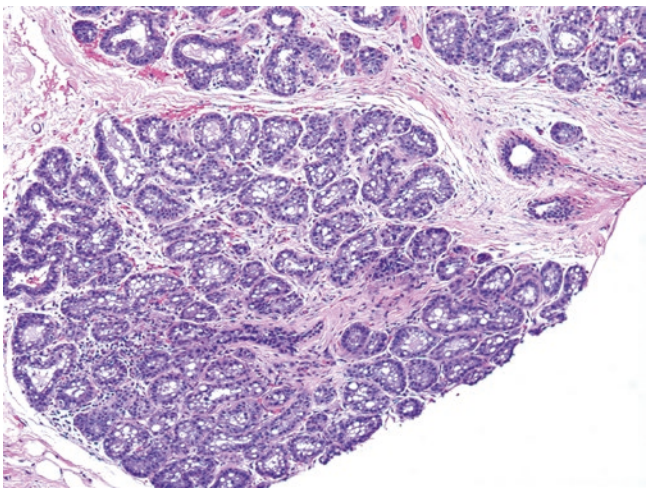

Fig. 3.9 Low-power magnification view of a lactational adenoma

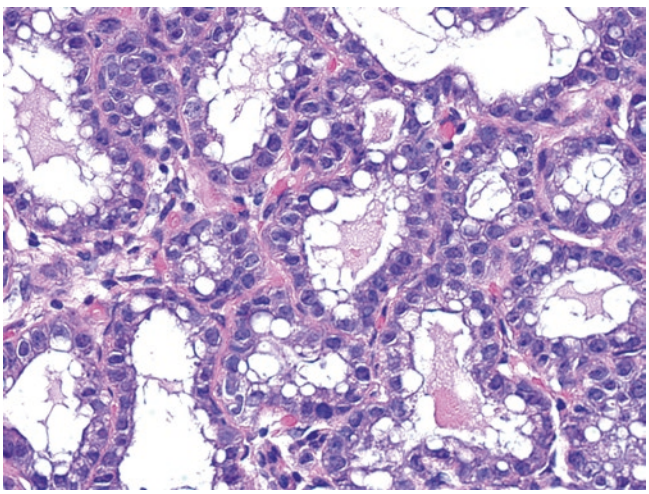

Fig. 3.10 High-power magnification view of a lactational adenoma with lactational changes within the secretory glands

Excision should be considered in patients who have evidence of growth, indeterminate histopathological findings (frequently reported as fibroepithelial lesions), complex fibroadenomas, symptoms such as pain or issues with cosmesis, or patient desire.

Surgical excision remains the most common method for removing fibroadenomas. These palpable lesions can be "shelled out" without excision of the surrounding normal breast tissue, and the incisions are planned such that they do not compromise the appearance of the breast postoperatively. The most frequent approaches to these lesions are through a periareolar or inframammary incision. Should the fibroadenoma be located a distance away from these sites, exposure and delivery of the fibroadenoma can be achieved using an anchoring stitch or Allis clamp to grasp the mass into view. When the fibroadenoma is non-palpable, intraoperative ultrasound guidance or wire localization aids excision.

Ultrasound-guided vacuum-assisted biopsy devices are emerging as a technique to excise smaller fibroadenomas. Advantages to this approach include its ability to be performed as an office procedure with image evidence of complete removal of the mass, a smaller required incision, preservation of breast cosmesis, and limited complications if the targeted lesion is <3 cm [11]. However, long-term data regarding outcomes and recurrence are not available.

Differential Diagnosis

It is important to distinguish between fibroadenomas and other mass lesions of the breast, particularly a phyllodes tumor, due to differences in surgical approach, amount of breast tissue excised, and patient counseling regarding risks of recurrence and need for further treatment. Although the phyllode tumor is beyond the scope of this section, it is discussed elsewhere in this book.

Intraductal Papilloma

Definition and Incidence

Intraductal papillomas are benign breast neoplasms that develop within a mammary duct and are composed of breast epithelium supported by underlying stroma and a branching fibrovascular core. They are so named due to their microscopic appearance, and they exhibit papillary architecture.

Benign intraductal papillomas are a rare entity with an incidence of 2–3% in the general population and are frequently diagnosed in women aged 30–55 [12]. By contrast, papillary lesions are observed in up to 5% of breast core needle biopsies [13]. The presence of atypical features may differentiate benign intraductal papillomas from lesions that confer a higher risk of malignancy.

Clinical Presentation

Intraductal papillomas are located in either the central or peripheral portion of the breast. They may be solitary or numerous; in the latter case, the term "papillomatosis" may be employed.

The most frequent symptom associated with a central papilloma is single-duct, spontaneous nipple discharge, which may be serous, greenish, or bloody. Among central or subareolar papillomas, 50% will be solitary, and up to 30% will be associated with bloody nipple discharge [14]. Less frequently, they may present as a palpable mass.

By contrast, peripheral papillomas are very frequently asymptomatic and detected incidentally on breast imaging. Peripheral papillomas, particularly when they are multiple or associated with atypia, are also more likely to be associated with malignancy [15].

Imaging

Central/subareolar papillomas are frequently mammographically occult [16]. However, in the presence of symptoms of a subareolar mass or nipple discharge, an ultrasound and ductogram will commonly reveal the cause.

The ultrasound appearance of an intraductal papilloma is an intraductal mass or complex cystic lesion and is often associated with a dilated duct. When a ductogram is performed, cannulation of the duct producing the pathological discharge will reveal an abnormality in 91% of patients with a papilloma. The findings are either a completely obstructed duct, duct expansion and distortion, intraductal filling defects, duct ectasia, or wall irregularity [17]. See Figs. 3.11 and 3.12.

Mammography detects peripheral papillomas more frequently than central papillomas due to the asymptomatic nature of the former. They appear as architectural distortions, nodular densities, breast masses with or without calcifications, or calcifications alone [18]. See Figs. 3.13 and 3.14.

Pathology

Intraductal papillomas are typically small lesions, measured in millimeters, that are not visible on gross examination unless associated with an enlarged duct. However, they can grow to several centimeters in size and will appear as a mass growing into the duct.

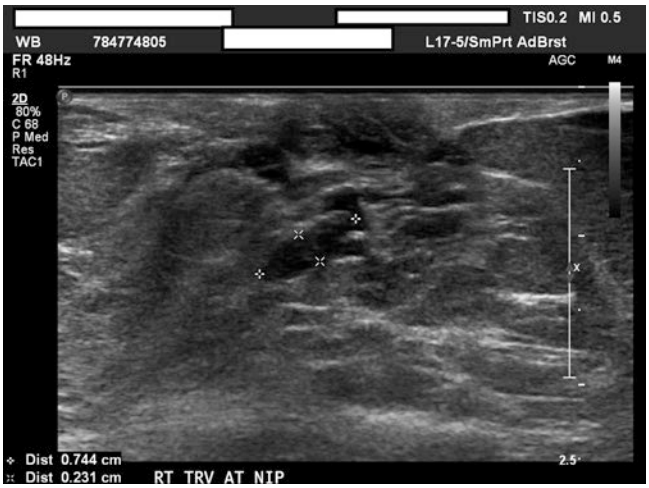

Fig. 3.11 Ultrasound picture of a centrally located intraductal papilloma. There is an intraductal hypoechoic mass measuring 7 × 2 mm

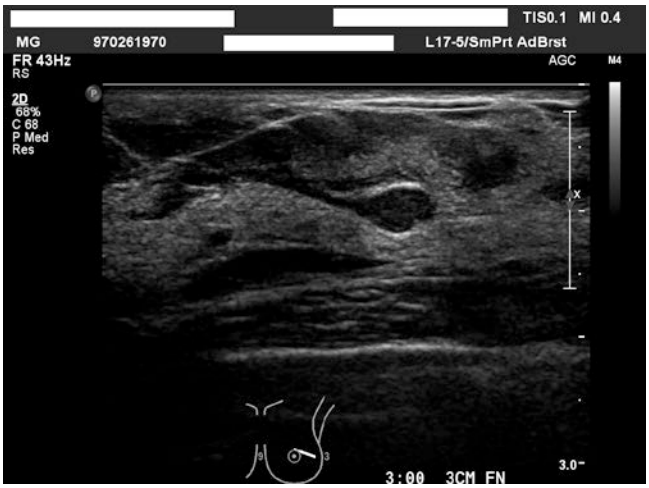

Fig. 3.13 Ultrasound picture of a peripherally located intraductal papilloma. There is an intraductal hypoechoic mass

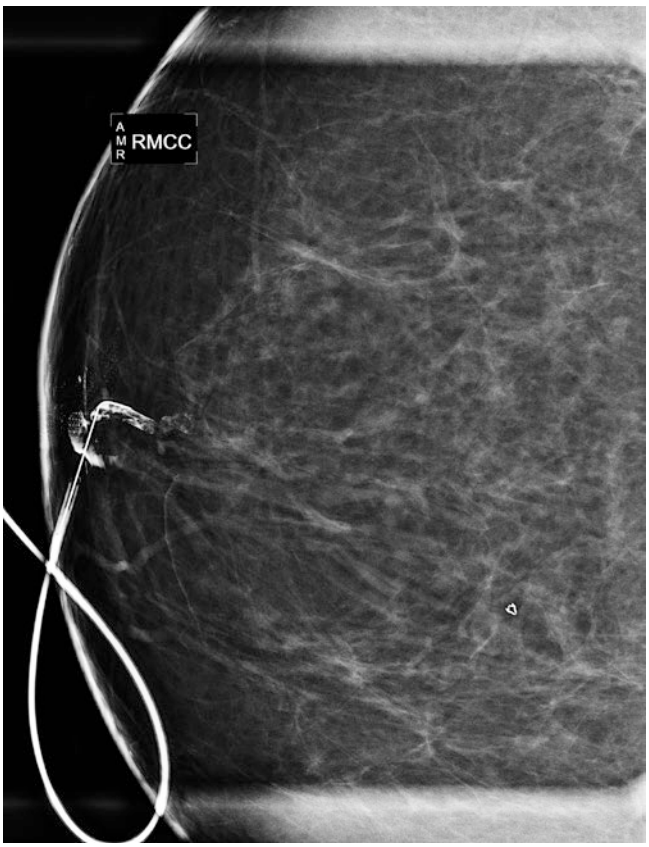

Fig. 3.12 Ductogram demonstrating partial filling of an approximately 1.5-cm span of a duct with numerous intraductal masses

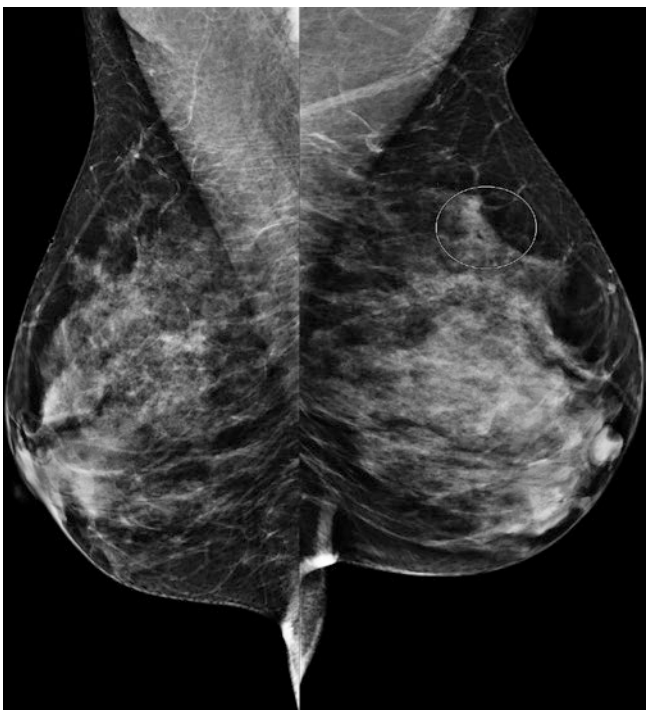

Fig. 3.14 Peripheral papilloma on mediolateral oblique mammogram, seen as an area of asymmetry in the left breast upper quadrant

Microscopically, peripheral and central papillomas are similarly composed of a stalk with a fibrovascular core and overlying myoepithelial and ductal epithelial cells. The ductal epithelium can also exhibit the same proliferative changes observed elsewhere in the breast. Hence, they may also be associated with ductal epithelial hyperplasia, atypical ductal hyperplasia, or ductal carcinoma in situ. See Figs. 3.15, 3.16, 3.17, 3.18, and 3.19.

Management

Surgical excision is still no longer recommended for all intraductal papillomas found on image-guided biopsy.

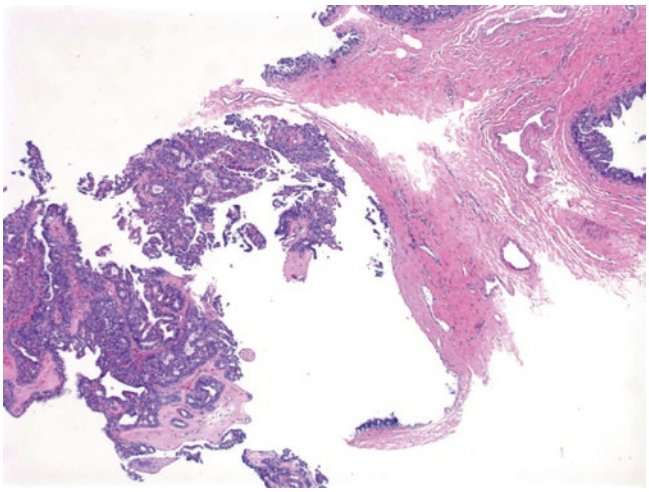

Fig. 3.15 Low-power magnification view of an intraductal papilloma with ductal epithelial hyperplasia

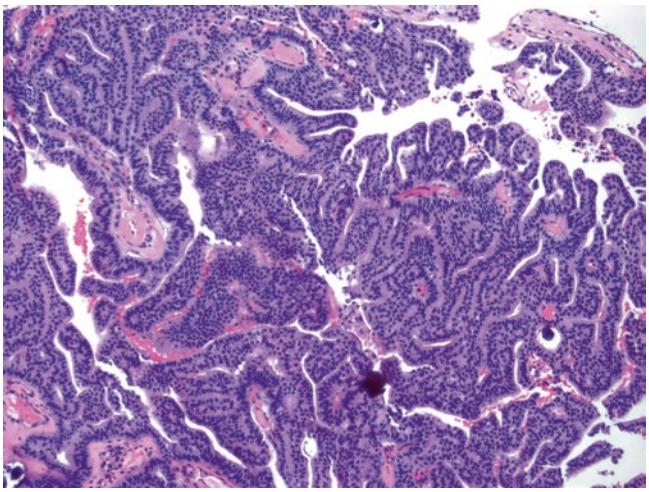

Fig. 3.18 High-power magnification view of an intraductal papilloma with atypia

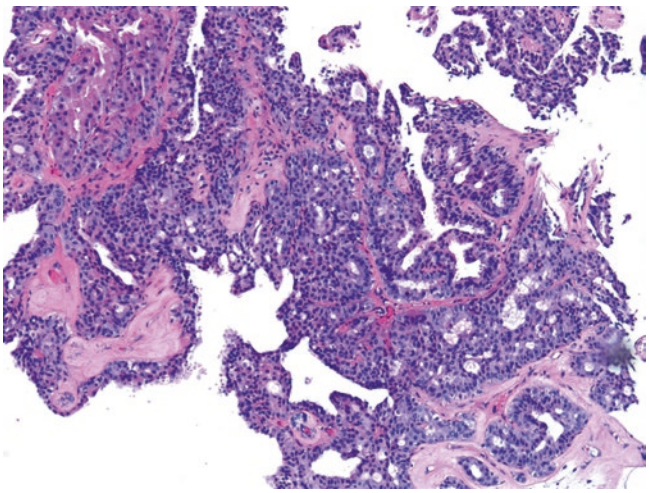

Fig. 3.16 High-power magnification view of an intraductal papilloma with ductal epithelial hyperplasia

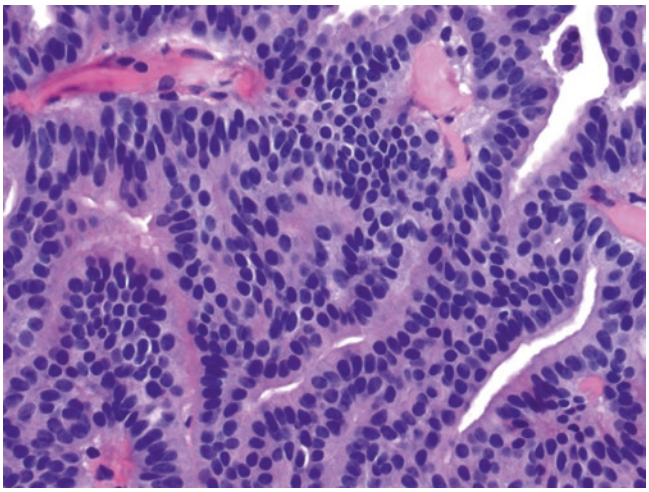

Fig. 3.19 A 400× view of an intraductal papilloma with atypia

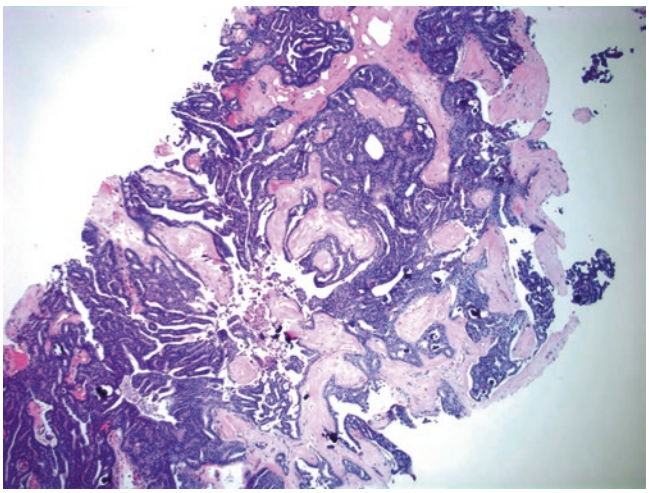

Fig. 3.17 Low-power magnification view of an intraductal papilloma with atypia

Several series have attempted to define the subset of patients with intraductal papillomas that can safely be observed. Characteristics such as the absence of associated calcifications [19], the microscopic size (complete papilloma excision on core needle biopsy) [20], the amount of tissue obtained at core biopsy [21], or the absence of atypia [22] have been investigated as predictive factors for a very low risk of upstaging and the current recommendations that these can be safely observed with surveillance imaging. However, the small sample size in these studies precludes recommendations on a large scale (Table 3.1).

Differential Diagnosis

Benign intraductal papillomas must be distinguished from intraductal papillomas with atypia, papillary ductal carcino-

Table 3.1 Intraductal papilloma studies and upstage rates

	N	Papilloma with atypia	Upstage to atypia, in situ, or invasive carcinoma (%)
Jaffer et al. [23]	104	0	16.4
Brennan et al. [24]	75	25 (33%)	6
Holley et al. [21]	128	0	18
Rizzo et al. [18]	276	49 (15%)	28.5
Kibil et al. [12]	62	12 (19.4%)	3
Fu et al. [25]	268	65 (24%)	23

mas, and encapsulated (intracystic) papillary carcinomas due to their differences in clinical behavior and management.

Lipoma

Definition and Incidence

A lipoma is a benign mass consisting of bland-appearing adipose tissue and is the most common soft tissue tumor in the body, with a prevalence of 2.1 per 1000 people [26]. Lipomas have been reported in virtually every area of the body, including the breast. Due to their relatively dull clinical course, much of the literature on breast lipomas are case reports on giant lipomas that pose an interesting scenario for workup and management. Therefore, the true incidence of breast lipomas in general may not be known, although the series of Lanng in 2004 reported an incidence of 4.6% [27].

Clinical Presentation

Breast lipomas are typically small, soft, and doughy or semi-firm, painless, mobile masses that are frequently well circumscribed. The term "giant" lipoma was coined by Sanchez et al. [28] and is used to describe a mass that has grown to at least 10 cm in size. Breast lipomas have been observed in both males and females.

Consultation is frequently sought due to symptoms such as pain or growth, concern for malignancy, or issues of cosmesis. See Figs. 3.20, 3.21, and 3.22.

As with any breast mass, the diagnostic workup includes breast imaging. Age, clinical features, and personal and family history should dictate the level of suspicion and attendant workup, which will frequently include a mammogram and breast ultrasound. Further imaging should be performed based upon the results of these tests.

Radiological Findings

A lipoma on mammography may have the appearance of a mass with a density similar to that of the surrounding breast

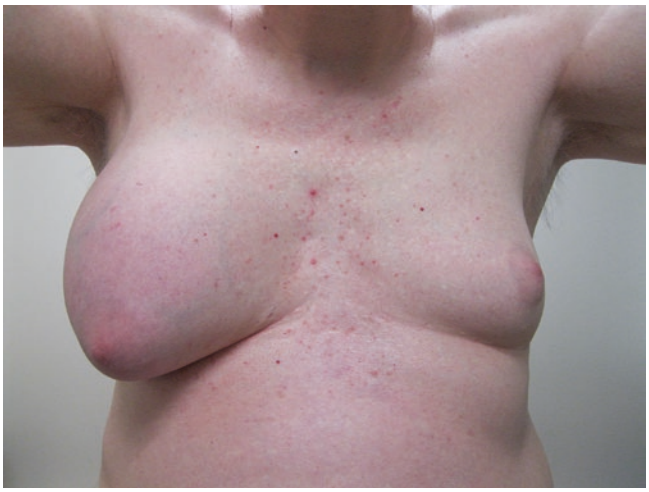

Fig. 3.20 Clinical picture of a man with gynecomastia and a right breast lipoma

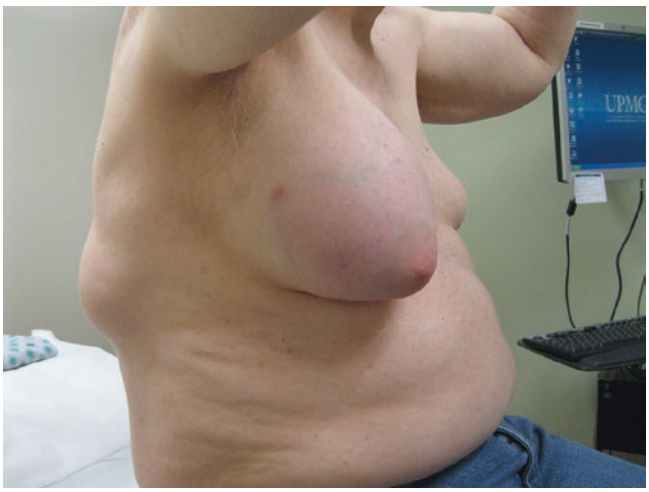

Fig. 3.21 Viewing from the side, there is also evidence of a lipoma in this patient's back

fat, possibly with a very thin surrounding capsule [29]. Occasionally, benign-appearing calcifications may also be observed within the mass and may represent fat necrosis [30]. See Figs. 3.22 and 3.23.

Ultrasound findings of a lipoma reveal an isoechoic or slightly hyperechoic mass with a thin surrounding echogenic capsule. There is also typically no posterior acoustic enhancement or shadowing [31].

Pathology

On gross examination of a breast lipoma, an encapsulated, smooth, fatty mass that may have lobulations is usually observed. Histologically, the specimen is composed of bland-appearing mature adipocytes. See Figs. 3.24 and 3.25.

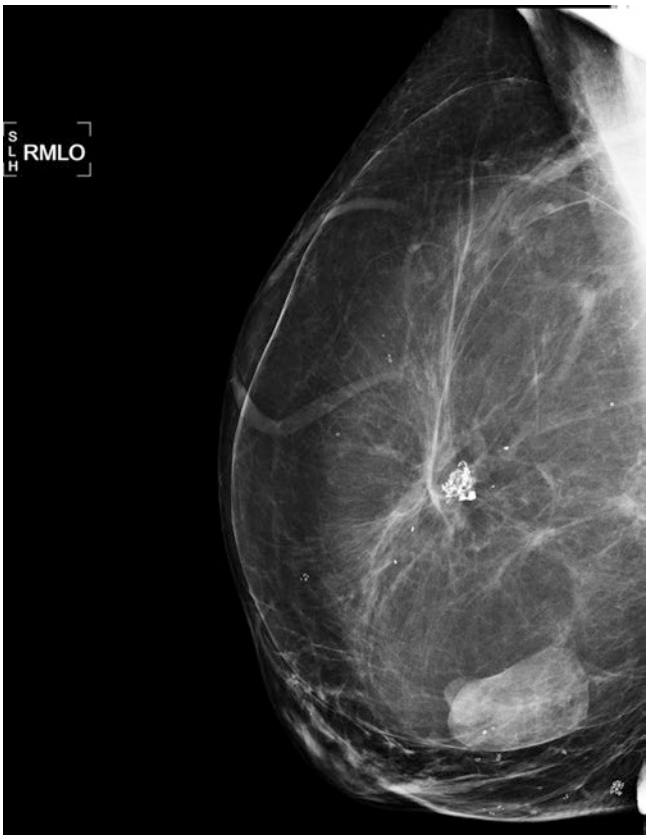

Fig. 3.22 Mediolateral oblique mammogram views of a large lipoma demonstrating density very similar to the surrounding breast fat, a very thin surrounding capsule, and benign-appearing calcifications within the mass that represent fat necrosis

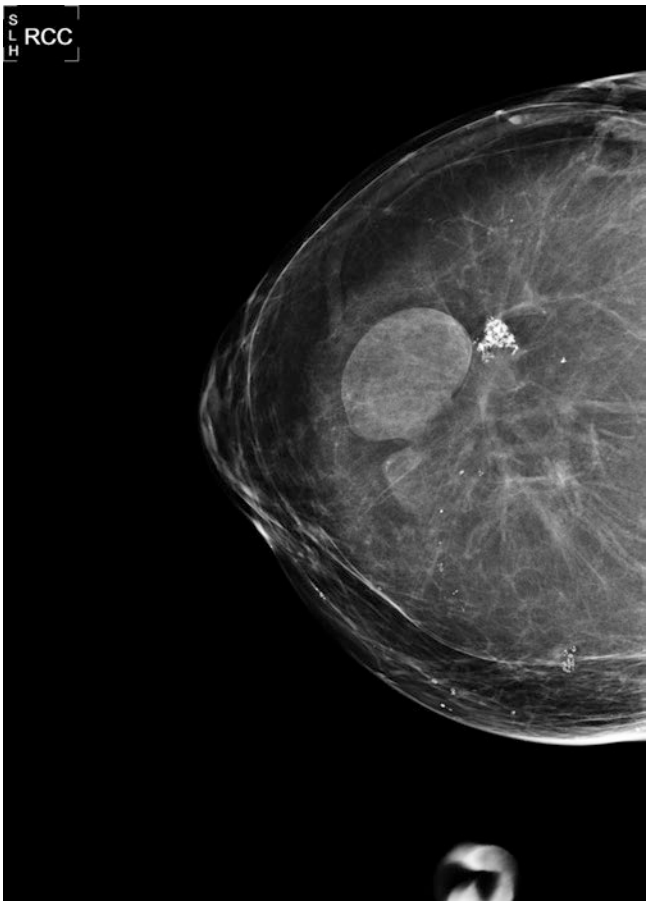

Fig. 3.23 Craniocaudal mammogram views of a large lipoma demonstrating density very similar to the surrounding breast fat, a very thin surrounding capsule, and benign-appearing calcifications within the mass representing fat necrosis

Management

Surgical excision for lipomas is curative although rarely necessary except for symptom control due to large size, diagnostic uncertainty, radiological-pathological discordance, or patient desire.

The use of liposuction for smaller lipomas has been described and can achieve removal with reduced risk of complications due to smaller incisions [32]. However, the practice has not been extended to the treatment of larger lipomas due to the higher likelihood of incomplete removal, subsequent recurrence, and increased incidence of complications such as hematomas [26]. This technique should also not be performed in situations of diagnostic uncertainty due to the difficulty that will arise in the pathological evaluation of the specimen.

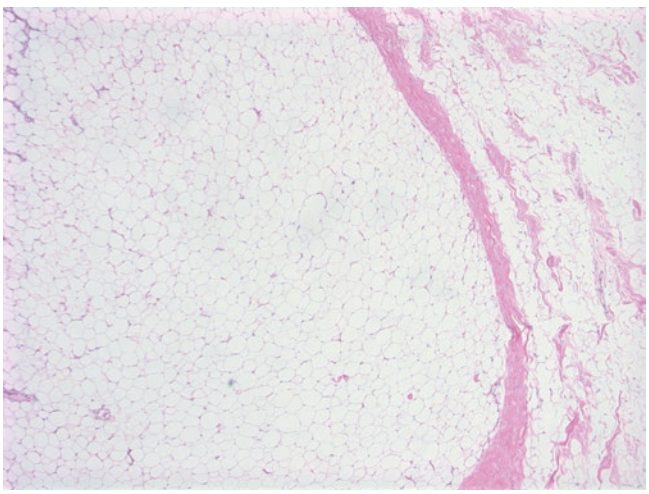

Fig. 3.24 Low-power magnification view of a lipoma composed primarily of adipocytes with a capsule

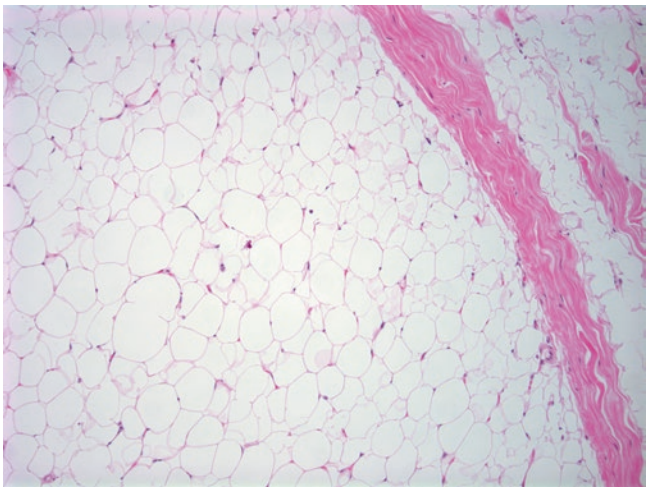

Fig. 3.25 High-power magnification view of a lipoma composed primarily of adipocytes with a capsule

Prognosis and Differential Diagnosis

As with lipomas in other locations, breast lipomas are benign and do not confer an increased risk of breast cancer when found in the breast. There is no current medical evidence to support the malignant degeneration of lipomas if left in situ.

Other considerations for soft tissue masses in the breast that may present similarly to lipomas include hibernomas, hematomas, hamartomas (discussed shortly), fat necrosis, and malignant processes such as sarcomas or other tumors of mesenchymal origin.

Hamartoma

Definition/Incidence

The earliest documented report on hamartomas in the breast was in the German literature by Prym in 1928, in which he described a benign mass called a "mastoma" [33]. Arrigoni et al. first used the term mammary hamartoma to refer to a well-circumscribed mass of benign breast tissue admixed with stromal tissue and fat that is without structural organization [34]. Hamartomas are considered rare breast tumors that encompass fibroadenolipomas, adenolipomas, chondrolipomas, and myoid hamartomas [35]. The literature referring to breast hamartomas is sparse, with 25 patients in the largest series reported to date [34].

As with lipomas, the true incidence of hamartomas is not known, but its diagnosis is increasing due to improvements in breast imaging. Breast hamartomas have also been reported in males [36, 37].

Clinical Presentation

A breast hamartoma typically presents as a well-circumscribed, mobile, soft, and non-tender breast mass. The palpable nature of the mass is what prompts consultation with a physician. However, they are also occasionally asymptomatic and detected on routine imaging.

Hamartomas have been diagnosed in women of varied ages, without any predilection for pre- or postmenopausal groups. The age range of hamartomas among various series is as young as 11 and as old as 76 [34, 38].

Imaging

On imaging, a hamartoma may be difficult to differentiate from other benign, solid tumors of the breast such as fibroadenomas.

Mammographic findings may include architectural distortions, asymmetric masses with mixed densities and pseudocapsules, or well-circumscribed nodules. Because hamartomas are masses of disorganized breast tissue with various stromal elements, they will typically have a density similar to that of the surrounding tissue and sometimes have been referred to as "breast-in-breast" lesions [39]. However, ultrasonographic findings may more specifically demonstrate a hypoechoic, homogenous mass with distinct borders and no posterior acoustic shadowing [40]. Similarly, they may also demonstrate heterogeneous internal echo patterns due to the differential amounts of breast and stromal tissue present in the tumor. Therefore, it is difficult to make a specific diagnosis of a hamartoma solely based on mammographic or ultrasound imaging. See Figs. 3.26, 3.27, and 3.28.

With the increasing use of MRI in breast imaging, hamartomas are more frequently being detected and characterized. Hamartomas exhibit a gradual enhancement pattern on time-signal intensity curves that differentiates them from malignant processes, which have more rapid enhancement patterns, particularly on dynamic contrast-enhanced MRIs [39].

Pathology

The gross appearance of a hamartoma is a smooth, lobulated mass with variable amounts of fat and fibrous tissue on sectioning. See Fig. 3.29.

Hamartomas can be further classified by their cellular composition. Adenolipomas will have disorganized benign glandular, adipose, and stromal elements that form a mass with a pseudocapsule or compressed tissue at the borders [41]. Chondrolipomas will contain benign hyaline cartilage admixed with breast lobules and adipose tissue. Myoid

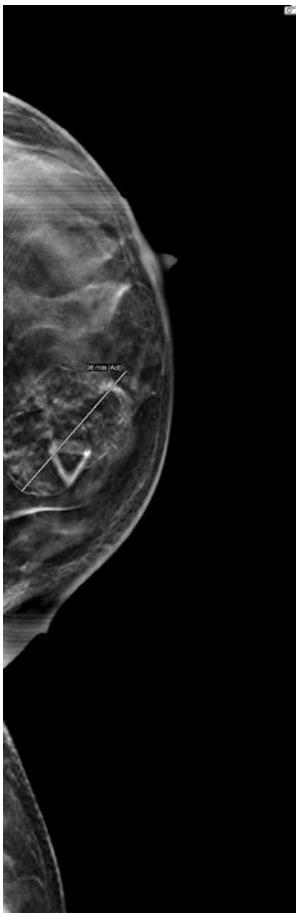

Fig. 3.26 Mammogram of a palpable 3.6-cm macrolobulated mass composed primarily of dense fat but with scattered areas of soft tissue density within it. This mass was identified as a hamartoma in a female, with the triangle marker overlying the palpable mass

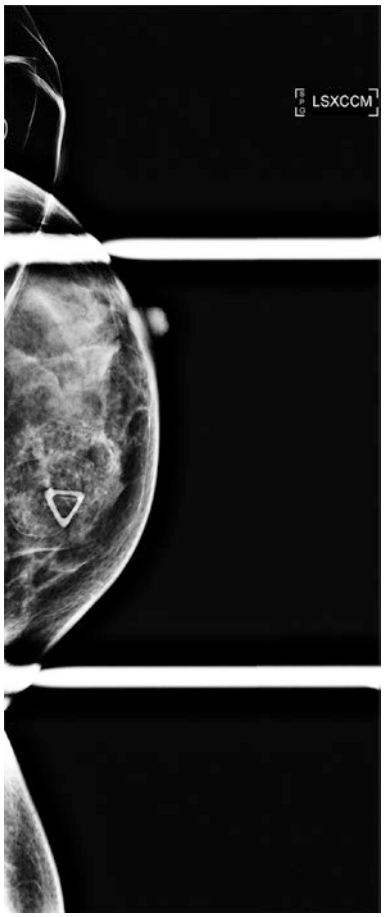

Fig. 3.27 Exaggerated and magnified craniocaudal mammographic view of a palpable hamartoma in a female, with a triangle marker overlying the palpable mass

hamartomas have an additional smooth muscle component [42, 43]. See Figs. 3.30, 3.31, and 3.32.

On occasion, other pathological changes are observed within hamartomas, such as apocrine metaplasia, usual or papillary hyperplasia of ductal epithelium, pseudoangiomatous stromal hyperplasia, cysts, and adenosis [34, 44]. Rare cases of associated ductal carcinoma have also been reported [45, 46].

Management

Fine needle aspiration has little role in the precise diagnosis of hamartomas due to the architectural features that are necessary to differentiate these lesions. However, a bland-appearing aspirate can suggest the benign nature of the hamartoma [47, 48].

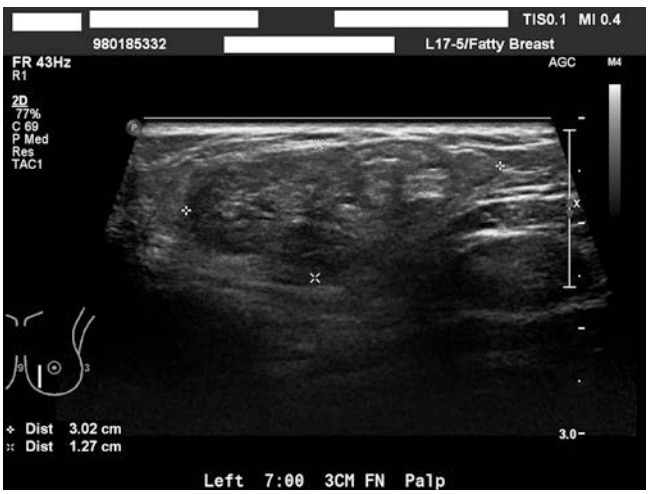

Fig. 3.28 Ultrasound of a hamartoma demonstrating a 3-cm circumscribed lesion that was isoechoic to normal fatty and fibroglandular tissue

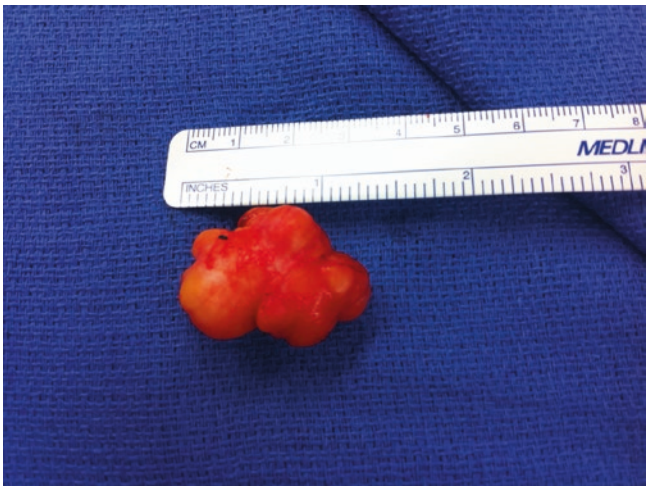

Fig. 3.29 Gross appearance of a hamartoma

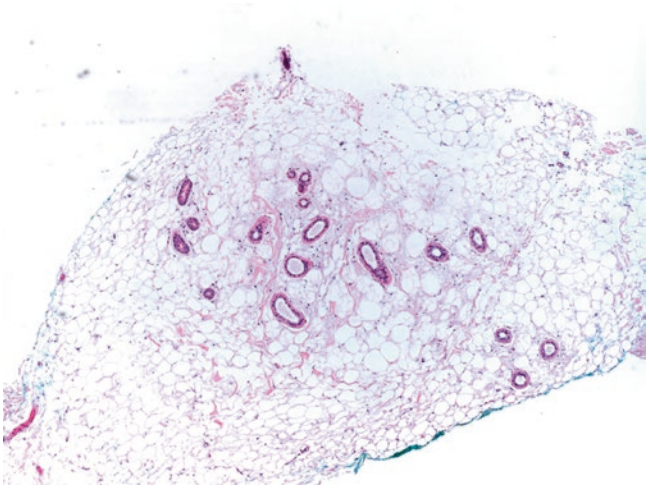

Fig. 3.30 Low-power magnification view of an adenolipoma with disorganized benign glandular, adipose, and stromal elements that form a mass with a pseudocapsule or compressed tissue at the borders

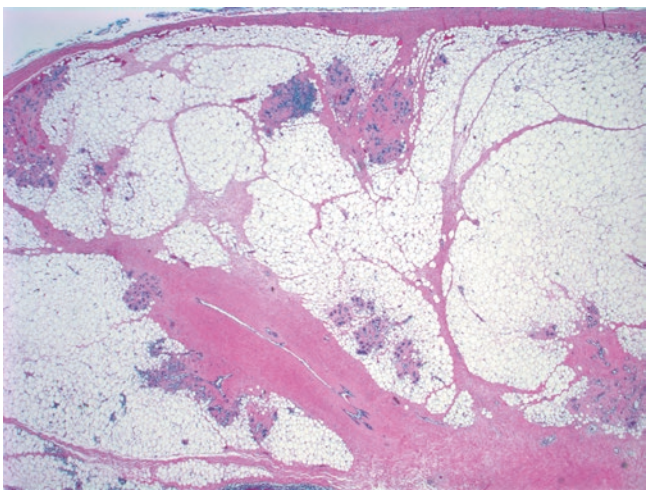

Fig. 3.31 High-power magnification view of an adenolipoma with disorganized benign glandular, adipose, and stromal elements that form a mass with a pseudocapsule or compressed tissue at the borders

Complete surgical excision is curative. There is no evidence for the need to obtain margins beyond the hamartoma, although rare cases of recurrence have been reported, presumably due to incomplete resection [34]. Pure breast hamartomas do not increase a patient's lifetime risk of breast cancer.

Granular Cell Tumor

Definition/Incidence

Granular cell tumors are rare, benign neoplasms of neural origin. Specifically, they are thought to be derived from Schwann cells. The lesion is still occasionally called an Abrikossoff tumor after the first description of a tongue mass in 1926 by Russian pathologist Aleksei Ivanovich Abrikosov [49]. They can arise in any organ of the body but most commonly occur in the skin, oral cavity, or digestive tract [50]. Approximately 5–8% of all granular cell tumors are found in the breast [51]. Granular cell tumors represent an estimated 1 of every 1000 breast neoplasms [52].

Granular cell tumors have been observed in both male and female breasts, and reports indicate that African Americans may be more prone to develop granular cell tumors [51].

Clinical Presentation

The clinical presentation of granular cell tumors can mimic a malignancy. Reports of granular cell tumors indicate that they can occur in any quadrant of the breast as a hard, non-tender, mobile mass; however, they may have a specific predilection for the medial breast quadrants due to their perineural origin along the path of the supraclavicular nerve [53, 54]. They infrequently cause overlying skin changes, fixation to the skin or chest wall, nipple retraction, or breast edema [55].

The most frequent age at presentation in women is the 40s or 50s, but the reported age range in Papalas' series is 19–70 years [56]. The youngest reported case in the literature is a 14-year-old girl [54]. In men, these tumors also tend to occur within the 40–50-year age group [51].

Imaging

The imaging appearance of a granular cell tumor may not always be helpful in indicating its benign nature or positively identifying it. The mammographic appearance may include smooth, rounded, or lobulated opacities suggestive of a benign process or an indistinct spiculated mass more suspicious of malignancy [53, 55]. Associated microcalcifications or lymphadenopathy has not been noted with pure granular cell tumors. See Figs. 3.33 and 3.34.

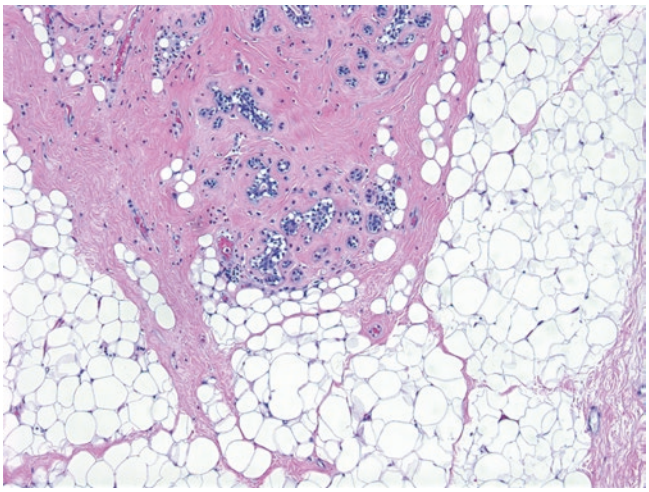

Fig. 3.32 High-power magnification view of an adenolipoma with disorganized benign glandular, adipose, and stromal elements that form a mass with a pseudocapsule or compressed tissue at the borders

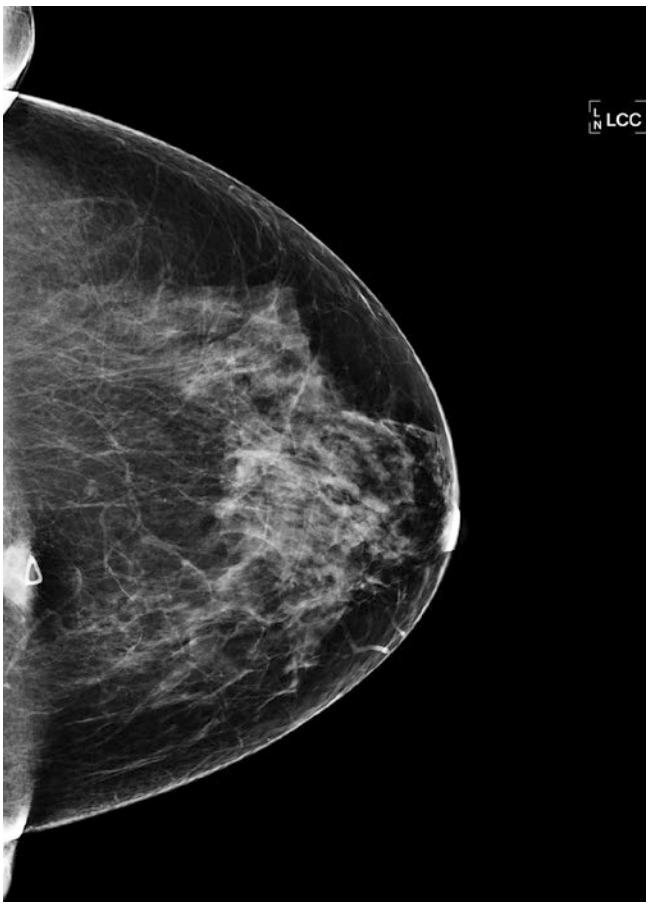

Fig. 3.33 Craniocaudal mammographic view of a palpable granular cell tumor in a female, with a triangle marker overlying the palpable mass, which is at the inframammary crease

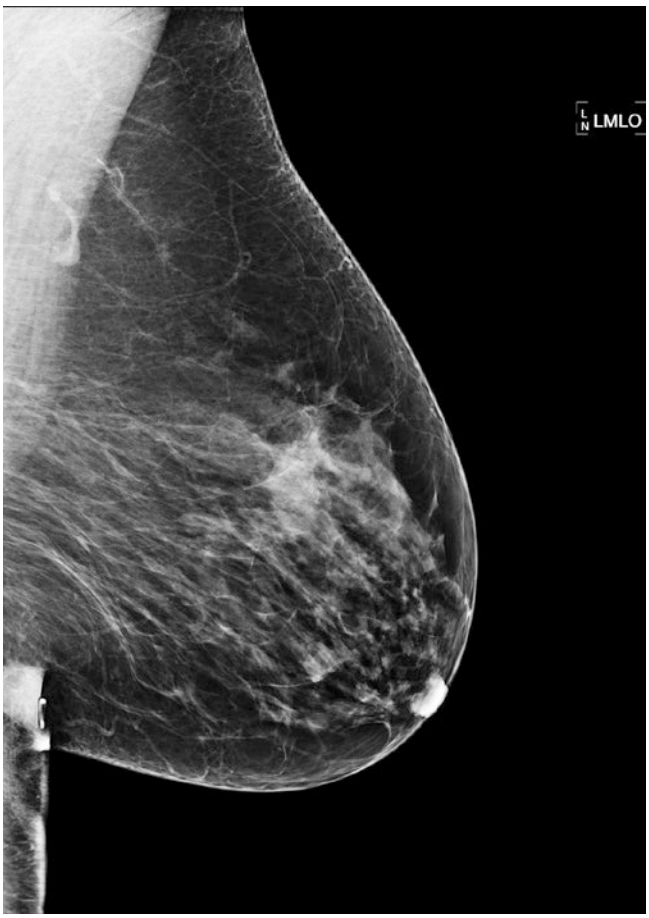

Fig. 3.34 Mediolateral oblique mammographic view of a palpable granular cell tumor in a female, with a triangle marker overlying the palpable mass, which is at the inframammary crease

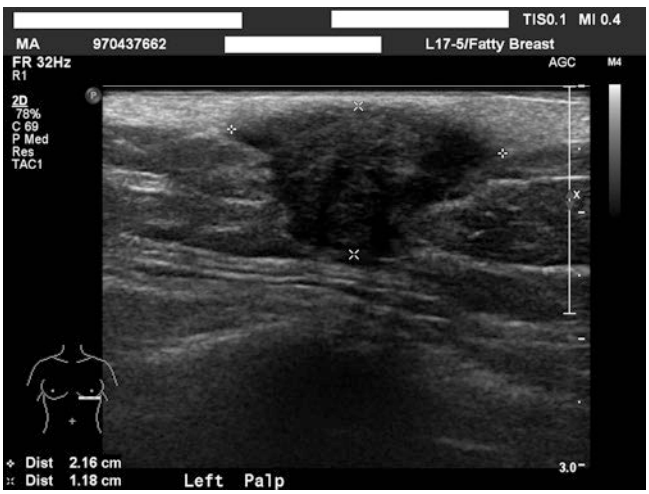

Fig. 3.35 Ultrasound picture of a granular cell tumor with a 2-cm hypoechoic, irregular mass involving the dermis

Ultrasonographically, granular cell tumors can appear as homogeneous or heterogeneously hypoechoic masses with indistinct borders and posterior acoustic shadowing or as anechoic lesions [50–57]. See Fig. 3.35.

Despite the upsurge in MRI use for breast imaging in recent years, there is very little in the literature regarding the appearance of a granular cell tumor with this modality. In addition, the reported MRI findings of granular cell tumors are also

variable. Irregular masses with low-to-high signal intensity on T1-weighted imaging, absent-to-high signal intensity on T2-weighted sequencing, and homogeneous or heterogeneous enhancement on contrast-enhanced T1-weighted imaging have been observed, rendering the MRI a nonspecific tool in identifying granular cell tumors [51, 52, 57].

Pathology

Gross examination of granular cell tumors reveals smooth-surfaced occasionally lobulated firm masses that are gray-white or tan in color. Lesions are generally less than 3 cm but grow up to 6 cm in size [58]. See Fig. 3.36.

Regardless of the site of excision, granular cell tumors appear pathologically similar on gross or histological examination. They are non-encapsulated tumors composed of polygonal cells that may be arranged in groups, sheets, or nests with a granular, eosinophilic cytoplasm and bland nuclei [59, 60]. See Figs. 3.37 and 3.38.

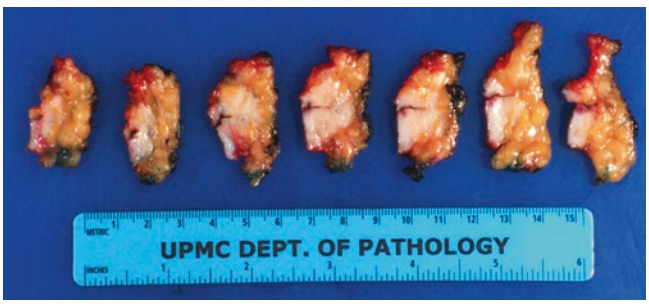

Fig. 3.36 Gross appearance of a granular cell tumor, sectioned. (Photo courtesy of Dr. Kandace McGuire)

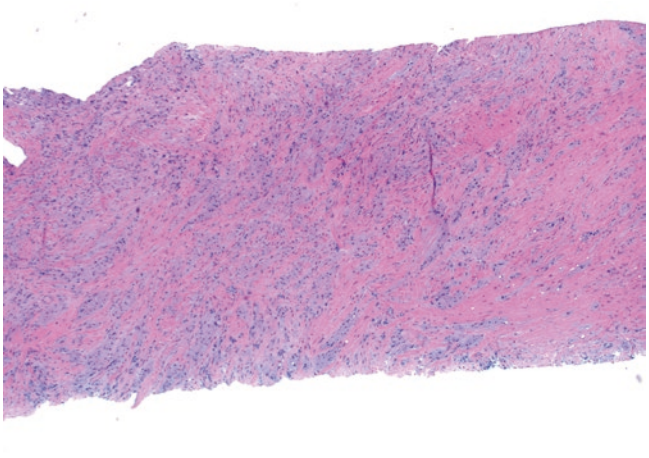

Fig. 3.37 Low-power magnification view of a granular cell tumor composed of polygonal cells that have eosinophilic cytoplasm and bland nuclei

The histological characteristics of granular cell tumors explain the theories behind the perineural origin of the tumor due to its microscopic similarities to Schwann cells, specifically its positive cytoplasmic and nuclear staining for S-100 protein [58–60].

See Fig. 3.39.

Malignant granular cell tumors are rare, occurring in 1–2% of all cases [49, 50, 60]. The histological criteria for malignant granular cell tumors include tumors >5 cm, areas of necrosis within the tumor, high mitotic activity, and nuclear pleomorphism [50, 60].

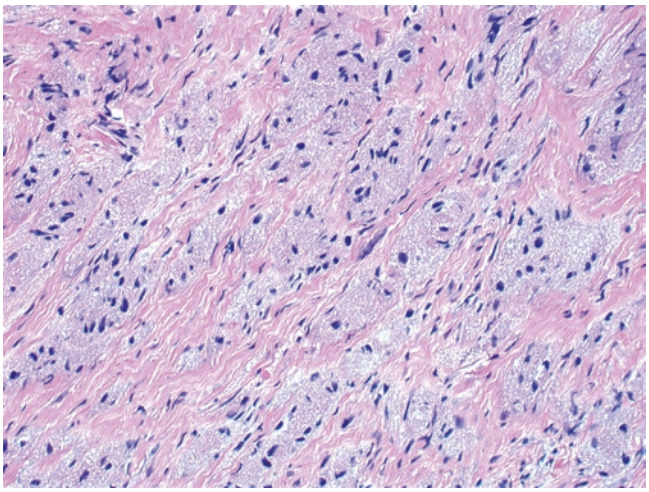

Fig. 3.38 High-power magnification view of a granular cell tumor composed of polygonal cells that have eosinophilic cytoplasm and bland nuclei

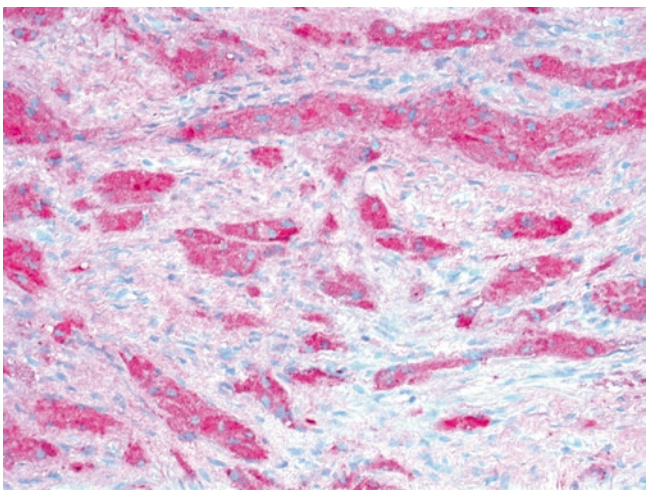

Fig. 3.39 High-power magnification view of a granular cell tumor composed of polygonal cells with positive cytoplasmic and nuclear staining for S-100 protein

Management

A needle biopsy is helpful for establishing the diagnosis of a granular cell tumor; however, wide local excision is still the recommended treatment to exclude coexisting malignant pathology. The lifetime recurrence of granular cell tumors after excision, even in the setting of positive and close margins, is extremely low [56].

There is no role for radiation or chemotherapy in the treatment of granular cell tumors. There is also no evidence of an increased lifetime risk of breast cancer in patients who have been diagnosed with a granular cell tumor.

Radial Scar

Definition/Incidence

A radial scar is a benign breast lesion of unknown origin [61, 62]. The term was first proposed by Dr. H. Hamperl, a pathologist at the University of Bonn, in 1975 [63]. He described the lesion as consisting of a "hyalinized sclerotic center containing abundant elastic and elastoid masses. These radiate into the periphery and enclose lobuli which reveal epithelial proliferation varying from simple hyperplasia with epithelial villi to the rather rare true papillomas [63]."

The term radial scar conventionally denotes pathological size and is used for lesions measuring up to 9 mm, whereas larger lesions are called complex sclerosing lesions [64]. However, other terms have been used to identify this pathological finding, including rosette-like lesions, proliferative centers, borderline breast tumor, sclerosing papillary proliferations, complex compound heteromorphic lesions, sclerosing lesions, benign sclerosing ductal proliferations, non-encapsulated sclerosing lesions, infiltrating epitheliosis, indurative mastopathy, and proliferating center of Aschoff [65]. The current literature most commonly utilizes radial scar and complex sclerosing lesions.

The true prevalence of radial scars is largely unknown due to its asymptomatic nature. Postmortem studies of women of various ages (17–93 years old) estimate the incidence of radial scars as 7–28% [66, 67]. In these reports, multiple radial scars were noted in over half of the women examined at autopsy, with bilateral radial scars in some cases. The estimated detection rate of radial scars as a result of mammogram screening is 0.03–0.09% [68, 69].

Clinical Presentation

Radial scars are now frequently detected in asymptomatic patients as a result of screening mammography but can also present as a painless, firm breast mass once it reaches a considerable size. The concern of carcinoma is always foremost when this clinical presentation is encountered, prompting a comprehensive breast workup and ultimately leading to surgical excision.

Radial scars can occur anywhere in the breast and have no particular predilection for any quadrant. In addition, radial scars or their larger counterpart, complex sclerosing lesions, has not been reported to fixate to the chest wall or involve the overlying skin.

Imaging

The mammographic appearance of radial scars has been well described in the literature according to criteria set forth by Tabar and Dean [70]. The observation of an architectural distortion with a central lucency, radiating, long thin spicules that vary in appearance on different projections in the absence of a palpable clinical finding suggests a radial scar. The absence of microcalcifications has also been noted as a feature of radial scars.

The similar appearance of a radial scar and carcinoma on mammography has led investigators to seek features that may aid the differentiation of these two entities on imaging. In addition, not all radial scars fit the classic criteria, with some lacking the features of a central lucency, others presenting solely with calcifications, and even others displaying similar features of conspicuity variation on different projections, precluding the ability to distinguish carcinoma from a radial scar [68, 71, 72]. These characteristics often lead to a mammographic classification of a suspicious lesion, which prompts further imaging and eventual biopsy. See Fig. 3.40.

Ultrasound provides little additional information for distinguishing radial scars from malignant lesions but can be utilized to more easily localize the lesion for surgical excision or facilitate percutaneous biopsy [61, 65, 68, 73]. The most frequent findings on ultrasound are hypoechoic masses or parenchymal distortion, but up to 1/3 of radial scars may not be visible by this modality [61, 73]. See Fig. 3.41.

MRI evaluation holds promise as an adjunctive test for distinguishing radial scars from malignancy, with the understanding that not all mammographically detected radial scars are visualized on MRI [61, 74]. However, pure radial scars that are appreciated on MRI may exhibit characteristics suggesting their benign nature. Several studies have been published to establish the high negative predictive value of the MRI for high-risk lesions, including radial scars specifically [75–77].

Pathology

The gross pathology of a radial scar reveals a firm lesion with a pale core, irregular edges, and yellowish radiating

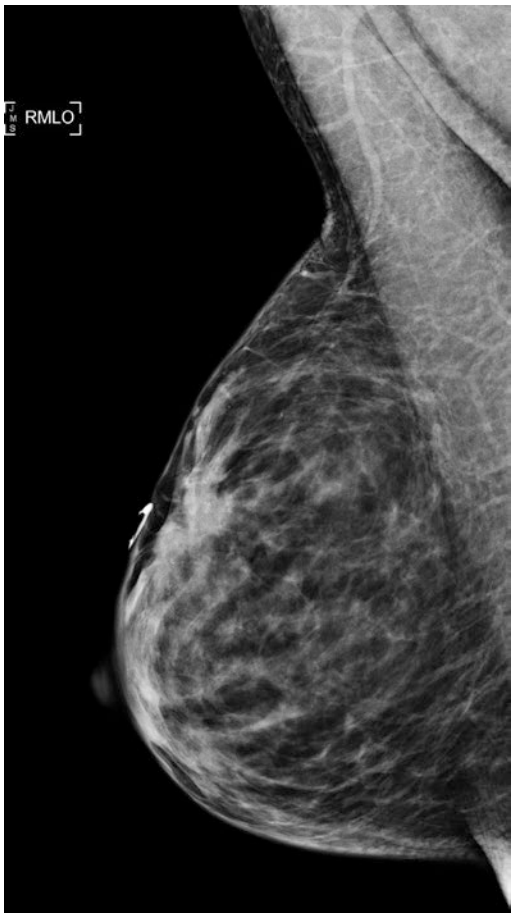

Fig. 3.40 Mediolateral oblique mammogram of a palpable radial scar. There is a spiculated mass with associated suspicious calcifications and a triangle marker denoting the area of the palpable mass

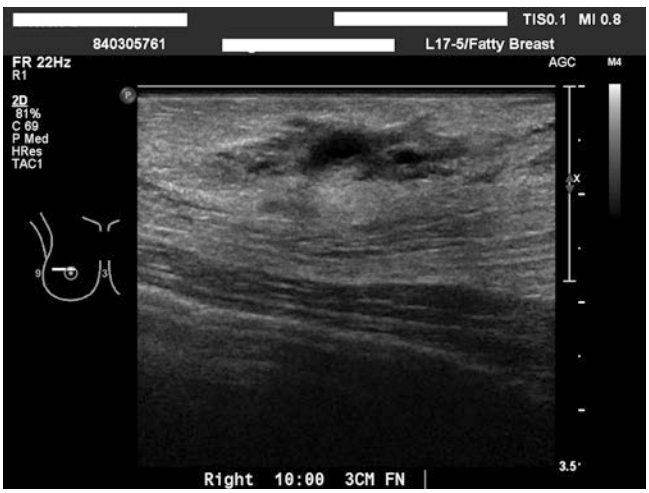

Fig. 3.41 Ultrasound findings of a radial scar denoting an irregularly shaped, irregularly marginated hypoechoic lesion, mimicking the appearance of a malignancy

streaks that appear to be infiltrating the surrounding normal breast tissue [65, 68]. These findings are also consistent with carcinomas; hence, gross examination alone is often not helpful in arriving at the diagnosis.

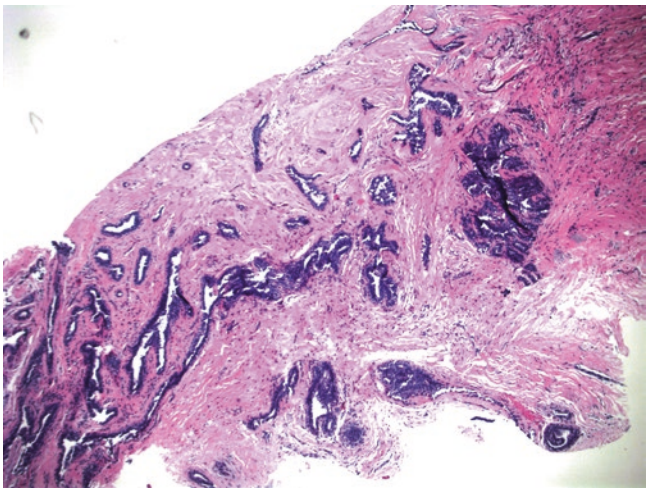

Fig. 3.42 Low-power magnification view of a radial scar with a fibroelastotic core and entrapped radiating ducts and lobules with variable levels of proliferation

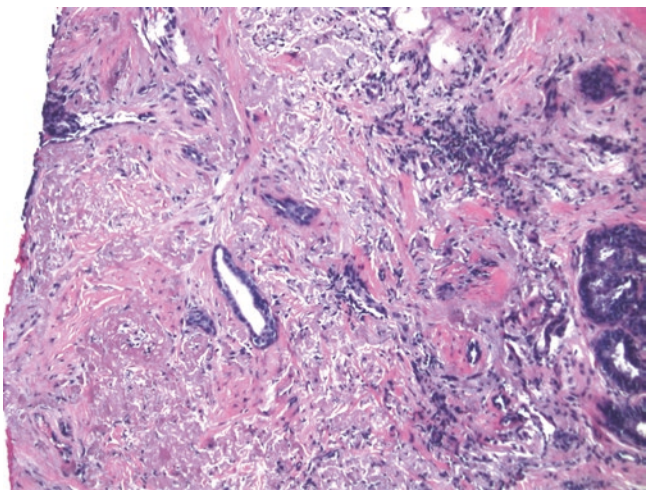

Fig. 3.43 High-power magnification view of a radial scar with a fibroelastotic core and entrapped radiating ducts and lobules with variable levels of proliferation

One clue that may point to a radial scar on gross examination is the finding of surrounding microcysts, which may be present in radial scars but are not typically seen in invasive disease [78].

Histologically, radial scars exhibit a fibroelastotic core with entrapped ducts and radiating ducts and lobules at varying levels of proliferation [65]. These will resemble the spokes in a wheel and are best appreciated on low-power magnification. Calcifications can also be appreciated within radial scars. In addition, radial scars can be associated with atypical lesions, lobular neoplasia, and in situ or invasive carcinomas. See Figs. 3.42 and 3.43.

It is often challenging to differentiate radial scars from invasive carcinomas, particularly tubular types, due to their similar appearance. The addition of immunohistochemical

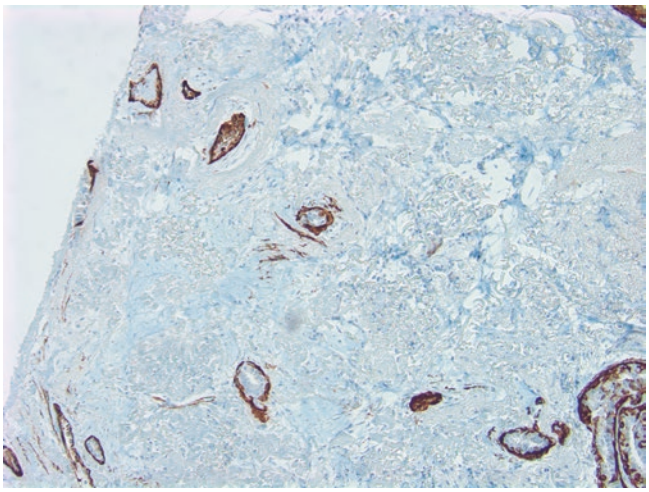

Fig. 3.44 High-power magnification view of a radial scar with positive smooth muscle myosin heavy chain staining

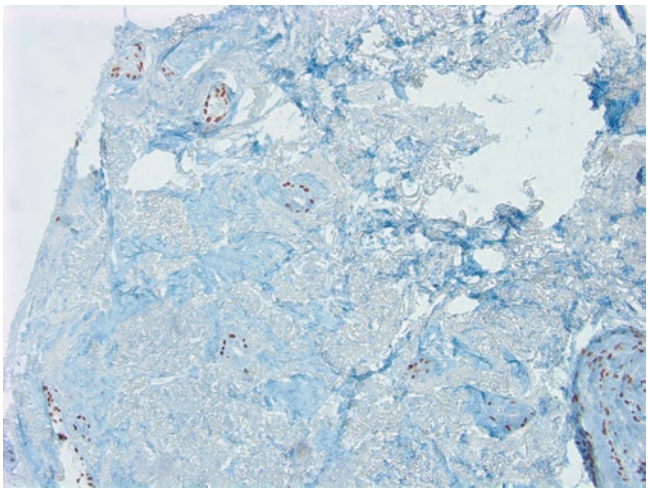

Fig. 3.45 High-power magnification view of a radial scar with positive p63 staining

staining for myoepithelial markers such as smooth muscle actin, calponin, smooth myosin heavy chain, or p63 can help distinguish these two entities. See Figs. 3.44 and 3.45.

Management

Surgical excision is still justified because of the variable upstage rate of radial scars diagnosed on percutaneous biopsy and because no imaging modality has been proven to reliably guarantee the benign nature of such lesions. A current review of the literature indicates an upstage rate of 0–32% (Table 3.2).

There may be a trend toward observation and follow-up, particularly in completely excised microscopic radial scars, radial scars without atypia, or radial scars that on MRI are non-enhancing based upon more recent evidence regarding the low likelihood of malignancy in these settings [75, 77, 82, 84].

Table 3.2 Studies of radial scars and rates of upstage to in situ or invasive carcinoma

	N = radial scar w/ and w/o atypia	Upstage to in situ or invasive carcinoma (%)
Brenner et al. [79]	157	8
Cawson et al. [80]	75	7
Lopez-Medina et al. [81]	43	19
Manfrin et al. [82]	117	32
Linda et al. [72]	62	8
Resetkova et al. [83]	19	0
Rahka et al. [84]	329	13
Lee et al. [77] (microscopic radial scars)	18	0
Bianchi et al. [85]	49	8
Toth et al. [69]	45	25
Linda et al. [75]	35	6
Morgan et al. [86]	67	9
Andacoglu et al. [87]	67	6

Differential Diagnosis and Breast Cancer Risk

The differential diagnosis for radial scars diagnosed on imaging varies widely from carcinoma to fat necrosis and even postoperative changes. A thorough history and physical examination should help eliminate or rule out these other causes.

With regard to its classification as a high-risk lesion, the evidence in the literature regarding future breast cancer risk in patients diagnosed with radial scars remains contradictory. Patterson in 2004, Berg in 2008, and Bunting in 2011 all report no increased incidence in breast cancer in women diagnosed with radial scars over follow-up periods of 5–17 years compared with the normal risk population [62, 88, 89]. However, in 2008, Manfrin reported an increased risk of breast cancer in women diagnosed with radial scars as a function of age [82]. However, this study did not follow these patients on a longitudinal basis but instead assessed risk at the time of radial scar diagnosis and concluded that older age conferred a higher risk of breast cancer. More recently, an update from the Nurses' Health Study also concluded that radial scars appear to be an independent risk factor for breast cancer. They identified 460 cases of radial scar with a mean follow-up period of 9 years. The risks for breast cancer in women >50 years and the development of hormone receptor-negative breast cancers among all women were higher [90].

Gynecomastia

Definition/Prevalence

Gynecomastia is defined as benign male breast enlargement that can be unilateral or bilateral, painless, or tender and is a result of glandular proliferation and local fat deposition. These characteristics differ from those of pseudogynecomastia, which is commonly observed in obese males and is characterized by excess fat deposition without glandular proliferation [91]. In addition to physical symptoms, gynecomastia can have a negative psychological impact on affected individuals, prompting patients to seek treatment [92, 93].

Gynecomastia is a common occurrence with three distinct peaks of incidence: the neonatal period, adolescence, and old age [91, 94, 95]. It is observed in approximately 75% of neonates [96]. Braunstein's 1993 review of gynecomastia in the adolescent age group revealed an incidence of 4–69% [94]. In older age, gynecomastia has been reported in up to 55% of autopsies, 57% of healthy older men, and 70% of hospitalized elder men [94–96].

Considering the high prevalence and varied causes of gynecomastia, understanding the etiology of the patient's gynecomastia is the clinician's best approach for successful management.

Etiology/Pathophysiology

The root cause of gynecomastia is an imbalance of estrogen and androgen levels in male breast tissue [91–97]. The male breast has both estrogen and androgen receptors. Estrogen promotes glandular proliferation, whereas androgen inhibits it. Disproportionate activity of estrogen relative to androgens in breast tissue leads to gynecomastia. This imbalance may have several origins, including increased circulating levels of estrogen produced by the adrenal glands or testes, increased peripheral aromatization of estrogen precursors, exposure to estrogen-like substances, medications that cause the release of more estrogen than androgen from sex hormone-binding globulin (SHBG), decreased androgen production by the testes or altered metabolism, a medication-induced shift of androgens from their receptors, or androgen receptor defects [91].

In the neonatal period, gynecomastia is a result of transplacental transfer of maternal estrogens, as evidenced by a small breast bud that is transient and spontaneously resolves over time [96]. No further treatment is required beyond reassurance.

Adolescent gynecomastia is typically noted at puberty, with a peak age of onset at 13 or 14, and results from peripheral aromatization of circulating androgens [98]. There is a 30-fold increase in testosterone concentration versus a three-fold increase in estrogen levels during the shift from prepuberty to puberty in boys, and a disproportionate increase in hormone levels during this phase can cause transient pubertal gynecomastia [99]. As with neonatal gynecomastia, this condition frequently resolves in 1–3 years, requiring only reassurance and surveillance as treatment. However, approximately 8% of adolescent gynecomastia will continue into adulthood [92]. Many of these patients will seek treatment due to psychological distress, including anxiety, embarrassment, and concern for malignancy, that can be associated with persistent gynecomastia [100].

Table 3.3 Causes of gynecomastia [91, 94, 101]

Idiopathic	–
Physiological	Neonatal period, adolescence, and older men
Medication induced	Antiandrogens Antibiotics Antihypertensives Chemotherapeutic agents Psychoactive agents Diuretics Cardiovascular drugs Gastrointestinal drugs Drugs of abuse
Endocrine dysfunction	Hypogonadism Hyperthyroidism Obesity
Endocrine tumors	Testicular tumors Adrenocortical tumors Tumors secreting ectopic β-hCG Pituitary tumors
Chronic diseases	Renal disease Cirrhosis HIV

Adult gynecomastia can be due to a variety of causes, and understanding the specific etiology facilitates the identification of the appropriate treatment. Gynecomastia in older age can be idiopathic or physiological and due to medications, chronic diseases such as cirrhosis or renal disease, endocrine tumors, or endocrine dysfunction [91]. The decrease in plasma testosterone in older men in association with increased peripheral conversion of androgens to estrogens may cause physiological or so-called senile gynecomastia [101]. Tables 3.3 and 3.4 illustrate the other causes of gynecomastia, including medications that cause gynecomastia.

Clinical Evaluation and Workup

Most cases of gynecomastia for which consultation is sought are either idiopathic (25%), acute, or persistent gynecomastia in puberty (25%) or due to medication (10–20%) [94, 96–98]. Other causes include cirrhosis or malnutrition (8%), hypogonadism (8%), and renal disease (1%) [94].

Table 3.4 Commonly used medications that cause gynecomastia [91, 94, 97, 101]

Antiandrogens	Bicalutamide, flutamide, nilutamide, finasteride, dutasteride
Antibiotics	Ketoconazole, isoniazid (rare) [97]
Gastrointestinal drugs	Histamine$_2$ blockers, omeprazole
Diuretics	Spironolactone
Cardiac drugs	Amiodarone, amlodipine, captopril, enalapril, nifedipine, digoxin, reserpine, verapamil, diltiazem
Chemotherapeutic agents	Methotrexate, cyclophosphamide, carmustine, etoposide, melphalan, cisplatin, vincristine, actinomycin D, procarbazine
Antipsychotics	Diazepam, tricyclic antidepressants, haloperidol, phenothiazine, olanzapine, ziprasidone
Recreational drugs	Alcohol, amphetamines, heroin, cocaine, marijuana, anabolic androgens (abuse in athletes)
Antiretrovirals	Efavirenz, saquinavir, indinavir, nelfinavir, ritonavir, lopinavir, stavudine
Others	Phenytoin, penicillamine, statins, theophylline

In approximately 3% of cases, gynecomastia is attributable to a testicular tumor [94, 96].

As with other conditions, a thorough history and physical examination are imperative in the evaluation of gynecomastia. Specific attention to underlying disease or medications known to cause gynecomastia may help identify the problem, although half of the cases are idiopathic or physiological.

A physical exam with the patient disrobed, in the sitting and supine position, and with hands raised and at the patient's side should be performed. In addition, the supraclavicular, infraclavicular, and axillary lymph node basins must be evaluated.

A distinguishing feature of gynecomastia is concentric enlargement of the breast as opposed to other types of breast masses seen in males, which will more often have an eccentric growth pattern. This growth pattern can be determined during palpation of the nipple areolar complex, where a disk of breast tissue can be palpated in the subareolar region [91, 95, 101, 102]. Gynecomastia is frequently bilateral, although up to 50% of cases are unilateral [95, 103]. This distribution is in contrast to pseudogynecomastia, which is observed in obese men and is characterized by excess fat deposition without concomitant ductal proliferation. In this situation, soft, bilaterally enlarged breasts are palpated without any distinct breast tissue in the subareolar region [95, 102]. In addition, breast masses that are not centrally located or have associated lymphadenopathy, skin changes, or nipple retraction must raise the suspicion for diseases other than gynecomastia. Nipple discharge is also uncommon in true gynecomastia [91].

Gynecomastia is frequently painless, asymptomatic, and detected incidentally on routine physical exams. However, it can be associated with pain, particularly in the setting of recent-onset gynecomastia [96].

It is imperative to rule out the possibility of carcinoma, particularly in unilateral cases and in patients who have a family history of breast cancer. The mammographic and sonographic characteristics of gynecomastia are dependent on the acuity or chronicity of the condition and described as having a nodular, dendritic, or diffuse glandular pattern [29, 102, 104]. In recent-onset gynecomastia (<1 year), the florid phase of proliferation of ducts and stromal tissue mammographically reveals a "fan-shaped" subareolar density that merges into the surrounding tissue, and sonography reveals a hypoechoic subareolar mass surrounded by fatty tissue [102, 104]. This stage is reversible because fibrosis has not yet occurred. The dendritic pattern is observed in the more chronic phase of gynecomastia in which irreversible fibrosis has occurred. The flame or cone-shaped subareolar density can be seen infiltrating the deeper, surrounding fat and can even permeate the upper outer quadrants of the breast on mammogram [29, 104]. The diffuse glandular pattern is observed in patients who are treated with high-dose estrogen therapy and have a mammographic and sonographic appearance similar to the female dense breast [29]. Any imaging characteristics that are suspicious warrant percutaneous biopsy and radiologic-pathologic correlation, as in women. The sensitivity and specificity for a mammogram in detecting breast malignancies in males are 92 and 90%, respectively [98, 102]. See Figs. 3.46, 3.47, and 3.48.

Adjunctive laboratory testing may be justified when imaging is benign, and there is a high suspicion that the gynecomastia is representative of an underlying pathology. Some of these tests include determining testosterone, β-hCG, luteinizing hormone, thyroid-stimulating hormone, and prolactin levels [91, 94, 95, 98]. Abnormal results should prompt consultation with an endocrinologist. In addition, liver and renal function testing may reveal chronic hepatic or kidney disease as the cause of the gynecomastia.

Pathology

Gross examination of a male breast with gynecomastia reveals a gray-white, rubbery mass in the subareolar region. As previously mentioned, there are two phases of gynecomastia: the florid (reversible) phase and fibrotic (irreversible) phase [105].

The florid phase is typically observed in the first year of onset. Histologically, there is benign proliferation of ductal epithelium and stromal elements, with periductal inflammation and surrounding edema without evidence of fibrosis [102, 105]. In the absence of fibrosis, nonsurgical

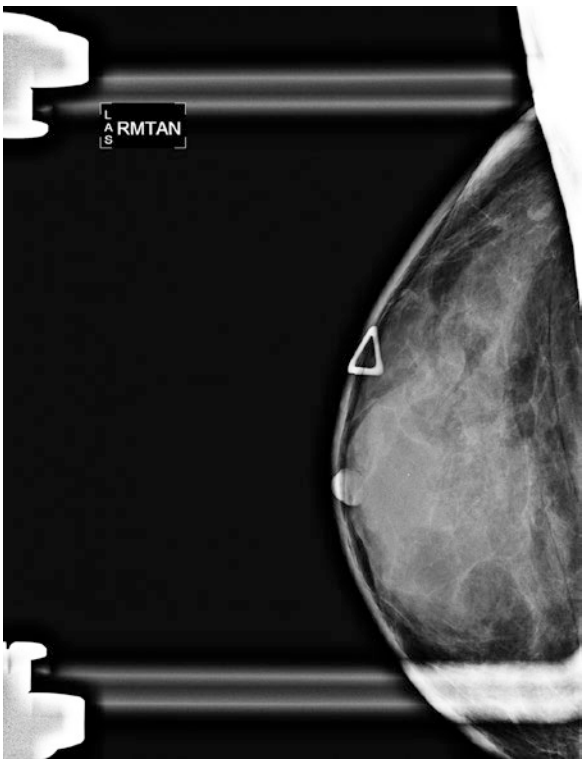

Fig. 3.46 Magnified tangential mammogram of gynecomastia. There is a large asymmetric density without a discrete mass, suspicious calcifications, or architectural distortions

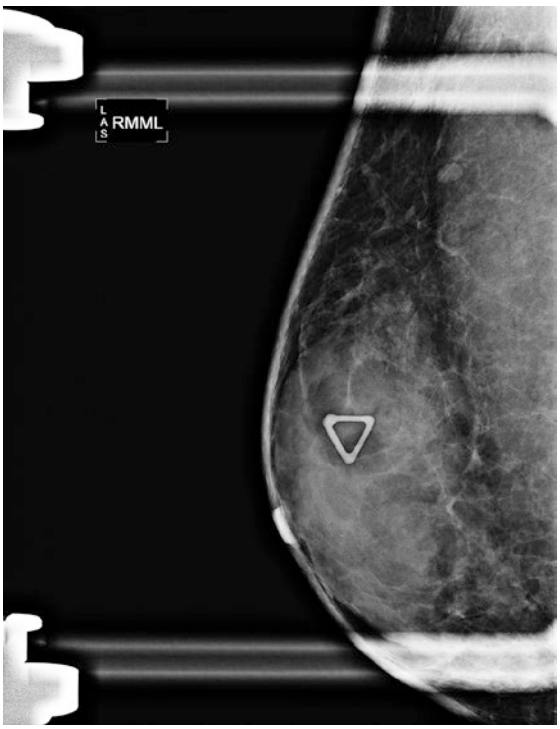

Fig. 3.47 Magnified mediolateral mammogram of gynecomastia. There is a large asymmetric density without a discrete mass, suspicious calcifications, or architectural distortions

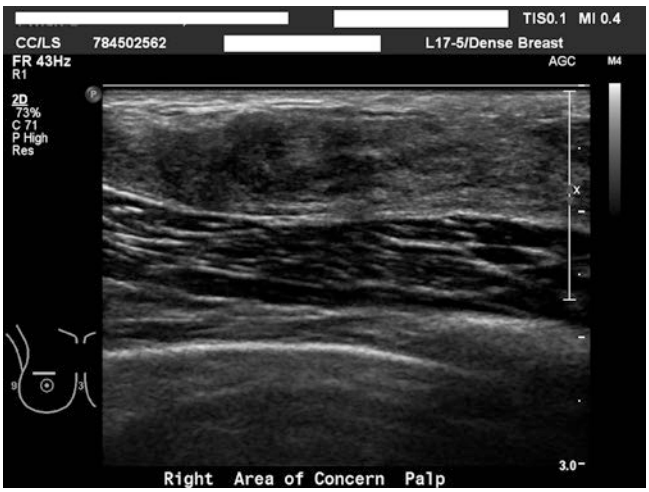

Fig. 3.48 Ultrasound findings of gynecomastia. There is absence of a focal mass but with findings of heterogeneous tissue with ill-defined hypoechoic areas

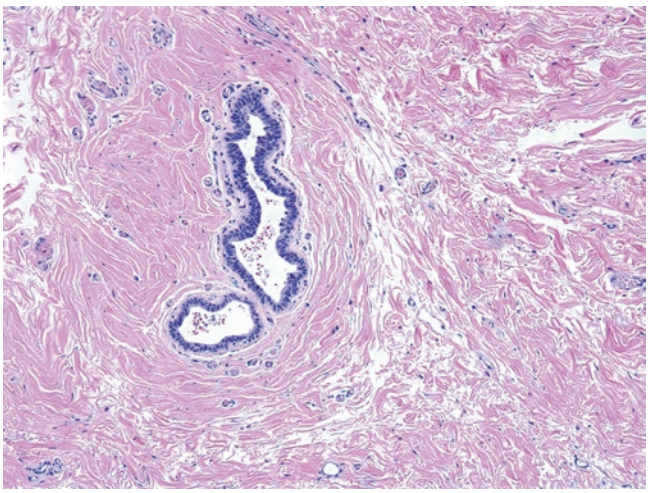

Fig. 3.49 High-power magnification view of gynecomastia with benign proliferation of ductal epithelium and stromal elements

treatment may be successful, and gynecomastia may be reversible [95]. See Fig. 3.49.

In the fibrotic phase, which can be observed beyond 6 months, there is minimal ductal proliferation and hyalinized periductal tissue [106]. If treatment is desired in this phase, only surgical options are feasible.

Management

Most patients with gynecomastia may experience a resolution of symptoms upon removal of the precipitating cause or treatment of the underlying condition.

When medication-induced gynecomastia precludes withdrawal of the drug due to medical necessity, tamoxifen therapy may reverse the condition. Small studies have indicated gynecomastia in up to 80% of patients and even prevention if used in a prophylactic manner, particularly in the setting of treatment for prostate cancer [107–109]. The US Food and Drug Administration, however, has not approved tamoxifen for this indication upon writing of this text [97]. Therefore, before initiating therapy, the patient must be counseled regarding current evidence, and the risk/benefit ratio must be considered on an individual basis.

Another strategy that deserves comment in the treatment and prophylaxis of gynecomastia induced by prostate cancer therapy is low-dose radiation. A few European studies have established the efficacy of radiotherapy in both treatment and prophylaxis, with minimal and transient side effects, making it an acceptable alternative for patients [96, 110]. However, large-scale studies and standardized regimens are lacking.

Surgery is still the mainstay of treatment for long-standing gynecomastia. It is the tenth most common procedure performed by plastic surgeons worldwide [106]. However, the types of procedures performed for gynecomastia are evolving and trending toward less invasive techniques with smaller incisions. The subcutaneous mastectomy with a periareolar or inframammary incision is still standard (with or without resection of excess skin), but the addition of liposuction may be beneficial in patients with a greater degree of ptosis [100, 103, 106].

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Epidemiology, Risk Factors, and Prevention

4

Soley Bayraktar and Banu K. Arun

Epidemiology of Breast Cancer

In the United States, breast cancer is the most frequently diagnosed cancer in women. In 2014, an estimated 232,670 new cases of invasive breast cancer were expected to be diagnosed in women in the United States, along with 62,570 new cases of noninvasive (in situ) disease. Approximately 40,000 women in the United States were expected to die in 2014 from breast cancer, although death rates have been decreasing since 1989, with larger decreases in women under 50 (American Cancer Society, www.cancer.org). Breast cancer accounts for 29% of all cancers diagnosed and 16% of all cancer deaths in US women [1].

Globally but particularly in developed countries, breast cancer is a major public health problem, with one million new cases diagnosed annually [2]. The lowest incidence rates of breast cancer are in Asian countries (10–15 cases/100,000 women) [2, 3]. However, incidence rates have increased rapidly in countries such as Japan, where major lifestyle changes have occurred in the last 50 years. When women from Asia or other low-risk areas migrate to an area of high risk, they gradually assume the risk of the high-risk population [4]. Interestingly, the difference in breast cancer incidence between Asian and Western populations is primarily the result of much lower incidence rates of postmenopausal breast cancer in Asian countries because premenopausal breast cancer rates are similar in Asian and Western countries [5]. These data suggest that the underlying genetic factors that primarily contribute to breast cancer in young women are similar in both populations, but hormone exposure and lifestyle factors that vary widely between continents may play an important role in defining postmenopausal breast cancer risk.

In the United States, approximately one in eight US women (approximately 12%) will develop invasive breast cancer over the course of her lifetime [6]. However, more than half of this risk is incurred after 60 years of age, and the risk of one in eight is not reached until 110 years of age [7] (Fig. 4.1). In addition, risk is very heterogeneous across the population. Therefore, individual risk assessment is considerably more useful than population risk in the development of clinical management strategies.

Breast cancer incidence rates in the United States began decreasing in 2000 after increasing for the previous two decades. They decreased by 7% from 2002 to 2003 alone. This decrease may be due in part to the reduced use of hormone replacement therapy (HRT) by women after the results of a large study called the Women's Health Initiative were published in 2002. These results suggested a connection between HRT and increased breast cancer risk. White women

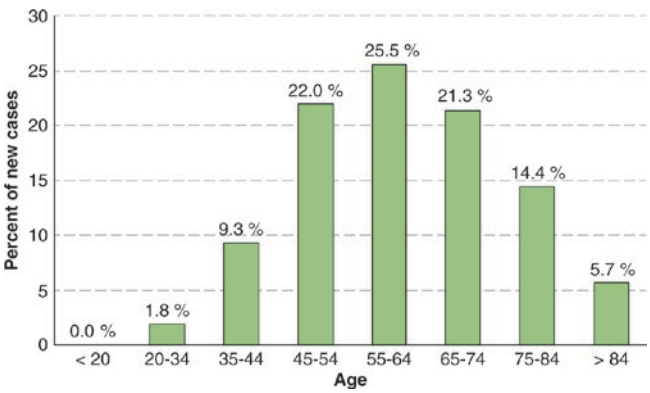

Fig. 4.1 Percent of new breast cancer cases by age group. (Surveillance, Epidemiology, and End Results [SEER], 2011, http://seer.cancer.gov/) [8]

S. Bayraktar (✉)
Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Department of Medical Oncology, Mercy Cancer Center, Ardmore, OK, USA

B. K. Arun
Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: barun@mdanderson.org

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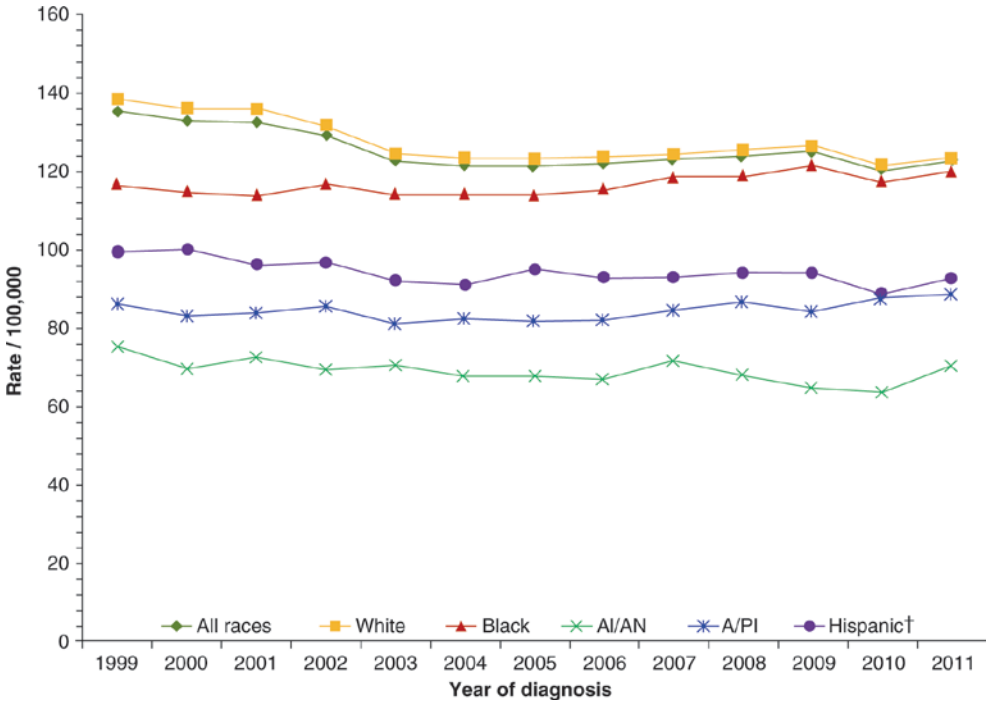

Fig. 4.2 Incidence rates* of female breast cancer by race and ethnicity, USA, 1999–2011. Combined data from the National Program of Cancer Registries as submitted to the Centers for Disease Control and Prevention (CDC) and from the Surveillance, Epidemiology, and End Results (SEER) program as submitted to the National Cancer Institute (NCI) in November 2013 [9]
*Rates are per 100,000 and are age adjusted to the 2000 US standard population (19 age groups Census P25–1130). Incidence rates are for state registries that meet USCS publication criteria for all years, 1999–2011. Incidence rates cover approximately 99% of the US population
†Hispanic origin is not mutually exclusive with other race categories (white, black, Asian/Pacific Islander, American Indian/Alaska Native)

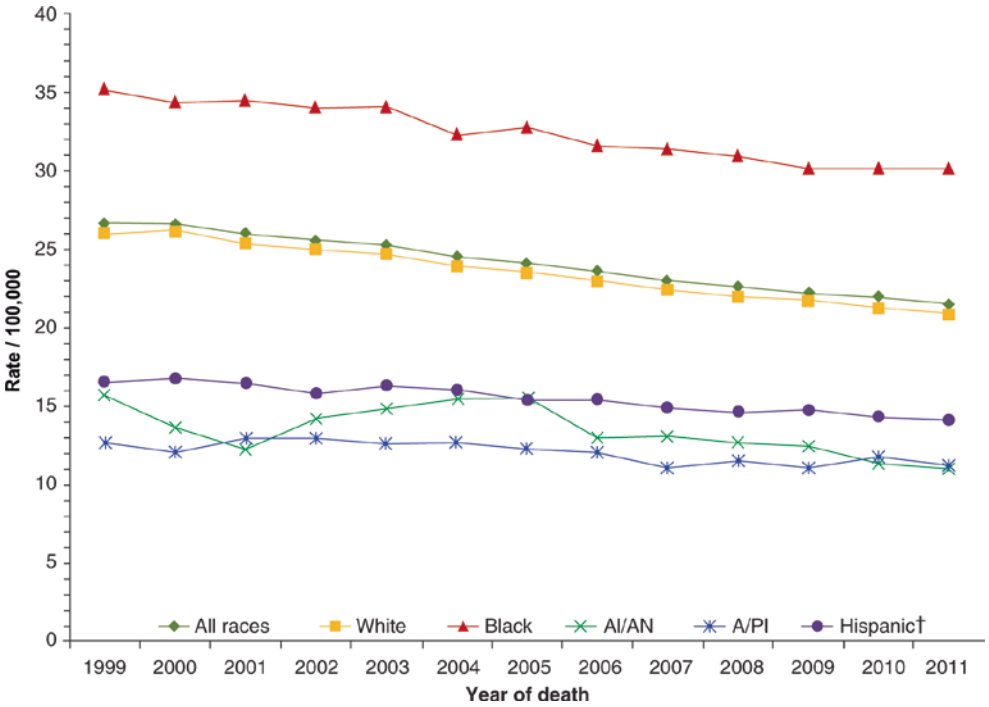

Fig. 4.3 Death rates* by race and ethnicity, USA, 1999–2011 US mortality files, National Center for Health Statistics and Centers for Disease Control and Prevention (CDC) [10]
*Rates are per 100,000 and are age adjusted to the 2000 US standard population (19 age groups Census P25–1130). Death rates cover 100% of the US population
†Hispanic origin is not mutually exclusive with other race categories (white, black, Asian/Pacific Islander, American Indian/Alaska Native)

are slightly more likely to develop breast cancer than African-American women (Fig. 4.2). However, in women under 45, breast cancer is more common in African-American women than white women. Overall, African-American women are more likely to die of breast cancer. Asian, Hispanic, and Native-American women have a lower risk of developing and dying from breast cancer [6] (Fig. 4.3).

Breast Cancer Risk Factors: Nongenetic and Inherited Genetic Factors

The risk factors that are associated with the development of breast cancer are summarized in Table 4.1 and are discussed briefly in the next section. Age, reproductive factors, personal or family history of breast disease, genetic

Table 4.1 Risk factors for breast cancer

Risk factor	Category at risk	Comparison category	Relative risk
Alcohol intake [11]	Two drinks per day	Nondrinker	1.2
Body mass index [12]	80th percentile, age 55 or greater	20th percentile	1.2
Hormone replacement therapy with estrogen and progesterone [13]	Current user for at least 5 years	Never used	1.3
Radiation exposure [14, 15]	Repeated fluoroscopy Radiation therapy for Hodgkin's disease	No exposure	1.6 5.2
Early menarche [16]	Younger than 12 years	Older than 15 years	1.3
Late menopause [17]	Older than 55 years	Younger than 45	1.2–1.5
Age at first childbirth [18, 19]	Nulliparous or first child after 30	First child before 20	1.7–1.9
Current age [20]	65 or older	Less than 65	5.8
Past history of breast cancer [21]	Invasive breast carcinoma	No history of invasive breast carcinoma	6.8
Other histologic findings [22]	Lobular carcinoma in situ Ductal carcinoma in situ	No abnormality detected	16.4 17.3
Breast biopsy [23]	Hyperplasia without atypia Hyperplasia with atypia Hyperplasia with atypia and positive family history	No hyperplasia	1.9 5.3 11
Cytology (fine needle aspiration) [24]	Proliferation without atypia Proliferation with atypia Proliferation with atypia and positive family history	No abnormality detected	2.5 4.9–5 18.1
Family history [25]	First-degree relative 50 years or older with postmenopausal breast cancer First-degree relative with premenopausal breast cancer Second-degree relative with breast cancer Two first-degree relatives with breast cancer	No first- or second-degree relative with breast cancer	1.8 3.3 1.5 3.6
Germline mutation [26]	Heterozygous for BRCA1, age < 40 Heterozygous for BRCA1, age 60–69	Not heterozygous for BRCA1	200 15

Adapted from Singletary [27]

predisposition, and environmental factors have all been associated with an increased risk of developing female breast cancer.

Age

The risk of developing breast cancer increases with age. According to the Surveillance, Epidemiology, and End Results (SEER) database, the probability of a woman in the United States developing breast cancer is 1 in 8 over a lifetime: 1 in 202 from birth to age 39 years of age, 1 in 26 from 40 to 59 years, and 1 in 28 from 60 to 69 years [1]. Young women who develop breast cancer appear to have worse disease-free survival (DFS) and overall survival (OS) and present with more aggressive-appearing biological characteristics than older women [28].

Personal History

A personal history of breast cancer is also a significant risk factor for the development of a second ipsilateral or contralateral breast cancer. In fact, the most common cancer among breast cancer survivors is metachronous contralateral breast cancer [29]. Factors associated with an increased risk of a second breast cancer include an initial diagnosis of ductal carcinoma in situ (DCIS), stage IIB, hormone receptor-negative cancers, and young age [30].

Breast Pathology

Proliferative breast disease is associated with an increased risk of breast cancer. Proliferative breast lesions without atypia, including usual ductal hyperplasia, intraductal papillomas, sclerosing adenosis, and fibroadenomas, confer only a small increased risk of breast cancer development, approximately 1.5–2 times that of the general population [31, 32]. Atypical hyperplasia, including both ductal and lobular, which are usually incidentally found during screening mammography, confers a substantial increased risk of breast cancer. Women with atypia have an approximately 4.3 times greater risk of developing cancer compared with the general population [23, 33].

Family History

A woman's risk of breast cancer is increased if she has a family history of the disease. In the Nurses' Health Study follow-up, women with a mother diagnosed before age 50 had an adjusted relative risk (RR) of 1.69, and women with a mother diagnosed at 50 or older had an RR of 1.37 compared with women without a family history of breast cancer. A history of a sister with breast cancer was associated with an increased RR of 1.66 if the diagnosis was made prior to age 50 and a RR of 1.52 if diagnosed after age 50 compared with patients without a family history [33]. The highest risk is associated with an increasing number of first-degree relatives diagnosed with breast cancer at a young age (younger than 50). Compared with women who had no affected relative, women who had one, two, or three or more affected first-degree relatives had risk ratios of 1.80, 2.93, and 3.90, respectively [34].

Endogenous Hormone Exposure and Reproductive Factors

Early Menarche

Early age at menarche is a risk factor among both pre- and postmenopausal women for developing breast cancer. Delay in menarche by 2 years is associated with a corresponding risk reduction of 10% [35]. Within the European Prospective Investigation into Cancer and Nutrition cohort, women who had early menarche (≤13 years) exhibited a nearly twofold increase in the risk of hormone receptor-positive tumors [36]. Among women with BRCA1 mutation-associated breast cancers, later age at menarche was associated with a later age at breast cancer diagnosis [37].

Parity and Age at First Full-Term Pregnancy

Nulliparous women are at an increased risk for the development of breast cancer compared with parous women. Young age at first birth has an overall protective effect, whereas relatively advanced age at first birth confers a relative risk of breast cancer greater than that of a nulliparous woman. Compared with nulliparous women, the cumulative incidence of breast cancer in women experiencing their first birth at age 20, 25, and 35 years was 20% lower, 10% lower, and 5% higher, respectively [38].

Breast-Feeding

Evidence suggests that breast-feeding has a protective effect against the development of breast cancer. Breast-feeding may delay the return of regular ovulatory cycles and decrease endogenous sex hormone levels. There is an estimated 4.3% reduction for every 1 year of breast-feeding [39].

Testosterone

High endogenous sex hormone levels increase the risk of breast cancer in both premenopausal and postmenopausal women. High levels of circulating testosterone in postmenopausal women have been linked to an increased risk of developing breast cancer (RR, 2.86–3.28) [40].

Age at Menopause

Later onset of menopause has also been associated with increased breast cancer risk. Every year of delay in the onset of menopause confers a 3% increase in risk, and every 5-year delay in the onset of menopause confers a 17% increase in the risk of breast cancer [35, 41].

Exogenous Hormone Exposure

Evidence suggests a relationship between the use of hormone replacement therapy (HRT) and breast cancer risk. Breast cancers related to HRT use are usually hormone receptor positive. When compared with patients who did not use HRT, breast cancer risk is higher in HRT users [42]. An international meta-analysis examining the risk of breast cancer with HRT found that in women who did not use HRT, the RR increased by a factor of 1.28 for each year older at menopause, comparable to the RR of 1.023 per year in women who used HRT or for those who ceased to use HRT up to 4 years previously [43].

In the Women's Health Initiative randomized controlled trial, combined estrogen plus progestin in postmenopausal women with an intact uterus significantly increased the risk of breast cancer, delayed breast cancer detection and diagnosis, and significantly increased breast cancer mortality. The study was terminated early because of increased mortality in the combined estrogen plus progestin group. By contrast, the use of estrogen alone by postmenopausal women without a uterus did not interfere with breast cancer detection and significantly decreased the risk of breast cancer [44]. Data from the Nurses' Health Study, however, suggest that women who use unopposed postmenopausal estrogen increase their risk of breast cancer by 23% at age 70 [45].

Timing and duration of HRT seem to be important factors associated with breast cancer risk as well. Breast cancer risk from exogenous hormone exposure is inversely associated with time from menopause. Women who initiate hormone therapy closer to menopause have a higher breast cancer risk [46]. Long-term (>5 years) combined HRT use has been associated with the highest risk, whereas short-term use of combined estrogen–progestin therapy does not appear to confer a significantly increased risk (RR = 1.023 per year) [43].

Lifestyle Factors

Modifiable risk factors, including the excessive use of alcohol, obesity, and physical inactivity, account for 21% of all breast cancer deaths worldwide [47].

Alcohol Consumption

Alcohol consumption has been significantly associated with increased breast cancer risk at consumption levels as low as 5.0–9.9 g per day, which is equivalent to three to six drinks per week (RR = 1.15; 95% CI, 1.06–1.24; 333 cases/100,000 person-years). Binge drinking, but not frequency of drinking, is associated with breast cancer risk after controlling for cumulative alcohol intake. Alcohol intake both earlier and later in adult life is independently associated with risk [48].

Physical Activity

Consistent physical activity reduces the risk of breast cancer in a dose-dependent manner, with modest activity conferring a 2% decrease in risk and vigorous activity conferring a 5% decrease in risk [49].

Obesity

Obesity, specifically in postmenopausal women, also increases a woman's risk of breast cancer. In the EPIC multicenter prospective cohort study, postmenopausal women who did not use HRT had an elevated breast cancer risk with increasing weight, body mass index (BMI), and hip circumference [42]. In this cohort, the multivariate RR was 1.28 for overweight women (BMI 25.0–29.9) and obese women (BMI > 30.0) compared with women in the normal weight range. Lean women on HRT are incongruously at an increased risk of breast cancer (RR = 2.04) compared with their overweight (1.93) and obese (1.39) counterparts [42].

Insulin resistance and hyperinsulinemia have been studied as risk factors for the comorbidities associated with obesity, including cardiovascular disease and diabetes. Insulin has anabolic effects on cellular metabolism, and human cancer cells overexpress the insulin receptor [50]. Hyperinsulinemia is an independent risk factor for breast cancer in nondiabetic postmenopausal women and may help explain the relationship between obesity and breast cancer [51].

Radiation

Radiation exposure from various sources, including medical treatment and nuclear explosion, increases the risk of breast cancer. Radiation to the chest wall for treatment of childhood cancer increases the risk of breast cancer linearly with chest radiation dose [52]. Survivors of childhood cancers who received therapeutic radiation are at a dose-dependent risk for the development of breast cancer, and those treated for Hodgkin's disease are at highest risk (RR = 7) [53]. Radiation effects on the development of female breast cancer were also demonstrated in Japan after the nuclear attacks on Hiroshima and Nagasaki [54] and positively correlated with age younger than 35 years at time of exposure. The incidence of breast cancer has also increased in areas of Belarus and Ukraine. A significant twofold increase was observed in the most contaminated areas around Chernobyl following the nuclear accident and manifested in women who were younger at the time of the exposure [55].

Mammographic Breast Density

Mammographic breast density (MBD), alone or in combination with other risk factors, is associated with an increased risk of breast cancer [56–58]. Percentage dense area (PDA) is the most common measurement of mammographic density. A four- to sixfold greater risk of breast cancer has been reported in women for whom more than 75% of the total area on mammogram is occupied by dense area [59]. In addition to PDA, the absolute dense area of the breast obtained during an assessment of PDA is an independent risk factor for breast cancer, and its inclusion in risk assessment tools has been proposed [60].

Genetic Predisposition

Approximately 20–25% of breast cancer patients have a positive family history, but only 5–10% of breast cancer cases demonstrate autosomal dominant inheritance [61, 62]. Genetic predisposition alleles have been described in terms of clinical significance [63]. High-risk predisposition alleles conferring a 40–85% lifetime risk of developing breast cancer include BRCA1 and BRCA2 mutations, mutations in TP53 resulting in Li–Fraumeni syndrome, phosphatase and tensin homolog (PTEN) resulting in Cowden syndrome, STK11 causing Peutz–Jeghers syndrome, neurofibromatosis (NF1), and (CDH-1) E-cadherin [64]. Half of the breast cancer predisposition syndromes are associated with mutations in BRCA1 and BRCA2. Women with BRCA1 or BRCA2 deleterious mutations have a significantly higher risk of developing breast cancer than the general population. Lifetime breast cancer risk ranges from 65 to 81% for BRCA1 mutation carriers and 45 to 85% for BRCA2 carriers [65–67]. Moderate-risk genes, including homozygous ataxia-telangiectasia (ATM) mutations [68], somatic mutations in tumor suppressor gene CHEK2, and the BRCA1 and BRCA2 modifier genes BRIP1 [69] and PALB2 [70], confer

a 20–40% lifetime risk of breast cancer. A study suggested an association between germline TP53 mutations and early-onset HER2-positive breast cancer [71]. Numerous low-risk common alleles have been identified, largely through genome-wide association studies [63], and the clinical implications of these mutations have not been determined.

Genetic Testing and Management of Patients with Hereditary Breast Cancer

Role of BRCA Genes

BRCA1 and BRCA2 function as tumor suppressor genes and are important in the maintenance of genomic stability through their role in DNA damage signaling and DNA repair. Both BRCA1 and BRCA2 have been implicated in mediating the repair of double-strand breaks by homologous recombination (HR) by interactions with RAD51. Upon DNA damage, BRCA1 associates with RAD51 and localizes to the damaged region; BRCA1 is then phosphorylated. BRCA2 functions downstream of BRCA1 by forming a complex with RAD51. The primary function of BRCA2 is to facilitate HR [72]. Cells deficient for BRCA1 or BRCA2 are unable to repair double-strand breaks via error-free HR, resulting in repair via the error-prone nonhomologous end-joining (NHEJ) pathway, which introduces chromosomal instability [73, 74]. During S-phase, the expression levels of BRCA1 and BRCA2 increase, indicating a function in maintaining genomic stability during the DNA replication process [75]. In addition to its role in HR, BRCA1 appears to have functions in DNA repair. BRCA1 is also part of the BRCA1-associated genome surveillance complex (BASC), which includes ATM, RAD50, MRE11, NBS1, and the mismatch repair proteins MLH1, PMS2, MSH2, and MSH6 [76]. BRCA1 is also involved in transcription-coupled excision repair, chromatin remodeling, and, together with BARD1, the ubiquitination process, through which proteins are tagged for degradation by the proteasome [72, 77].

A germline mutation in *BRCA1* or *BRCA2* only represents the first hit in the classical Knudson's two-hit hypothesis, whereas the second inactivating somatic mutation often involves deletion of the wild-type allele, termed loss of heterozygosity (LOH). LOH is present in the majority (80%) of tumors arising from mutation carriers [78, 79]. By contrast, small somatic mutations involving a single or few bases are very rare [80]. Another somatic inactivation mechanism, epigenetic silencing by promoter methylation, has been reported for *BRCA1* in 9–13% of sporadic breast tumors and up to 42% of non-*BRCA1*/*BRCA2* hereditary breast tumors, leading to reduced *BRCA1* expression [81, 82]. By contrast, *BRCA1* promoter methylation is rare in tumors from *BRCA1* and *BRCA2* mutation carriers [83], and *BRCA2* promoter methylation in general is seldom observed in both sporadic and hereditary breast cancers [84]. Genetic testing for BRCA mutations is now widely available, and multiple professional societies have published guidelines for testing and management. Genetic testing trends include utilization of multigene panels that take advantage of next-generation sequencing and testing for low- and moderate-penetrance susceptibility genes [85].

BRCA1- and BRCA2-Associated Breast Cancers

Up to 10% of breast cancers result from specific genetic mutations in BRCA1 and BRCA2, which are associated with hereditary breast/ovarian cancer syndrome; CHEK2 and p53, which are associated with Li–Fraumeni syndrome; and PTEN, which is associated with Cowden syndrome [86]. Families carrying genetic mutations in the abovementioned genes exhibit an apparently dominant inheritance pattern and are often characterized by early age of onset and overrepresentation of ovarian, bilateral breast, and male breast cancers [87].

Early reports suggested that germline mutations in the genes BRCA1 and BRCA2 were responsible for the majority of hereditary breast cancers, although more recent studies have demonstrated that mutations in the two genes only account for 25–28% of the family risk [88, 89]. However, additional BRCA1/BRCA2 mutations likely remain undetected by the screening methods used today. Women carrying a BRCA1 or BRCA2 germline mutation also have increased risk of developing ovarian cancer and fallopian tube cancer. In addition, BRCA1/BRCA2 mutation carriers also have increased risk of other cancer types, such as male breast cancer, prostate cancer, pancreatic cancer, gastrointestinal cancers (e.g., gall bladder, bile duct, and stomach), and melanoma [90, 91]. In a large study by the Consortium of Investigators of Modifiers of BRCA1/BRCA2 (CIMBA), the median age of breast cancer diagnosis was 40 years among BRCA1 and 43 years among BRCA2 mutation carriers [92].

Although germline mutations in BRCA1 and BRCA2 confer high risk of breast and ovarian cancers, the penetrance of these genes is incomplete. The risk of developing breast cancer by the age of 70 is 45–87% in BRCA1 and BRCA2 mutation carriers. For ovarian cancer, the risk is 45–60% among BRCA1 mutation carriers and 11–35% among BRCA2 mutation carriers [66, 67, 93]. However, the penetrance depends on several different factors, including the type of mutation and exogenous factors.

The majority of invasive breast cancers that arise in BRCA1 and BRCA2 carriers are invasive ductal carcinomas (IDCs) (80%) [94]. A higher frequency of BRCA1 tumors is classified as medullary carcinomas compared with sporadic tumors (9% versus 2%, respectively) [92, 95]. Notably, 11% of medullary carcinomas carry BRCA1 germline mutations [96]. By contrast, an excess of invasive lobular and tubular

carcinomas has been reported for BRCA2 tumors relative to BRCA1 tumors [95]. BRCA1 tumors are more frequently high grade compared with sporadic tumors [97]. Most BRCA2 tumors are grade 2/3 with high mitotic rates.

A recent study examining pathology data from 4325 BRCA1 and 2568 BRCA2 mutation carriers reported that 78% of tumors arising in BRCA1 carriers were estrogen receptor (ER) negative, while only 23% of tumors arising in BRCA2 mutation carriers were ER negative. Furthermore, HER2 overexpression was only observed in approximately 10% of tumors from mutation carriers. Consequently, 69% of the BRCA1 tumors were triple negative (TN), which was true for only 16% of the BRCA2 tumors [92]. In contrast to BRCA1 tumors, BRCA2 tumors seem to be more similar to sporadic tumors with respect to the expression of IHC markers. Most BRCA2 breast tumors exhibit a luminal phenotype featuring overexpression of ER, progesterone receptor (PR), and cytokeratins CK8 and CK18 [98].

Recent studies have observed preinvasive lesions both in prophylactic mastectomy specimens from mutation carriers and in normal breast tissue adjacent to breast cancers [99]. Among BRCA1-/BRCA2-associated breast cancers, 59% had at least one associated preinvasive lesion compared with 75% of controls. Preinvasive lesions were more prevalent in BRCA2 mutation carriers than in BRCA1 mutation carriers (70% versus 52%, respectively). The most common preinvasive lesion in both groups was DCIS; 56% of BRCA1-/BRCA2-associated breast cancers and 71% of the sporadic breast cancers had adjacent intraductal disease, respectively [99]. These findings suggest that BRCA1-/BRCA2-associated breast cancers progress through the same intermediate steps as sporadic breast cancers and that DCIS should be considered part of the BRCA1/BRCA2 tumor spectrum.

While most studies indicate a similar prognosis for women with hereditary breast cancers compared with age-matched women with sporadic breast cancers [100–106], other studies have reported worse survival outcomes [107–111]. Lee et al. [112] reported similar survival rates in BRCA1 mutation carriers with TN disease compared with noncarriers. Confirming those findings, Bayraktar et al. [113] observed a 50% prevalence of deleterious BRCA1/BRCA2 mutations in high-risk women diagnosed with TN breast cancer. Overall prognosis of TN breast cancer in BRCA carriers and noncarriers was not significantly different within the first 5 years following initial diagnosis.

Genetic Counseling

A cancer genetic counseling risk assessment typically involves collecting a three- to four-generation family medical history (pedigree), which should include information such as current age/age at death, personal cancer history for each individual (cancer type, age at diagnosis, and pathology and treatment, if known), environmental exposures/lifestyle factors, and ethnicity [114]. The information obtained about the individual's personal and family medical history should then be used to estimate her cancer risk and the likelihood of a hereditary cancer syndrome. The National Comprehensive Cancer Network (NCCN) has guidelines regarding who should be offered genetic testing on the basis of personal and family history. Women who meet one or more of the following familial/hereditary breast cancer risk criteria should be referred to a cancer genetic counselor for further evaluation: individuals from a family with known mutations that increase their risk of breast cancer (*BRCA1*, *BRCA2*, CDH1, STK11, and TP53) or genes associated with breast cancer, a family history of two or more primary breast cancers in a single individual, two or more members with breast primaries on the same side of the family, first- or second-degree relative ≤45 years of age with breast cancer, one or more primary ovarian cancers on the same side of the family, family history of male breast cancer, or one or more family members on the same side of the family with an aggressive early-onset cancer in addition to breast cancer [115]. Recently, BRCA mutation testing for women with TN breast cancers who were younger than 50 years at diagnosis was found to be a cost-effective strategy and was adopted into current guidelines for genetic testing [116]. Moreover, women who have DCIS and a family history of ovarian cancer or who have BRCAPRO scores ≥10% have a high rate of BRCA positivity regardless of age at diagnosis [117]. The prophylactic contralateral mastectomy rate among patients with DCIS who undergo BRCA genetic testing is high [118]. Factors associated with increased likelihood of prophylactic contralateral mastectomy among this group are age, BRCA positivity, and a family history of ovarian cancer. Therefore, high-risk patients with DCIS may be appropriate candidates for genetic testing for BRCA mutations in the presence of predictive factors even if they do not have invasive breast cancer.

Risk Assessment Models

Several models have been developed to predict an individual's lifetime risk for a specific cancer and/or their risk of having a genetic mutation. Empiric models of breast cancer risk assessment include the Claus, Gail, Tyrer–Cuzick, and BRCAPRO models. Each model incorporates different risk factors and thus may be utilized to provide a range of risk estimates.

The Claus model estimates a woman's lifetime risk for breast cancer based on her family history of breast cancer in first- and/or second-degree relatives [119]; however, the model does not include any other risk factors. The American Cancer Society guidelines for recommending MRI include

women with a lifetime risk for breast cancer of 20–25% or greater, as defined by models that largely depend on family history, such as the Claus model [120]. These guidelines, however, are based on trials that did not consider the Gail model or "tissue risks" (ADH, ALH/LCIS). Therefore, Hollingsworth and Stough suggest that the current guidelines do not account for risk factors that increase the likelihood of mammography failure; thus, a different approach may be needed to determine MRI candidates [121].

The Gail model estimates a woman's risk for breast cancer based on her age and personal risk factors, such as age at menarche, age at first live birth, and biopsy history; however, this model inadequately utilizes family history because only first-degree relatives are considered [122]. This model has been extensively validated in US populations; however, limited data are available related to the use of this model internationally. Pastor-Barriuso et al. recently evaluated the predictive accuracy of the Gail model in a Spanish cohort and found that the original Gail model cannot be applied to populations with varying rates of invasive breast cancer; however, a recalibrated version of the model provides more unbiased risk estimates [123].

The Tyrer–Cuzick model (also known as IBIS) incorporates personal and family history risk factors and can be utilized to calculate a woman's risk of breast cancer and the likelihood of a BRCA mutation [124]. The BOADICEA model was designed to predict the probability of BRCA1/BRCA2 mutation and to provide breast and ovarian cancer risk estimates, and it uniquely accounts for the possibility of genetic modifiers and the polygenic component of breast cancer risk [125–127]. Several studies have assessed the validation and accuracy of these risk prediction tools in specific, high-risk cohorts. The IBIS model was well calibrated for women of Marin County, California, a population with high rates of breast cancer [128]. In addition, the BOADICEA and IBIS risk models were validated in a high-risk population of Israeli women, which revealed that the BOADICEA model has better predictive value and accuracy for 10-year breast cancer risk than the IBIS model [129].

BRCAPRO predicts the probability of a BRCA mutation based on an individual's personal and family history of cancer and can be utilized for an affected or unaffected patient [130, 131]. Several validation studies in various populations have indicated that BRCAPRO may accurately measure the probability of identifying a BRCA mutation; thus, the tool is widely utilized in clinical practice [132–134]. One recent study, however, examined the accuracy of BRCAPRO specifically in patients with a personal history of ovarian cancer and reported that the model significantly underestimated BRCA1/BRCA2 mutations in individuals with high-grade serous ovarian cancer at BRCAPRO scores less than 40%, suggesting a benefit of universal testing in this patient population [135]. Another recent study evaluated the performance of the BRCAPRO and BOADICEA models in Italian cancer genetics clinics and reported that using these models at the commonly used 10% threshold for testing missed 25% of carriers; these data question the strict utilization of these models for risk assessment [136]. A study of a German patient cohort supported the use of BRCAPRO and BOADICEA for decision making related to BRCA1/BRCA2 genetic testing; however, the model calibration may need to be improved for this specific population [137]. Ready et al. [138] demonstrated that the BRCAPRO model overestimated the relative contribution of bilateral breast cancer to the likelihood of detecting a BRCA1 or BRCA2 mutation. In that study, bilateral breast cancer did not appear to be a good indicator of mutation status, particularly for women whose age at first diagnosis was >40 years. Clinical providers should proceed with caution when strictly using risk prediction tools, such as BRCAPRO, to determine if an individual should undergo genetic testing. Considerations of demographics (e.g., ethnicity), cancer histology and tumor markers, relatives with cancer beyond first and second degrees of relatedness, limited family structure, and the possibility of misreported or unknown family history should all be considered during risk assessment.

Currently, there is a lack of validated models to predict an individual's lifetime risk for gynecological malignancies such as endometrial and ovarian cancer. Pfeiffer et al. recently derived and validated a model to predict a woman's absolute risk for developing breast, endometrial, and ovarian cancers [139]. The model incorporates several risk factors, such as parity, menopausal status, age at menopause, BMI, smoking history, and family history. The validation study demonstrated expected to observe cancer ratios of 1.00 (95% CI, 0.96–1.04) for breast cancer and 1.08 (95% CI 0.97–1.19) for ovarian cancer; however, the number of endometrial cancers was significantly overestimated. There is currently a significant lack of literature related to risk prediction models for endometrial and ovarian cancer. Further research is needed to develop tools that can be utilized for individualized risk assessment to allow management recommendations to be made in the context of a woman's personal and family history risk factors.

Risk-Reducing Surgical Interventions in Women with BRCA Mutation-Associated Breast Cancer

Prophylactic Contralateral Mastectomy

In addition to the risk of ipsilateral recurrence (IPR), breast cancer patients with a deleterious BRCA1 mutation have up to a 43.4% 10-year risk of contralateral breast cancer (CBC), while BRCA2 mutation carriers have up to a 34.6% 10-year risk [140]. Importantly, studies have shown that prophylactic

bilateral mastectomy results in up to a 97% risk reduction of CBC [141–143]. The decision for prophylactic bilateral mastectomy vs. ipsilateral mastectomy should be based on the type of surgery the patient is undergoing for the treatment of the primary breast cancer diagnosis and, of course, the patient's choice. If the patient is undergoing lumpectomy for ipsilateral breast cancer, then prophylactic contralateral mastectomy may not be the best option for that patient considering cosmesis. For patients who do not want to have contralateral prophylactic mastectomy, breast cancer screening with MRI every 6 months should be offered, and tamoxifen can be used as a chemoprevention in ER-positive breast cancer types.

Similar to the risk of IPR, the risk of CBC in BRCA mutation carriers is increased in patients who are diagnosed at a younger age [144–146]. However, the impact of other cancer therapies on the risk of CBC in BRCA mutation carriers is controversial. While some studies have reported a 50–60% reduction in the risk of CBC with adjuvant chemotherapy [147, 148], other studies have shown no impact of adjuvant chemotherapy [141, 146, 149]. Likewise, some studies have reported that tamoxifen reduces the risk of CBC by 50–70% in BRCA mutation carriers [148, 150, 151], whereas other studies have not reported a significant reduction [141, 146, 147, 149, 152]. However, most studies have reported that oophorectomy reduces the risk of CBC in BRCA mutation carriers by 50–70%, with the greatest benefit observed if the surgery is performed before the age of 50 [141, 144, 148, 150].

The above findings justify the practice of offering the option for risk-reducing surgery to the intact breast (before any breast cancer diagnosis) in women with BRCA mutations. Despite the significant reduction in the risk of CBC associated with prophylactic contralateral mastectomy in BRCA mutation carriers, the procedure has not currently been found to improve survival, although studies have been limited by short follow-up [142, 153].

Risk-Reducing Salpingo-Oophorectomy

BRCA mutation carriers have a well-established enhanced risk of ovarian cancer [66, 154, 155]. Risk-reducing salpingo-oophorectomy (RRSO) clearly reduces the risk of tubal–ovarian cancer [156, 157] and also reduces breast cancer [158]. A meta-analysis of ten studies reported a significant reduction in the risk of CBC and ovarian cancer in BRCA mutation carriers who had undergone RRSO [159] (Table 4.2). Overall, RRSO was associated with a 51% reduction in breast cancer risk (HR, 0.49; 95% confidence interval (CI) 0.37–0.65). Similar risk reductions were observed in BRCA1 (HR 0.47; 95% CI 0.35–0.64) and BRCA2 (HR 0.47; 95% CI 0.26–0.84) mutation carriers. RRSO was also associated with a significant risk reduction of ovarian–fallopian tube cancer (HR 0.21; 95% CI 0.12–0.39).

Table 4.2 Use of pharmacological interventions for breast cancer risk reduction: ASCO Clinical Practice Guidelines 2014

Intervention
Pharmacological interventions for breast cancer risk reduction, including selective estrogen receptor (ER) modulators and aromatase inhibitors
Key recommendations
Tamoxifen (20 mg per day orally for 5 years) should be discussed as an option to reduce the risk of invasive breast cancer, specifically ER-positive breast cancer, in premenopausal or postmenopausal women age 35 years at increased risk of breast cancer or with lobular carcinoma in situ (LCIS). Tamoxifen is not recommended for use in women with a history of deep vein thrombosis, pulmonary embolus, stroke, or transient ischemic attack; during prolonged immobilization; or in women who are pregnant, may become pregnant, or are nursing mothers. Tamoxifen is not recommended in combination with hormone therapy
Raloxifene (60 mg per day orally for 5 years) should be discussed as an option to reduce the risk of invasive breast cancer, specifically ER-positive breast cancer, in postmenopausal women age ≥35 years at increased risk of breast cancer or with LCIS. It should not be used for breast cancer risk reduction in premenopausal women. Raloxifene is not recommended for use in women with a history of deep vein thrombosis, pulmonary embolus, stroke, or transient ischemic attack or during prolonged immobilization
Exemestane (25 mg per day orally for 5 years) should be discussed as an alternative to tamoxifen or raloxifene to reduce the risk of invasive breast cancer, specifically ER-positive breast cancer, in postmenopausal women age ≥35 years at increased risk of breast cancer or with LCIS or atypical hyperplasia. Exemestane should not be used for breast cancer risk reduction in premenopausal women
For tamoxifen and raloxifene, the most favorable risk–benefit profile is seen in women with the greatest risk of developing breast cancer
Discussions with patients and healthcare providers should include both the risks and benefits of each agent under consideration

Because BRCA1 carriers more typically have hormone receptor-negative breast cancer, the efficacy of RRSO in reducing the risk of ovarian and breast cancer has been questioned in the past. In a multicenter, prospective cohort study by Domchek et al. [153], salpingo-oophorectomy was associated with a reduction of ovarian cancer and ovarian cancer mortality, a reduction of subsequent breast cancer and breast cancer mortality, and a reduction of overall mortality. In this study of 2482 women with BRCA mutations, RRSO was associated with a significant (85%) reduction (HR = 0.15; 95% CI, 0.04–0.63) in the risk of ovarian cancer among BRCA1 mutation carriers with a history of breast cancer. Among BRCA2 mutation carriers with and without a history of breast cancer, no cases of ovarian cancer were observed after RRSO. Most importantly, RRSO was associated with a significant mortality benefit in BRCA mutation carriers, both in patients with and without history of breast cancer. In the first group, RRSO reduced all-cause mortality by 70% (HR = 0.3; 95% CI, 0.17–0.52), and interestingly, breast cancer-specific mortality was reduced by 65%

(HR = 0.35; 95% CI, 0.19–0.67), despite a lack of benefit in breast cancer incidence.

The optimal timing of prophylactic oophorectomy is still controversial. However, the risk of second primary breast cancer development or ipsilateral breast cancer recurrence cumulatively increases with younger age at initial diagnosis (<40 vs. >50 years of age) [145]. A few studies have shown that the survival outcomes are better if prophylactic oophorectomy is performed before 40 years of age [161, 162]. However, surgical menopause in premenopausal women is associated with long-term side effects, including increased risks of osteoporosis, heart disease, high cholesterol, and hot flashes [163]. Therefore, the associated side effects and the resultant decreased quality of life should be weighed against the benefits of this procedure. In summary, both prophylactic bilateral mastectomy and RRSO result in a reduction of breast cancer in mutation carriers, and the risk of CBC in women with BRCA mutations who undergo both procedures is less than 2% [142, 143, 164]. A detailed discussion with the patient regarding the surgical risk-reducing intervention and its long-term side effects is central to the management of mutation carriers.

Cancer Risk Management Decisions of Women with BRCA Variants of Uncertain Significance

One result of BRCA genetic testing is a variant of uncertain significance (VUS). VUSs are changes in the BRCA genes that may or may not be associated with an increased risk of cancer. Women with a VUS are a growing population, making up to 21% of patients who undergo genetic testing. Because the cancer risks associated with VUSs are unknown, cancer risk management recommendations are difficult, and patients must make decisions regarding risk management for cancers that they may or may not be at risk for. A recent study described the risk management decisions (RMDs) undertaken by women who have a BRCA VUS [165]. Women who had a BRCA VUS and a personal history of breast cancer appeared to be more aggressive in their RMD than women who had a VUS but no personal history of breast or ovarian cancer. Given the prognostic uncertainty and high rate of reclassification for women with a VUS, individualizing counseling and directing efforts toward surveillance, chemoprevention, or salpingectomy are currently recommended.

Systemic Therapy Options for Women with BRCA Mutation-Associated Breast Cancer

Cytotoxic Chemotherapy

Traditionally, for those who develop breast or ovarian cancer, systemic therapy has been selected similarly to those with sporadic cancers, and the choice of chemotherapy (adjuvant or neoadjuvant as appropriate), endocrine therapy, and radiation has been based on ER/PR/HER2 status, lymph node involvement, and the size of the tumor. Studies have reported survival outcomes of BRCA carriers that are equivalent to those of patients with sporadic breast cancer after appropriate treatment [102, 106]. However, the approach to treatment is changing based on the recent data suggesting unique patterns of sensitivity and resistance to systemic therapies in BRCA mutation-associated breast cancers [166–171].

Due to the involvement of the BRCA1/BRCA2 protein products in DNA repair mechanisms, BRCA mutational status may impact sensitivity to different chemotherapeutic agents [172–174]. In vitro studies have demonstrated that BRCA1-defective cell lines are sensitive to DNA-damaging agents, such as platinum, and are relatively resistant to taxanes compared with BRCA-competent cell lines [175, 176]. Several subsequent clinical studies have supported these preclinical findings [166, 167, 169]. Byrski et al. reported a remarkable pathological complete response (pCR) rate of 80% in a small prospective trial evaluating neoadjuvant cisplatin in BRCA1 mutation-associated breast cancer [166]. The promising neoadjuvant data with cisplatin initiated a randomized phase III trial comparing carboplatin to docetaxel in metastatic BRCA mutation-associated breast cancer (NCT00321633) and a smaller phase II trial evaluating cisplatin for metastatic BRCA1 mutation-associated breast cancer. Early results from the phase II trial have been encouraging, with 46% of women achieving a complete response and 26% of women achieving a partial response. However, a recent study from MD Anderson demonstrated that BRCA1 carriers had a high pCR to neoadjuvant anthracycline–taxane-based chemotherapy (pCR in 46% of BRCA1 carriers vs. 22% of noncarriers) [168]. Of note, the sensitivity to single-agent taxanes was low in this study. Interestingly, BRCA mutation status and ER negativity were independently associated with higher pCR rates. Similarly, in another study among women with metastatic breast cancer, hormone receptor-negative BRCA1 mutation-associated breast cancer patients had lower response rates and shorter time to progression with a taxane-containing regimen compared with hormone receptor-negative sporadic breast cancer controls [177]. Regarding the current efficacy of other cytotoxic agents and targeted therapy, most studies have been directed against TN breast cancer and have assessed the roles of combinations of ixabepilone and cetuximab, gemcitabine and erlotinib, and paclitaxel and cetuximab [178, 179]. The specific responses to these combinations in BRCA mutation-associated breast cancers have not been analyzed.

PARP Inhibitors

With advances in molecularly targeted therapy in solid tumors, an appealing targeted therapy for BRCA1/BRCA2

carriers, poly(ADP-ribose) polymerase (PARP) inhibitors, has also been developed. PARP proteins play a role in single-strand DNA repair; when PARP is inhibited, single-strand breaks cannot be repaired, leading to double-strand breaks at the replication fork [180, 181]. Because BRCA1 and BRCA2 proteins are critical in double-strand DNA repair, combining PARP inhibition with tumors that have defective BRCA1 or BRCA2 proteins exerts a synergistic lethal effect [182, 183]. This hypothesis has been supported by in vitro studies showing enhanced cytotoxicity in BRCA1- and BRCA2-deficient cells compared with cells with wild-type BRCA proteins [184, 185].

In a phase I study of 60 patients, of whom 22 were BRCA carriers, patients were treated with two different dose levels of the PARP inhibitor olaparib, and the maximum tolerated dose (MTD) was determined to be 400 mg orally twice daily. The most common side effects were grade 1 or 2 nausea, vomiting, fatigue, dysgeusia, and anorexia. Myelosuppression (anemia or thrombocytopenia) was also observed in a few patients [171]. All patients had a partial response, according to the Response Evaluation Criteria in Solid Tumors (RECIST), with responses lasting 20–80 weeks in the 19 *BRCA* mutation carriers with ovarian, breast, or prostate cancer who could be evaluated for tumor response.

Phase II multicenter, multinational studies that examined breast and ovarian cancers independently were then conducted in mutation carriers. Both used the phase I MTD, 400 mg twice daily and 100 mg twice daily, because this dose was the lowest dose at which an antitumor effect was seen in the phase I trial. The primary end point for both studies was the objective response rate (ORR). Among breast cancer patients, the ORR for those in the 400-mg arm was 41% (11/27), with an additional 44% (12/27) of women achieving stable disease. For those with metastatic breast cancer on 100-mg olaparib, 22% (6/27) had partial responses, and an additional 44% (12/27) of the patients achieved stable disease. These results were particularly impressive because the patients had undergone a median of three prior chemotherapy regimens. Similar results were observed in the ovarian cancer study, with an ORR of 33% (11/33) in the 400-mg arm. The most common side effects were nausea and fatigue [186, 187]. Both studies demonstrated the efficacy and tolerability of olaparib in BRCA1 or BRCA2 mutation carriers with breast or ovarian cancer. These data resulted in a paradigm shift assuming that BRCA1 or BRCA2 carriers have differential susceptibility to systemic therapy compared with noncarriers. Multiple studies are ongoing and examining different PARP inhibitors with the hope that BRCA1/BRCA2 mutation status will provide a targeted group for these agents.

In a randomized phase II trial, the intravenous PARP inhibitor iniparib was evaluated in combination with gemcitabine and carboplatin for the treatment of women with TN metastatic breast cancer [188]. The arm that was treated with the combination regimen had greater clinical benefit and improved progression-free survival and overall survival when compared with the chemotherapy alone arm [189]. Unfortunately, the results of a randomized phase III trial comparing iniparib plus chemotherapy to chemotherapy alone in TN metastatic breast cancer were disappointing, with no demonstration of survival benefit when used as a first-line treatment in the metastatic setting. Of note, iniparib was not specifically evaluated in BRCA mutation-associated breast cancer in this study.

Endocrine Therapy

Two studies have demonstrated that adjuvant use of tamoxifen reduces the risk of IPR or CBC in BRCA1/BRCA2 carriers, regardless of ER status [151, 190]. A small retrospective study comparing outcomes in early-stage BRCA mutation-associated and sporadic breast cancer treated with endocrine therapy observed a lower OS in BRCA carriers, suggesting relative resistance to adjuvant endocrine therapy with tamoxifen [191]. These results, however, require confirmation, and the use of adjuvant endocrine therapy is recommended in patients with BRCA mutation-associated ER-positive breast cancer. Currently, there are no data regarding outcomes with aromatase inhibitors as adjuvant endocrine therapy in BRCA mutation-associated breast cancer.

Increased Surveillance

Current screening recommendations for the asymptomatic BRCA mutation carrier encompass examination, imaging, and laboratory evaluation. Surveillance for female carriers emphasizes screening techniques for breast and ovarian cancers.

There is general agreement that women with a higher lifetime risk of breast cancer, such as that conferred by a *BRCA* mutation, should undergo earlier and more frequent screening, with additional imaging modalities considered. A consolidated summary of current screening recommendations published by the National Comprehensive Cancer Network (NCCN), American Cancer Society (ACS), American College of Radiology (ACR), and other national organizations for the asymptomatic, female, *BRCA* mutation carrier includes the following [192, 193]:

- Monthly breast self-exam (BSE) beginning at the age of 18 years
- Semiannual clinical breast exam (CBE) beginning at the age of 25 years
- Alternating annual mammograms with annual breast magnetic resonance imaging (MRI) beginning at the age of 25–30 years or individualized based on the earliest age of cancer onset in the family [194]

While risk-reducing prophylactic oophorectomy is more effective in preventing ovarian cancer in these women compared to general population, some may not opt to pursue this intervention until after their childbearing years. In the absence of more effective screening methods, transvaginal ultrasound (TVU) and CA-125 levels continue to be recommended and endorsed by national organizations for women who are at high risk for hereditary breast and ovarian syndromes [195]. Current NCCN screening guidelines for *BRCA* mutation carriers who are not undergoing prophylactic oophorectomy include the following:

- Semiannual concurrent pelvic exam, TVU, and CA-125 antigen determination beginning at the age of 35 years or 5–10 years earlier than the youngest age at which any family member was diagnosed with ovarian cancer

Mutations in the tumor suppressor genes *BRCA1* and *BRCA2* place male and female carriers at increased risk for a number of other cancers, notably pancreatic, melanoma, colorectal, and other gastrointestinal tumors. Further research is needed to define the association of specific *BRCA* mutations with these cancers. No expert consensus or evidence-based guidelines exist regarding screening for these cancers. Some literature and investigational studies support considering the following additional surveillance modalities [196–198]:

- Pancreatic: annual endoscopic ultrasound, beginning at the age of 50 years or 10 years prior to the earliest pancreatic cancer diagnosis in the family
- Melanoma: annual full body skin and ocular exam
- Colorectal: population screening guidelines, beginning at the age of 50 years and continuing until 75 years old
- Annual fecal occult blood testing
- Sigmoidoscopy every 5 years or colonoscopy every 10 years

Breast Cancer Prevention

Due to the rising incidence of breast cancer and because several of the risk factors are non-modifiable, strategies for the primary prevention of breast cancer represent an important area of interest. In this section, we will review the different approaches directed at reducing the incidence of breast cancer.

Pharmacotherapy (Chemoprevention)

The effects of various pharmacologic agents on the incidence of invasive breast cancer (IBC) and noninvasive breast cancer have been investigated in several prospective randomized clinical trials [199]. In this review, we will discuss the role of selective estrogen receptor modulators (SERMs) such as tamoxifen, raloxifene, arzoxifene, and lasofoxifene and aromatase inhibitors (AIs) such as exemestane. Table 4.2 provides a summary of the prior (2009) ASCO guidelines and updated recommendations.

Tamoxifen

National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention Trial (P1)

The NSABP-P1 trial, which was initiated in 1992, randomized 13,388 women to receive either 20 mg of tamoxifen or a placebo daily for 5 years [200]. After a median follow-up of 54.6 months, a 49% reduction in the risk of IBC was observed in the patients who were treated with tamoxifen (RR = 0.51; 95% confidence interval [CI], 0.39–0.66). The cumulative incidence of IBC through 69 months was 43.4 versus 22.2 per 1000 women in the placebo and tamoxifen groups, respectively. Tamoxifen was effective across all age groups, in patients with a history of LCIS or atypical ductal hyperplasia, and in those with any category of predicted 5-year risk. Tamoxifen reduced the occurrence of IBC in ER-positive tumors by 69% (RR = 0.31; 95% CI, 0.22–0.45), but no significant difference in the occurrence of ER-negative tumors was observed.

The increased incidence of endometrial cancer, stroke, deep venous thrombosis, and cataracts was noted in patients treated with tamoxifen, with most cases occurring in women who were >50 years of age. All endometrial cancers in the tamoxifen group were International Federation of Gynecology and Obstetrics stage I. In 2005, the NSABP provided the 7-year follow-up results of the above study, which continued to demonstrate a reduced incidence of both IBC (RR = 0.57; 95% CI, 0.46–0.70) and noninvasive breast cancer (RR = 0.63; 95% CI, 0.45–0.89) [201]. A 32% reduction in osteoporotic fractures was also noted with tamoxifen.

Italian Tamoxifen Prevention Study

The Italian Tamoxifen Prevention Study randomized 5408 women who had previously undergone a hysterectomy to receive tamoxifen or placebo [202]. The initial results of the trial failed to demonstrate an overall benefit of tamoxifen after a median follow-up of 46 months; however, after 11 years of follow-up, the investigators observe a significant reduction in the incidence of ER-positive breast cancer among women at high risk (defined as women taller than 160 cm, with at least one intact ovary, with no full-term pregnancy before the age of 24 years, and younger than age 14 years at menarche) treated with tamoxifen compared to women not treated with tamoxifen (6.26 versus 1.50 per 1000 woman-years; RR = 0.24; 95% CI, 0.10–0.59) [203].

The Royal Marsden Hospital Tamoxifen Chemoprevention Trial

The Royal Marsden Hospital Tamoxifen Chemoprevention Trial, which randomized 2494 women aged 30–70 years who also had a family history of breast cancer to tamoxifen or placebo, failed to demonstrate a decreased incidence of ER-positive breast cancer (30 cases in the tamoxifen arm versus 39 in the placebo arm; hazard ratio [HR] = 0.77; 95% CI, 0.48–1.23) [204]. In 2007, the investigators provided an update to this trial with an extended follow-up of 20 years, which revealed a significant decrease in the risk of ER-positive breast cancer in the tamoxifen arm (23 cases in the tamoxifen arm and 47 in the placebo arm; HR = 0.48; 95% CI, 0.29–0.79) [205]. The adverse events observed with tamoxifen in the European trials were similar to the NSABP-P1 trial.

International Breast Cancer Intervention Study (IBIS-I)

Another trial testing the efficacy of tamoxifen among women at increased risk of breast cancer in the United Kingdom, Australia, and New Zealand was initiated in 1992 [206]. With a median follow-up of 49.6 months, the investigators determined that tamoxifen decreased the incidence of breast cancer by 32% (RR = 0.68; 95% CI, 0.50–50.92). With further follow-up (up to 96 months), the incidence continued to be lower in the tamoxifen group (27% reduction in IBC; RR = 0.73; 95% CI, 0.58–50.91) [207]. Similar to the NSABP-P1 experience, the benefit of tamoxifen was only observed in ER-positive tumors, and an increased risk of thromboembolic events with tamoxifen was reported. However, in contrast to the NSABP-P1 results, the use of HRT for postmenopausal symptoms at the lowest possible dose was permitted in the trial, and the increased risk of endometrial cancer with tamoxifen was not significant.

In 2003, an overview of the abovementioned tamoxifen prevention trials was published, and there was no reduction in ER-negative IBC; however, there was a significant 48% decrease in the incidence of ER-positive IBC [208]. The consensus of endometrial cancer and venous thromboembolic events had a RR of 2.4 and 1.9, respectively; women aged 50 years or older had an increased risk. Overall, there was no effect on all-cause mortality, but there was a high degree of heterogeneity across the various trials.

Several studies have demonstrated that tamoxifen decreases MBD [209–211]. A case–control study nested within the IBIS-I showed a 10% or greater reduction in breast density at the 12- to 18-month mammogram in 46% of women in the tamoxifen group [212]. These women had a 63% reduction in breast cancer risk (odds ratio [OR] = 0.37; 95% CI, 0.20–0.69; $P = 0.002$). The women who experienced less than a 10% reduction in breast density with tamoxifen had no risk reduction (OR = 1.13; 95% CI, 0.72–1.77; $P = 0.60$). Similar reductions in MBD in the placebo group were not associated with decreased risk of breast cancer; hence, the authors concluded that a 12- to 18-month change in MBD was a good predictor of the response to tamoxifen for the prevention of breast cancer.

Raloxifene

Raloxifene is an oral, second-generation SERM that has estrogenic effects on the bone, lipid metabolism, and blood clotting and antiestrogenic effects on the breast and uterus. The US Food and Drug Administration (FDA) initially approved raloxifene for the prevention and treatment of osteoporosis in postmenopausal women [213].

The Multiple Outcomes of Raloxifene Evaluation (MORE) Trial

In this trial, 7705 postmenopausal women with osteoporosis were randomly assigned to receive raloxifene (60 mg or 120 mg per day) or placebo [214]. The initial results of this trial reported a 30% reduction in the risk of vertebral fractures, which was associated with an increase in bone mineral density in the spine and femoral neck, but the incidence of non-vertebral fractures was not significantly different. The incidence of IBC, which was a secondary end point of the study, was decreased by 76% during the 3 years of treatment and by 72% after 4 years of treatment with raloxifene. The number needed to treat (NNT) to prevent one case of breast cancer was 126 [215, 216]. Similar to the tamoxifen trials, the benefit of raloxifene was limited to ER-positive breast cancer, and an increased risk of venous thromboembolism was observed (RR = 3.1; 95% CI, 1.5–6.2). In contrast to tamoxifen, raloxifene did not increase the risk of endometrial cancer (RR = 0.8; 95% CI, 0.2–2.7).

The Continuing Outcomes Relevant to Evista (CORE) Trial

This double-blind, placebo-controlled study investigated the efficacy of an additional 4 years of raloxifene compared with placebo in decreasing the incidence of IBC in women who had participated in the MORE trial [217]. The primary breast cancer analysis included 5213 patients (3996 who had completed MORE when CORE began and 1217 who were still participating in MORE when CORE began). The 4-year incidences in the raloxifene group of IBC and ER-positive IBC were reduced by 59% and 66%, respectively. Over the 8 years of both trials, the incidences of IBC and ER-positive IBC were reduced by 66% (HR = 0.34; 95% CI, 0.22–0.50) and 76% (HR = 0.24; 95% CI, 0.15–0.40), respectively, in patients who received raloxifene.

The Study of Tamoxifen and Raloxifene (STAR) Trial (NSABP-P2)

This double-blind, randomized controlled trial included 19,747 postmenopausal women aged 35 years and older with an increased risk of breast cancer [218], which was defined as a personal history of LCIS or a 5-year predicted risk for IBC of at least 1.66% as determined by the Gail model. Women with a history of cerebral vascular accidents, transient ischemic attack, pulmonary embolism, deep venous thrombosis, uncontrolled diabetes, uncontrolled hypertension, or atrial fibrillation were excluded from the study. Women were randomly assigned to receive 20 mg of tamoxifen per day plus a placebo or 60 mg of raloxifene per day plus a placebo for a 5-year period. The primary end point was the development of biopsy-proven IBC. The secondary end points of the trial included the incidence of noninvasive breast cancer, uterine cancer, cardiovascular events, stroke, transient ischemic attack, pulmonary embolism, deep venous thrombosis, osteoporotic fractures, cataracts, life, and death from any cause. There was no difference between the effects of tamoxifen and raloxifene on the incidence of breast cancer. There were 163 cases of IBC in the women assigned to the tamoxifen group, compared with 168 cases in the raloxifene group. The rate per 1000 woman-years was 4.3 in the tamoxifen group and 4.4 in the raloxifene group (RR = 1.02; 95% CI, 0.82–1.28). The pathological characteristics of the tumors did not differ between the treatment groups with respect to the distribution by tumor size, nodal status, or ER level. The incidence of noninvasive breast cancer was lower in the tamoxifen group (1.51 per 1000 women) compared with the raloxifene group (2.11 per 1000 women); however, this difference did not reach significance. There were fewer cases of uterine malignancies in the raloxifene group (23 cases) compared with the tamoxifen group (36 cases), although this difference was also not significant. Similarly, no significant differences between the two groups were observed regarding the incidence of stroke, transient ischemic attack, and osteoporotic fractures at the hip, spine, and radius. However, a 30% decrease in the incidence of pulmonary embolism and deep venous thrombosis was noted in the raloxifene arm (100 versus 141 events in the raloxifene versus tamoxifen groups, respectively; RR = 0.70; 95% CI, 0.54–0.91). Fewer women who received raloxifene developed cataracts (RR = 0.79; 95% CI, 0.68–0.92). Mortality was similar in the two groups (101 deaths in the tamoxifen group versus 96 in the raloxifene group; RR = 0.94; 95% CI, 0.71–1.26). Based on the data from STAR and other raloxifene trials, the FDA approved raloxifene for the prevention of IBC in postmenopausal women who are at increased risk of breast cancer or in postmenopausal women with osteoporosis.

An updated analysis of the STAR trial was performed in 2010 with a median follow-up time of 81 months [219]. There continued to be no significant difference in the incidence of IBC between tamoxifen and raloxifene (RR = 1.24; 95% CI, 1.05–1.47). There were 137 cases of noninvasive breast cancer in the raloxifene group and 111 cases in the tamoxifen group (RR = 1.22; 95% CI, 0.95–91.59); as such, the difference between the two groups was smaller than that in the original report. In contrast to the initial study, there was a significant decrease in the risk of endometrial cancer with raloxifene (RR = 0.55; 95% CI, 0.36–30.83). In addition, significant reductions in the incidence of thromboembolic events (RR = 0.75; 95% CI, 0.60–60.93) and uterine hyperplasia (RR = 0.19; 95% CI, 0.12–10.29) were reported. No significant mortality differences between raloxifene and tamoxifen were noted.

Additional SERMS

The Postmenopausal Evaluation and Risk Reduction with Lasofoxifene (PEARL) study randomly assigned 8556 postmenopausal women with osteoporosis to receive a placebo or either 0.25 mg or 0.5 mg of lasofoxifene per day [220]. A significant reduction in the incidence of ER-positive breast cancer (HR = 0.19; 95% CI, 0.07–0.56) was reported in women assigned to 0.5 mg of lasofoxifene per day. In addition, the incidences of vertebral and nonvertebral fractures, coronary heart disease events, and stroke were also reduced in this group. A smaller effect on the incidence of ER-positive IBC was noted with 0.25 mg of lasofoxifene per day.

The investigational SERM arzoxifene has also been evaluated in postmenopausal women with breast cancer. The GENERATIONS trial was a large, multicenter, double-blind, placebo-controlled study that compared daily dosing of 20 mg of arzoxifene to placebo in 9354 postmenopausal women with osteoporosis or low bone mass [221, 222]. The median follow-up was 48 months. The incidence of IBC was decreased in women assigned to the arzoxifene group (22 cases versus 53 in the placebo group; HR = 0.41; 95% CI, 0.25–0.68). This reduction was primarily observed in women with ER-positive breast cancer, similar to the results for other SERMs.

Role of Aromatase Inhibitors

High aromatase levels in breast tissues and high circulatory estrogen levels are known risk factors for IBC [223]. Anastrozole, letrozole, and exemestane decrease the circulating estrogen levels in postmenopausal women by inhibiting the enzyme aromatase, which catalyzes the conversion of androgens to estrogens. The role of AIs in the adjuvant treatment of postmenopausal women with receptor-positive IBC is well established [224]. A 37–55% reduction in the incidence of CBC has been reported with the use of AIs in clinical trials [225–227]. The main side effects of AIs include arthralgia and accelerated bone resorption, and its

overall safety profile is relatively more favorable compared with tamoxifen.

The NCIC CTG MAP.3 Trial

The NCIC CTG MAP.3 trial was a prospective trial that investigated the role of exemestane in reducing the incidence of IBC in postmenopausal women who were at increased risk [228]. This double-blind trial randomized 4560 postmenopausal women who had at least 1 risk factor (age >60 years, Gail 5-year risk score >1.66%, prior atypical ductal or lobular hyperplasia or LCIS, or DCIS with mastectomy) to receive either 25 mg of exemestane per day or placebo. The median age of women who participated in the trial was 62.5 years, and the median Gail risk score was 2.3%. The investigators reported a reduction in the incidence of IBC in women assigned to the exemestane group (11 cases) compared with those in the placebo group (32 cases) at a median follow-up of 35 months. A 65% relative reduction in the annual incidence of IBC (0.19% versus 0.55%; HR = 0.35; 95% CI, 0.18–0.70; $P = 0.002$) with exemestane was reported. The NNT to prevent 1 case of IBC with exemestane therapy was 94 in 3 years. The annual incidence of IBC plus DCIS (20 in the exemestane group and 44 in the placebo group) was 0.35% and 0.77% in the exemestane and placebo groups, respectively (HR = 0.47; 95% CI, 0.27–0.79). Adverse events were experienced by 88% of women in the exemestane group and 85% in the placebo group; hot flashes and arthritis were the most common adverse events in both groups. There were no significant differences between the two groups regarding secondary end points, such as new osteoporosis, skeletal fractures, cardiovascular events, and cancers other than IBC. No treatment-related deaths were reported. Women taking exemestane reported slightly worse menopause-related quality-of-life events compared with placebo (7% more overall).

IBIS-II Trial

IBIS-II is a multicenter, randomized, double-blind, placebo-controlled phase III trial that evaluated the AI anastrozole in postmenopausal women at high risk for breast cancer (family history, atypical hyperplasia or LCIS, nulliparity or age ≥30 at first birth, mammographic opacity covering at least 50% of the breast) [229]. Anastrozole (1 mg/day) was associated with a 53% reduction in the incidence of IBC and DCIS (primary end point) compared with placebo after a median follow-up of 5 years (HR = 0.47; 95% CI, 0.32–68.0; $P < 0.0001$). Similar to most chemoprevention trials, the protective effect of anastrozole was observed in ER-positive IBC, with no significant effect in the ER-negative subgroup. The total mortality was 0.9% for both arms. Interestingly, a reduction in the incidence of skin, gastrointestinal, and gynecological cancers as well as other cancers was noted in the anastrozole group (2% versus 4% in the placebo group; RR = 0.58; 95% CI, 0.39–0.85). A significant increase in the incidence of musculoskeletal events, such as aches and pain, vasomotor symptoms, dryness of the eyes, and hypertension, was observed in the anastrozole arm. Bone fractures occurred in 7.7% of those on placebo compared with 8.5% of women receiving anastrozole. Based on the results of this trial, anastrozole may be an effective chemopreventive option for postmenopausal women.

Recently, a meta-analysis based on individual participant data from nine randomized prevention trials using tamoxifen, raloxifene, arzoxifene, and lasofoxifene was reported [230]. These trials included the Royal Marsden Hospital Tamoxifen Trial, IBIS-I, NSABP-P1, Italian Tamoxifen Prevention Study, MORE/CORE, RUTH, STAR, PEARL, and GENERATIONS. The median follow-up time was 65 months. Overall, a 38% reduction in the incidence of breast cancer (including DCIS) was noted (HR = 0.62; 95% CI, 0.56–0.69), with the largest reduction in the first 5 years of follow-up compared with years 5–10. The estimated 10-year cumulative incidence was 6.3% in the control group and 4.2% in the SERM group. A total of 42 women would need to be treated to prevent one breast cancer event in the first 10 years of follow-up. A significant overall reduction of 31% in the incidence of DCIS was reported, with a 38% reduction in the tamoxifen trials but no effect for raloxifene.

The investigators noted a significant reduction in all breast cancers and ER-positive breast cancers with 0.5 mg of lasofoxifene per day compared with placebo; however, there was a nonsignificant increase in the incidence of ER-negative IBC (HR = 1.43; 95% CI, 0.43–1.66) and a nonsignificant decrease for DCIS (HR = 0.76; 95% CI, 0.26–2.21) with lasofoxifene (both 0.5 mg and 0.25 mg per day). Similarly, arzoxifene decreased overall IBC and ER-positive breast cancer incidences by 58% and 70%, respectively. No effect was noted on ER-negative breast cancers, while there was a small reduction in DCIS (HR = 0.30; 95% CI, 0.08–1.09). Overall, a higher rate of endometrial cancer was noted in women receiving a SERM compared with placebo (HR = 1.56; 95% CI, 1.13–2.14; $P = 0.007$). This increase was limited to the first 5 years of follow-up and primarily to the tamoxifen trials. No increase in the incidence of endometrial cancer was observed in the raloxifene trials. An increased risk was also observed with arzoxifene (HR = 2.26; 95% CI, 0.70–7.32; $P = 0.2$).

An overall increase in the incidence of venous thromboembolic events was noted, with both tamoxifen and raloxifene demonstrating a similar risk (OR = 1.60; 1.21–2.12; $P = 0.001$ versus OR = 1.45; 1.18–1.76; $P < 0.0001$). The rate was higher for arzoxifene and lasofoxifene. Overall, no side effects of myocardial infarction, stroke, or transient ischemic attack were noted for SERMs. The authors reported a 34% reduction in vertebral fractures and a smaller reduction of non-vertebral fractures.

American Society of Clinical Oncology (ASCO) Clinical Practice Guidelines

In July 2013, ASCO updated its clinical practice guidelines for the use of pharmacological agents to reduce the incidence of breast cancer [160]. The recommendations included a discussion of the use of tamoxifen (20 mg per day) in women (35 years or older) who are at increased risk of breast cancer. In postmenopausal women, raloxifene (60 mg per day for 5 years) and exemestane (25 mg per day for 5 years) may be an alternative to tamoxifen. An increased risk of breast cancer was defined as a 5-year projected absolute risk of breast cancer of 1.66% (using the National Institute of Cancer Breast Cancer Risk Assessment Tool [122] or an equivalent measure) or women with LCIS. The use of tamoxifen or raloxifene was not recommended for women with a history of deep venous thrombosis, pulmonary embolism, stroke, or transient ischemic attack; during prolonged immobilizations; in women who are pregnant or may become pregnant; or in nursing mothers. Discussions with patients and healthcare providers should include the risks and benefits of the agents under consideration.

Currently, there are no data from phase III randomized trials on the protective effect of raloxifene and AIs in BRCA1/BRCA2 mutation carriers. Anastrozole is currently being studied as a prevention agent in a large phase III trial, and IGFBP-1 is being evaluated as a surrogate end point biomarker in prospective breast chemoprevention studies [231]. There are limited data on the effectiveness of tamoxifen for the reduction of breast cancer risk in BRCA1/BRCA2 mutation carriers. In the NSABP-P1, 19 of the 288 women who developed breast cancer had BRCA1/BRCA2 mutations. A significant effect on breast cancer risk was not observed with tamoxifen in women with BRCA1 (RR = 1.67; 95% CI, 0.32–10.70) or BRCA2 (RR = 0.38; 95% CI, 0.06–1.56) mutations [232].

Interest is now focused on developing agents with a broader spectrum of preventive activity, particularly with regard to ER-negative breast cancer subtypes. A number of phase I and II trials using tissue-derived surrogate end point biomarkers (SEBs) as outcomes have been implemented. These smaller trials address prevention not only of ER-negative but also ER-positive breast cancers because approximately 50% of the latter are resistant to the estrogen-targeting drugs used in the large trials [233].

The Role of Diet and Nutrition

The association between various dietary factors and the risk of breast cancer has been controversial due to the lack of randomized prospective studies. An international panel of the World Cancer Research Fund and the American Institute for Cancer Research concluded that alcohol intake increases the risk of breast cancer for all age groups [234]. Some of the mechanisms postulated include carcinogenic metabolites of alcohol, such as acetaldehyde or oxygen radicals, interference with folate or estrogen metabolism, and several nutrient deficiencies associated with alcohol intake [235]. Some studies have demonstrated a 10% increase in the risk of breast cancer for every 10 g of alcohol consumed per day [236, 237]. Interestingly, the excess risk due to alcohol consumption may be reduced or mitigated by adequate folate consumption [11, 238, 239]. In addition, the role of dietary fat as a possible risk factor for IBC has been considerably investigated, and a nonsignificant increase in the rate of breast cancer (6–11%) was reported in women who consume excess dietary fat [234]. In the Women's Health Initiative Randomized Controlled Dietary Modification trial, a nonsignificant decrease in breast cancer risk was noted (RR = 0.91; 95% CI, 0.83–1.02) in women with a reduced intake of animal fat [240]. Similarly, a large prospective study demonstrated a small increase in the risk of IBC with increased intake of dietary fat [234]. Red meat intake has also been linked to breast cancer risk. A modest association between the two was reported in a meta-analysis of case–control and cohort studies; however, this association was not observed in a pooled analysis of prospective studies [241, 242]. An increased breast cancer risk was observed among women with high red meat intake in the UK Women's Cohort Study (12% increase risk per 50-g increment of meat per day) [243]. The influence of BMI on the risk of breast cancer has also been well characterized. Women with a higher BMI are at a lower risk of breast cancer before menopause but have an increased risk in the postmenopausal stage [234]. The prospective Nurses' Health Study II, in which 116,000 women have been followed since 1989, has prespecified objectives to assess the role of risk factors such as dietary fiber, saturated and unsaturated fat, plasma levels of insulin-like growth factor, low-dose oral contraceptive pills, breast-feeding, and physical activity among younger nurses [244]. In summary, there is currently no conclusive evidence based on randomized controlled trials that a specific dietary intervention or weight loss will decrease the risk of developing IBC.

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Breast Imaging and Image-Guided Biopsy Techniques

5

Marie Ganott, Brandy Griffith, and Scott M. Rudzinski

Mammography

Screening Mammography

Observational studies of screening mammography show that deaths due to breast cancer are reduced by 30–40% among women who undergo screening mammography compared to those who do not [1, 2]. The American College of Radiology recommends beginning annual screening mammography at age 40 for women with average risk for developing breast cancer [3, 4]. The probability of a mammogram detecting breast cancer in a woman age 40 years and older is much higher than the risk of mammography causing breast cancer. The cancer detection rate of screening mammography is approximately 2–7 per 1000 screened women depending on the patient population [5]. Mammography provides the lowest dose of radiation among imaging modalities that utilize radiation to image the breasts.

Mammography can be performed using a film-screen or digital technique. The diagnostic accuracies of film-screen and digital mammography are similar in the general screening population. However, digital mammography is superior to film-screen mammography in women under the age of 50, in premenopausal or perimenopausal women, and in women with dense breasts (heterogeneously or extremely dense) on mammography [6]. Digital mammography allows easier access to and storage of images and uses a lower dose of radiation [7]. Therefore many breast imaging centers have converted to digital mammography despite the increased expense of digital mammographic units and the high-resolution monitors required for viewing images. The DMIST (Digital Mammographic Imaging Screening Trial) demonstrated breast doses with digital mammography to be 22% lower per view than those with film-screen mammography [7]. Two-view digital and film-screen mammograms have a mean average glandular dose of 3.7 mGy and 4.7 mGy, respectively [8].

A screening mammographic examination is performed on an asymptomatic individual to detect clinically unsuspected breast cancer. The standard mammographic views are craniocaudal (CC) and mediolateral oblique (MLO) (Fig. 5.1). A specially trained technologist skilled in mammographic positioning obtains the X-ray images while the patient's breast is tightly compressed between a compression plate and the image receptor. The images are then typically reviewed on special workstations designed for mammography if acquired digitally or on a light box designed for viewing film if recorded on special mammography film. Computer-aided detection (CAD) is often utilized, in which application of a computer algorithm to the images marks potentially suspicious lesions and calcifications that may have been missed on the initial mammogram review.

Diagnostic Mammography

A diagnostic mammogram is performed in several circumstances. It is utilized on a patient with clinical signs or symptoms of breast disease and on an individual for whom further evaluation has been requested due to an abnormal screening mammogram or for follow-up of prior imaging findings [3]. In addition, women with a personal history of breast cancer treated with breast conservation may choose to have diagnostic mammographic imaging. The patient waits in the department, while the radiologist reviews the images; additional mammographic views and/or ultrasound (US) may be obtained at that time in order to evaluate findings or

M. Ganott, M.D. (✉)
Department of Radiology, University of Pittsburgh Medical Center, Magee-Womens Hospital, Pittsburgh, PA, USA
e-mail: mganott@mail.magee.edu

B. Griffith, D.O.
Department of Radiology, The Ohio State University, Wexner Medical Center, Columbus, OH, USA
e-mail: Brandy.Griffith@osumc.edu

S. M. Rudzinski, M.D.
Windsong Radiology Group, Williamsville, NY, USA
e-mail: srudzinski@windsongradiologygroup.com

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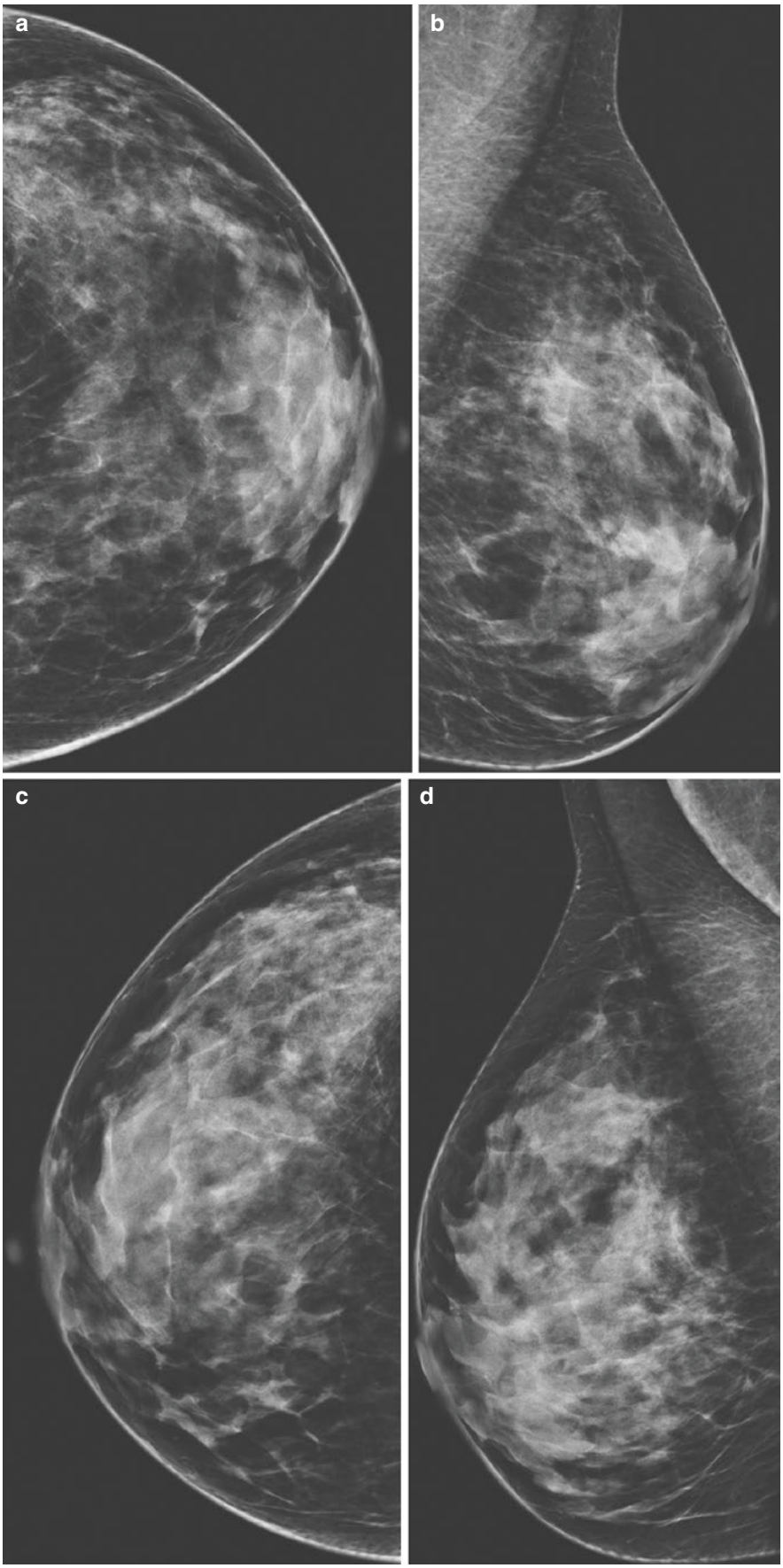

Fig. 5.1 Normal screening mammogram in a 45-year-old asymptomatic female. (**a**–**d**) Bilateral digital craniocaudal and mediolateral oblique views demonstrate heterogeneously dense breast tissue

symptoms. Because the appearance of normal breast tissue varies on mammography, comparison to prior mammograms is extremely valuable and can allow the radiologist to detect a subtle developing malignancy.

Additional mammographic views enable further characterization of a lesion. Mammographic views, including true lateral, rolled CC, exaggerated CC, tangential, and cleavage views, are used to help image more of the breast tissue and determine the location of a lesion. Spot compression views and tomosynthesis evaluate the margins of a mass and help to distinguish summation of normal tissue from a true mass. Spot compression uses a smaller paddle to focally compress the breast, spread out the overlapping tissue, and improve lesion conspicuity. Masses with microlobulated or indistinct margins are worrisome for malignancy, and masses with spiculated margins are highly suspicious for malignancy (Fig. 5.2). However, while circumscribed oval or round masses are usually benign, malignant tumors can have this appearance (Figs. 5.3 and 5.4). A mass is described as obscured

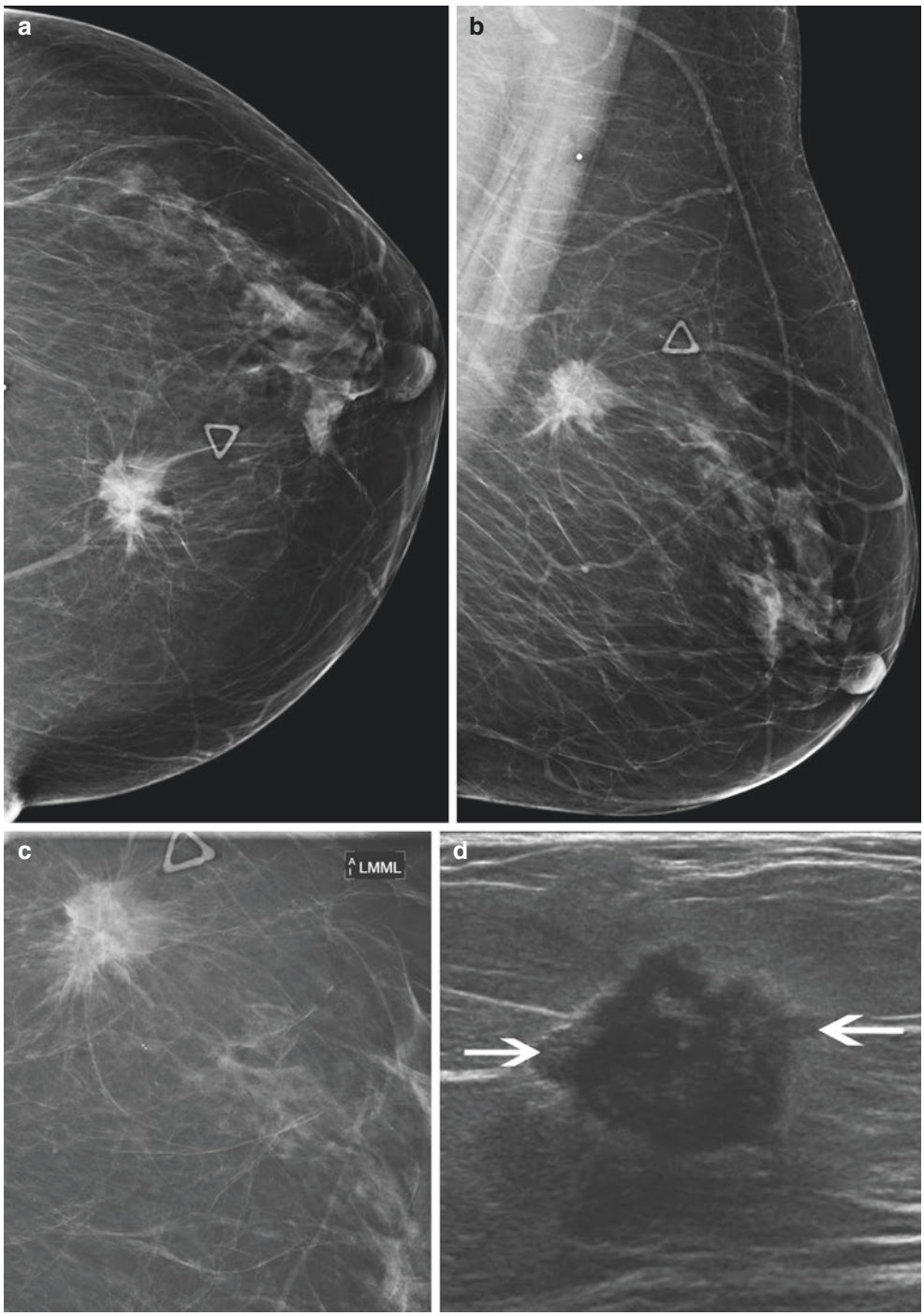

Fig. 5.2 A 60-year-old female with a palpable lump in the left breast. (**a**) CC, (**b**) MLO, and (**c**) magnified mediolateral mammograms demonstrate a spiculated mass in the upper inner breast corresponding to the palpable lump (*triangle marker*). (**d**) Ultrasound demonstrates an irregular hypoechoic mass (*arrows*). Percutaneous ultrasound-guided core biopsy revealed high-grade infiltrating duct carcinoma

when its margins are hidden by glandular tissue and can only be adequately visualized with additional views or ultrasound.

Architectural distortion refers to radiating spicules from a central point without an obvious mass or may manifest as focal retraction of the breast tissue. Most

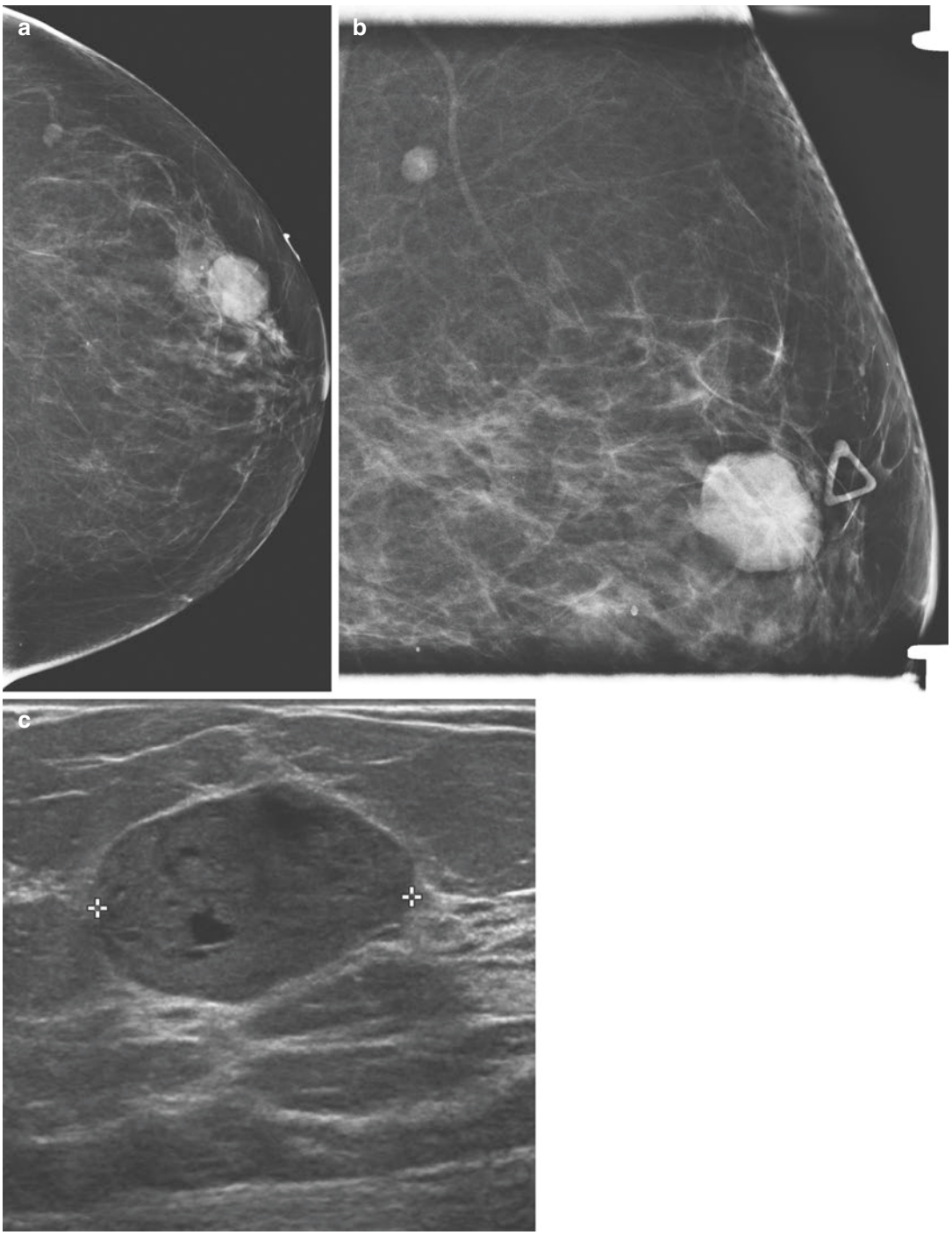

Fig. 5.3 A 51-year-old female with a palpable lump in the left upper outer quadrant. (**a**) Craniocaudal and (**b**) spot compression mediolateral mammograms of the left breast demonstrate a circumscribed oval mass corresponding to the area of palpable concern (*triangle marker*). (**c**) Ultrasound demonstrates a circumscribed slightly hypoechoic mass (calipers) oriented parallel to the chest wall. Percutaneous ultrasound-guided core biopsy revealed a fibroadenoma

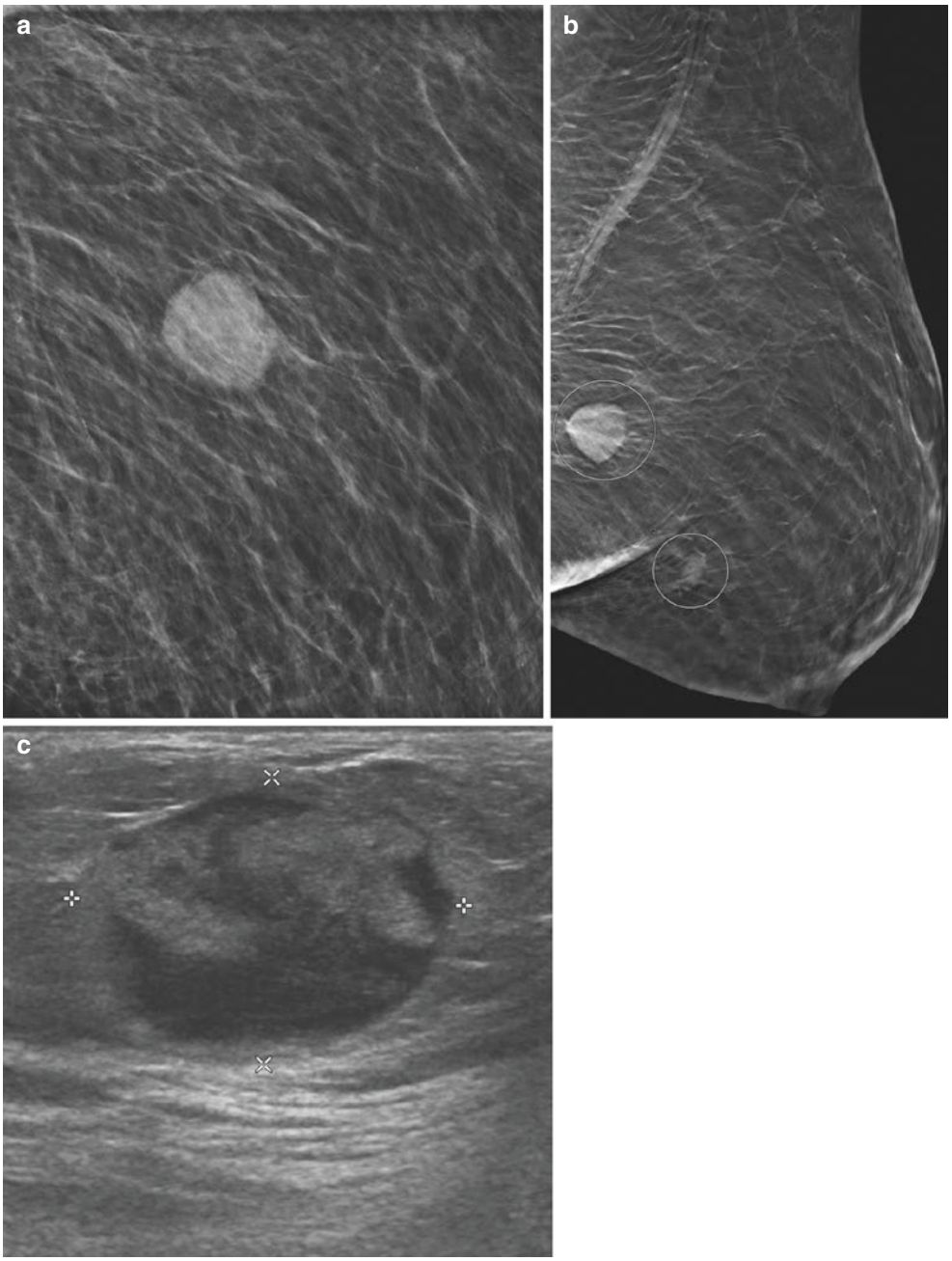

Fig. 5.4 A 73-year-old asymptomatic female recalled from screening mammography. (**a**) Spot compression mediolateral left breast mammogram confirms a circumscribed dense mass. (**b**) Mediolateral tomosynthesis demonstrates this mass and a second circumscribed mass (circles). (**c**) Ultrasound of the larger mass (calipers) demonstrates heterogeneous echotexture. Ultrasound-guided core biopsy of both masses demonstrated high-grade invasive duct carcinoma. Diffuse metastatic disease was found on subsequent PET/CT

commonly, architectural distortion is secondary to a surgical scar but can be a sign of a subtle invasive carcinoma, radial scar, ductal carcinoma in situ (DCIS), focal fibrosis, and other less common lesions. Distortion is best seen on magnified views or tomosynthesis images and is often subtle on ultrasound. Image-guided biopsy of distortion is performed unless it is due to a surgical scar (Fig. 5.5).

Asymmetry is reported when an area of tissue density is seen on only one view and is usually an area of glandu-

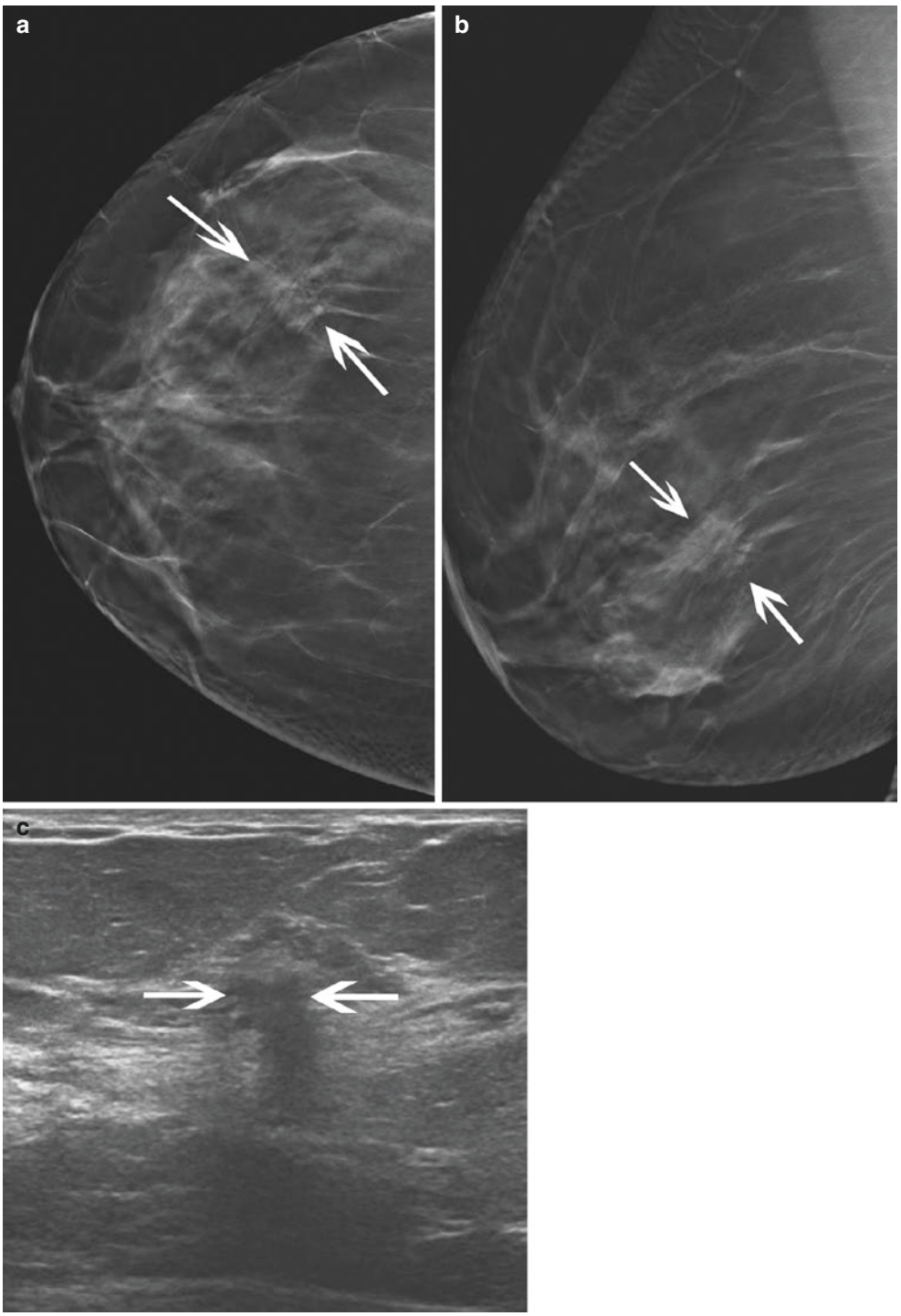

Fig. 5.5 (**a**) Craniocaudal and (**b**) mediolateral tomosynthesis images of the right breast demonstrate an area of architectural distortion (*arrows*) in the central outer right breast. (**c**) Ultrasound demonstrates a corresponding irregular hypoechoic lesion (*arrows*) with acoustic shadowing. Percutaneous ultrasound-guided biopsy revealed a radial scar

lar tissue or a summation artifact from overlapping breast tissue. A focal asymmetry observed on both views but without the convex borders and conspicuity of a mass is also usually an area of glandular tissue. These areas are evaluated with additional views or tomosynthesis and compared with prior mammograms. If new when compared with prior studies, these areas are concerning for malignancy and require additional investigation including ultrasound and potential biopsy (Fig. 5.6).

Magnification views are typically used to evaluate microcalcifications; however these mammographic views may also be useful in characterizing the margins of a mass and architectural distortion. Certain calcification morphologies are typically benign, including skin, vascular, rim, dystro-

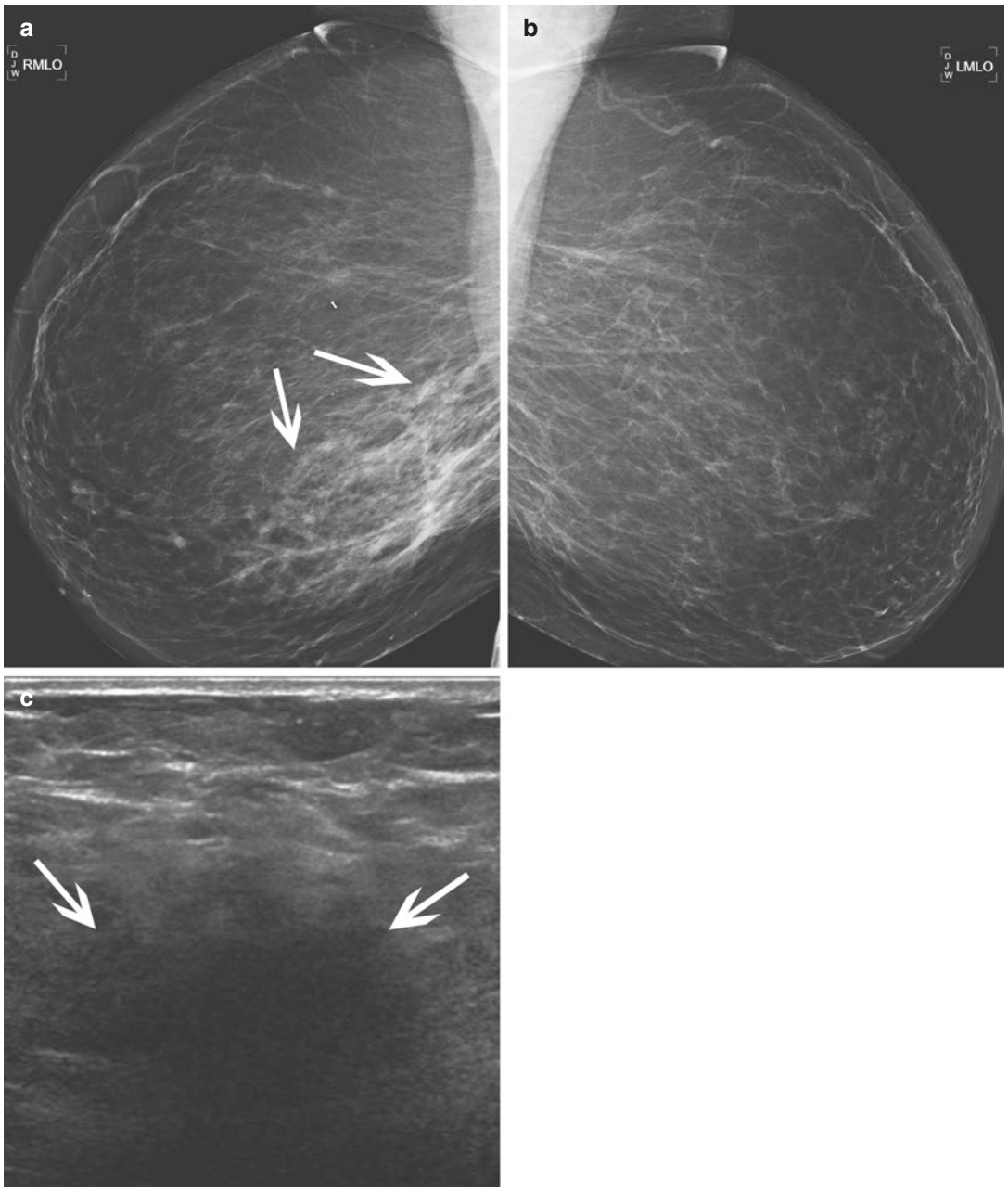

Fig. 5.6 (**a**) Right and (**b**) left MLO views demonstrate a new asymmetry (*arrows*) in the posterior inferior aspect of the right breast. Incidentally noted is a biopsy clip from a prior benign biopsy in the right breast. (**c**) Ultrasound demonstrates an irregular hypoechoic mass with indistinct margns (*arrows*). Subsequent ultrasound-guided biopsy revealed intermediate-grade ductal carcinoma in situ

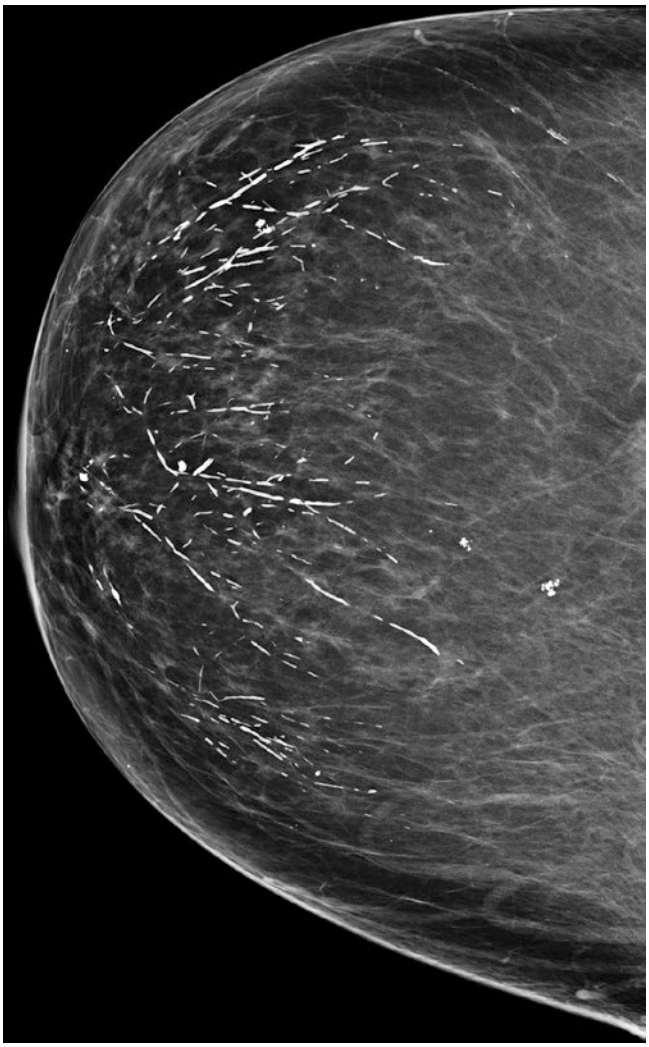

Fig. 5.7 CC view of the right breast demonstrates classic large rodlike (also called secretory) calcifications, which are dense, well defined, and often described as "cigar" shaped. They are in a ductal distribution directed toward the nipple and are usually bilateral

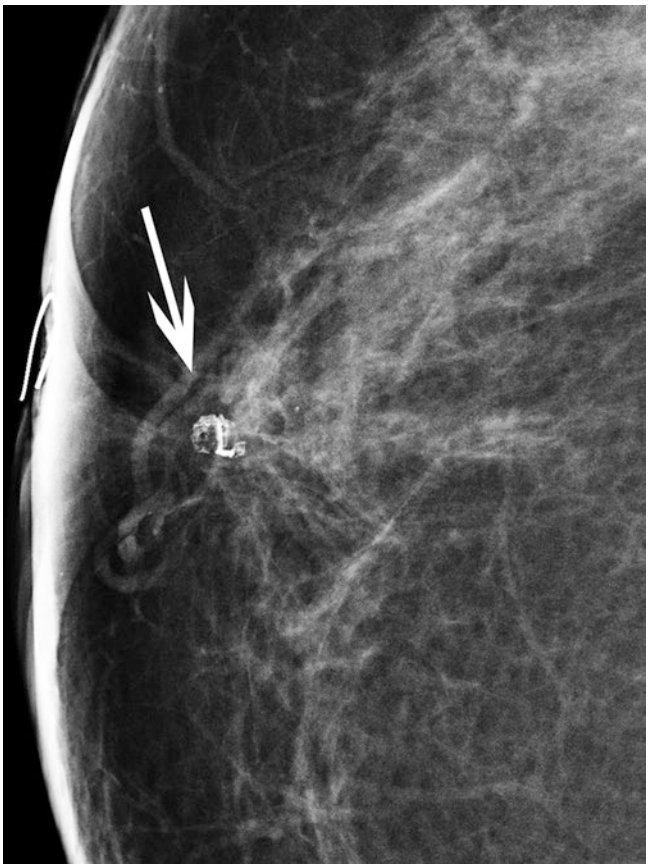

Fig. 5.8 41-year-old female with a history of right breast cancer 5 years ago, post-lumpectomy and radiation: CC mammogram of the right breast demonstrates postsurgical changes (*arrow*) from the prior lumpectomy in the right retroareolar breast with skin retraction and coarse calcifications typical of fat necrosis

phic, round, large rodlike, and milk of calcium (Figs. 5.7 and 5.8). Circumscribed masses containing coarse popcorn-like calcifications are characteristic of calcifying fibroadenomas. Calcifications that are layered in a meniscal fashion on true lateral views represent milk of calcium in microcysts. Calcifications that are categorized as not classically benign include punctate, coarse heterogeneous, and amorphous, while fine pleomorphic and fine linear calcifications are often observed with malignancy. The distribution of calcifications is also an important factor in characterizing calcifications as suspicious or benign. The distribution may be described as diffuse, regional, grouped or clustered, linear, or segmental. Pleomorphic and linear calcifications in a segmental or ductal distribution are highly suggestive of DCIS (ductal carcinoma in situ) and will undergo biopsy, typically with stereotactic guidance, as calcifications are not reliably visualized with US (Fig. 5.9). Calcifications that cannot be categorized definitively as benign or suspicious are reported as "indeterminate" and are usually also recommended for stereotactic biopsy. Calcifications believed to be "probably benign," indicating a 2% or less chance of malignancy, are usually recommended for observation, with follow-up mammography at 6-, 12-, and 24-month intervals if stable.

Reporting

Radiologists use the American College of Radiology Breast Imaging Reporting and Data System (BI-RADS®) as a guide to standardize reporting, breast imaging terminology, assessment, and follow-up recommendations for mammog-

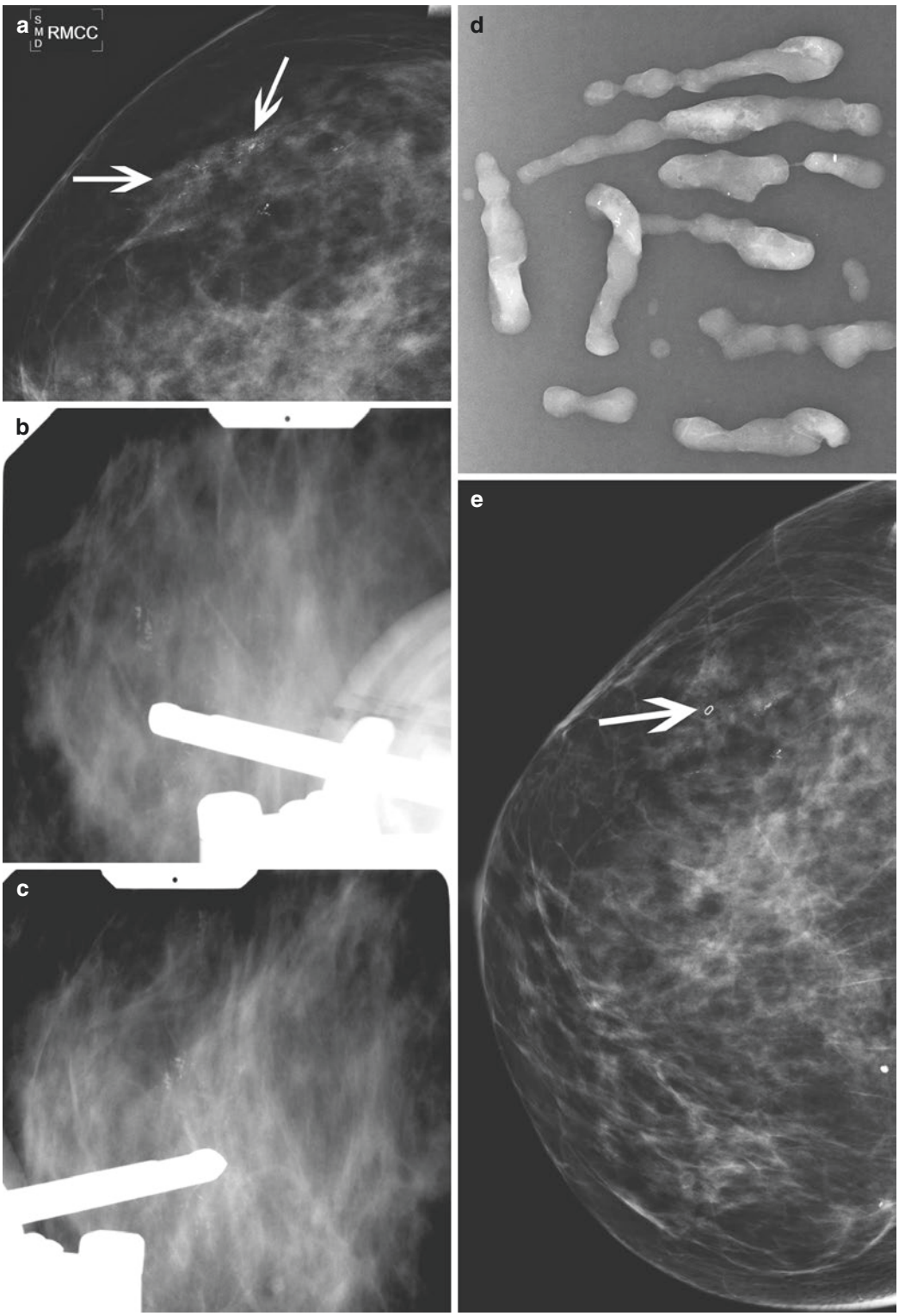

Fig. 5.9 A 57-year-old asymptomatic female recalled from screening mammogram. (**a**) Magnified CC view of the right breast demonstrates pleomorphic calcifications (*arrows*) in the outer breast in a segmental distribution. (**b**, **c**) Stereotactic biopsy is performed utilizing paired images taken at 15° of obliquity. (**d**) A specimen radiograph of the cores confirms the targeted calcifications were sampled in the biopsy. (**e**) CC view of the right breast demonstrates the clip (*arrow*) placed at the site of biopsy. Pathology revealed high-grade ductal carcinoma in situ

Table 5.1 Concordance between BI-RADS® assessment categories and management recommendations

Assessment	Management	Likelihood of cancer
Category 0: Incomplete – need additional evaluation and/or prior mammograms for comparison	Recall for additional imaging and/or comparison with prior examination (s)	N/A
Category 1: Negative	Routine mammography screening	Essentially 0% likelihood of malignancy
Category 2: Benign	Routine mammography screening	Essentially 0% likelihood of malignancy
Category 3: Probably benign	Short-interval (6-month) follow-up or continued surveillance mammography	>0% but ≤2% likelihood of malignancy
Category 4: Suspicious	Tissue diagnosis	>2% but <95% likelihood of malignancy
Category 4A: Low suspicion for malignancy		>2% but ≤10% likelihood of malignancy
Category 4B: Moderate suspicion for malignancy		>10 to ≤50% likelihood of malignancy
Category 4C: High suspicion for malignancy		>50 to <95% likelihood of malignancy
Category 5: Highly suggestive of malignancy	Tissue diagnosis	≥95% likelihood of malignancy
Category 6: Known biopsy-proven malignancy	Surgical excision when clinically appropriate	N/A

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raphy, US, and MRI of the breast [3]. A BI-RADS rating indicating the overall assessment of the imaging evaluation and the radiologist's recommendation for follow-up is required at the end of each mammography report. These assessment categories and recommendations are presented in Table 5.1.

The mammography report should include (and in some states is required by law) a comment on the density of the breast tissue. Breast density is affected by age, hormone replacement therapy, menstrual cycle phase, parity, body mass index, lactation, and familial predisposition. Dense tissue is more common in younger women; however, predominantly fatty or predominantly dense breast tissue can be found in women of any age. Breast density is typically subjectively categorized by the interpreting radiologist based on the amount of glandular tissue observed on mammography. Computer-assisted software programs may also be used to assess breast density. The categories are almost entirely fat (<25% glandular), scattered fibroglandular (25–50%), heterogeneously dense (50–75%), and extremely dense (>75% glandular) (Figs. 5.1 and 5.10). In the first two categories, 80% of breast cancers are identified by mammography. Only 30–60% of breast cancers may be seen on film-screen mammograms in heterogeneously and extremely dense breasts [9]. Digital mammography improves tissue contrast, increasing cancer detection in dense tissue to up to 70%, compared to film-screen mammography [6].

The risk of breast cancer is increased in women with mammographically dense breasts [10]. Women with extremely dense breast tissue (>75%) have a four to six-fold increased risk of developing breast cancer compared to those women with little or no dense tissue [11–14]. In addition, dense breast tissue lowers the sensitivity of mammography due to the ability of dense tissue to obscure tumors [15, 16].

Imaging of the Male Breast

Gynecomastia is the most common reason for males to undergo breast imaging. It may present as a subareolar mass or breast enlargement that may be painful. Although these symptoms are often unilateral, initial evaluation is with bilateral mammography to allow comparison of the breast tissue. There are three mammographic patterns of gynecomastia: early nodular, late dendritic, and diffuse glandular. The early nodular pattern occurs when the gynecomastia has been present for less than 1 year and is evident as a subareolar flame-shaped or fan-shaped density (Fig. 5.11). The late dendritic appearance typically occurs after 1 year and is a flame-shaped, subareolar mass with radiating linear tissue extending into the posterior adipose tissue. The diffuse glandular pattern is due to exogenous estrogen (prostate cancer treatment, transsexuals) and is dense nodular parenchyma [17]. US is not always necessary to evaluate gynecomastia unless the mammographic appearance is atypical. Sonography of gynecomastia may reveal discrete round or hypoechoic nodular retroareolar tissue or irregular hypoechoic tissue that extends into the adjacent tissue [18] (Fig. 5.11).

Breast cancer in a man most commonly presents as a painless palpable subareolar mass. Up to 85% of male breast cancers are invasive ductal carcinoma, as the male breast lacks lobules, except in states of excess estrogen such as during treatments for prostate cancer and transsexuals taking estrogen [19–21]. DCIS occurs in up to 50% of male breast cancers [19, 22]. The mammographic appearance of

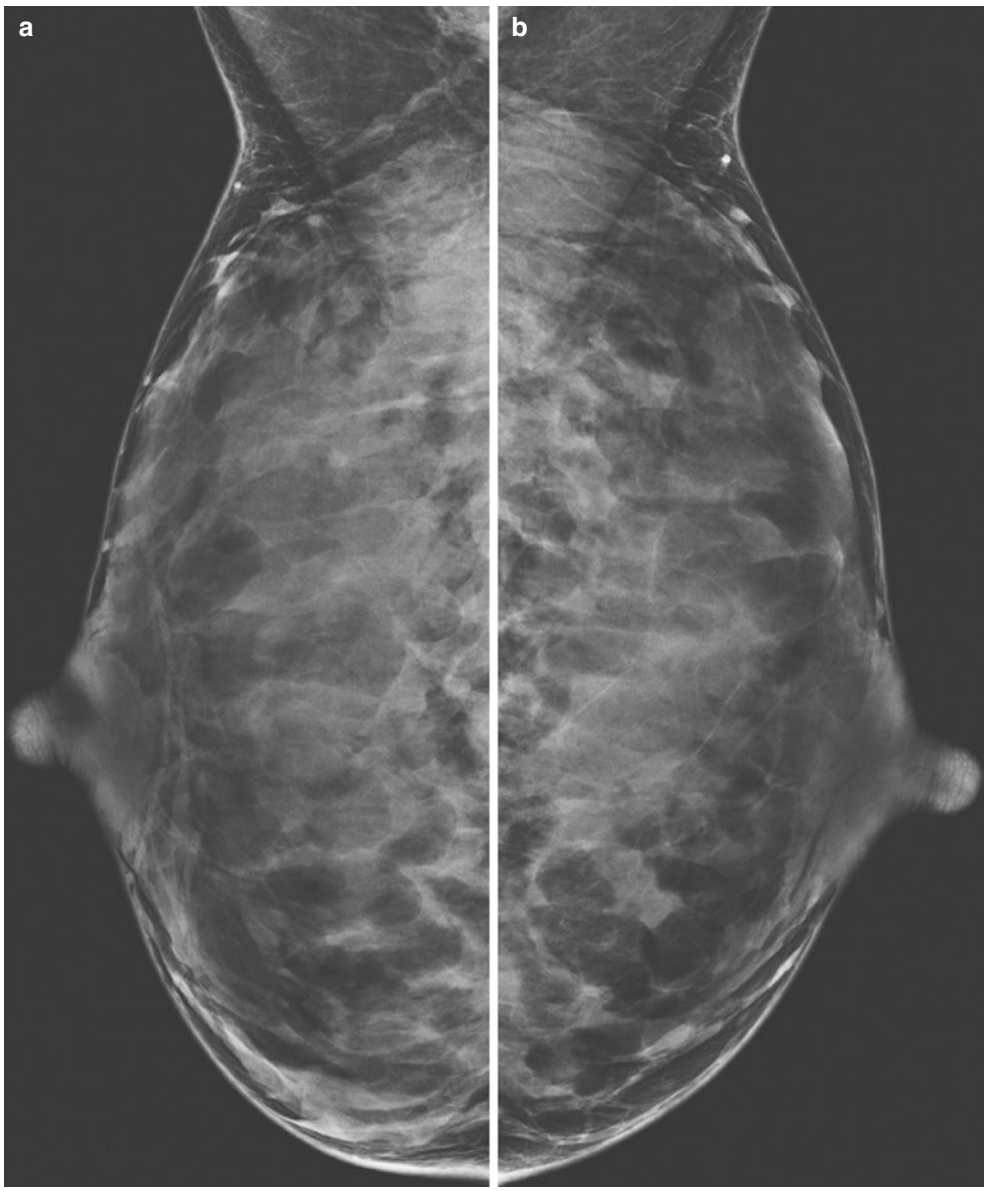

Fig. 5.10 A 35-year-old asymptomatic female for high-risk screening ultrasound due to strong family history and positive pathogenic BRCA-1 gene mutation. (**a**) Right MLO and (**b**) left MLO mammograms demonstrate extremely dense breasts

male breast cancer is that of a high-density round or oval mass with spiculated, lobulated, or indistinct margins (Fig. 5.12). Calcifications are less commonly seen than in females, occurring in only 13–30% of cases. The calcifications are coarser and less linear than those seen associated with female breast cancer [19, 23]. The mass may be accompanied by skin thickening, skin or nipple retraction, and axillary adenopathy, as in female breast cancer. The differential diagnosis includes gynecomastia, fat necrosis, and metastatic disease.

The ultrasound features of invasive ductal carcinoma are typically that of a solid hypoechoic subareolar irregular mass with spiculated or microlobulated margins [24, 25]. Less commonly the mass may be oval and circumscribed (Fig. 5.12).

High-Risk Screening

Women are considered to be at high risk for developing breast cancer if their estimated lifetime risk is 20% or greater based on family history, if they have a known or suspected *BRCA* or other high-risk genetic mutations, or if they had mantle radiation therapy to the chest prior to age 30. Lifetime risk is calculated by using one of several formulas, such as BRCAPRO, Claus, Gail, and Tyrer-Cuzick [26, 27].

The Society of Breast Imaging and American College of Radiology recommend that women with a known *BRCA1* or *BRCA2* gene mutation or other high-risk genetic syndrome or those who have not been tested but have a first-degree relative with a known *BRCA* mutation have annual mammograms starting by age 30, but not before age 25. The

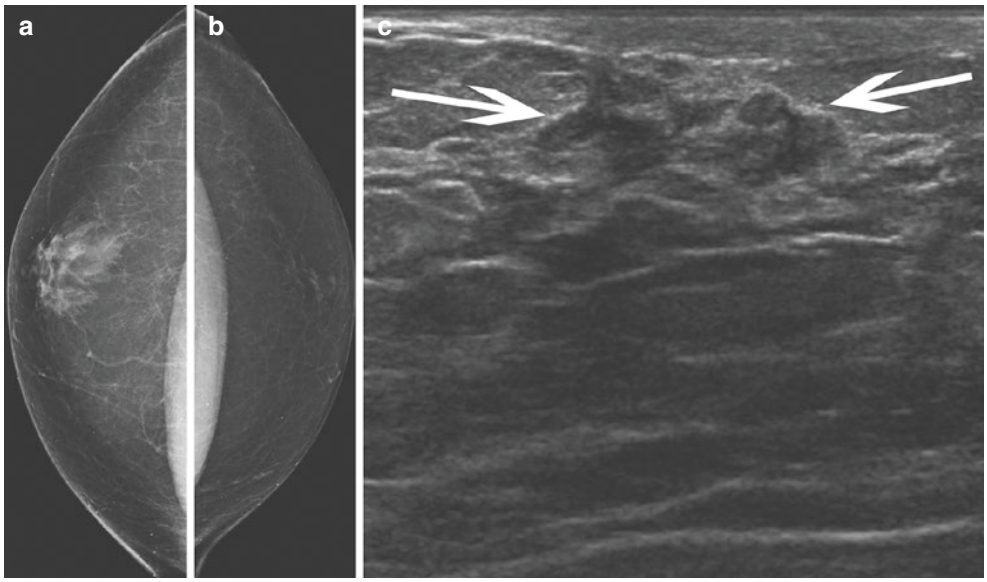

Fig. 5.11 A 50-year-old male presenting with right breast pain beneath the nipple for 1 year. (**a**) Right CC and (**b**) left CC mammograms demonstrate flame-shaped density in the right retroareolar region, consistent with gynecomastia. (**c**) Ultrasound of the right retroareolar region demonstrates dispersed subareolar hypoechoic regions (*arrows*) consistent with breast tissue of gynecomastia

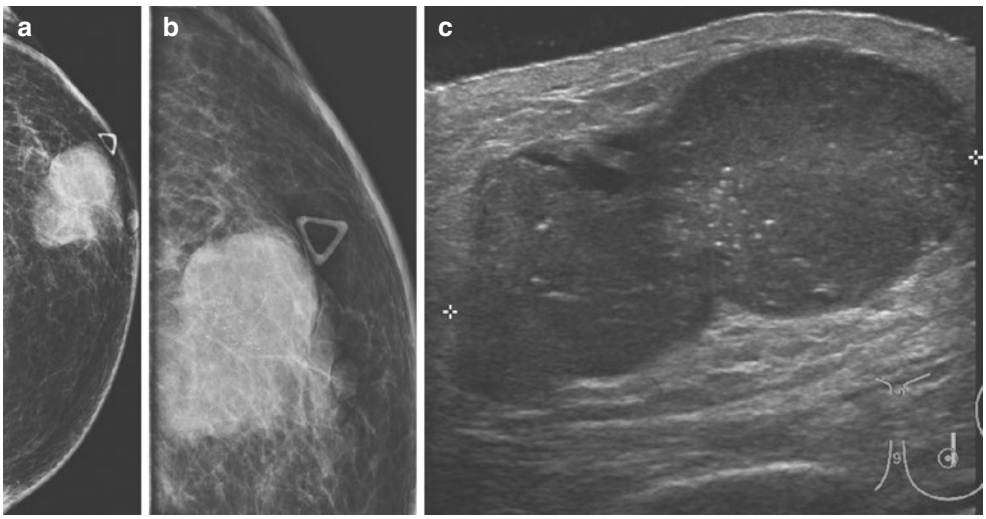

Fig. 5.12 An 87-year-old male with a palpable left breast mass. (**a**) Left CC and (**b**) left magnification CC views demonstrate a multilobulated retroareolar mass with partially circumscribed and partially indistinct margins and associated pleomorphic calcifications. (**c**) Ultrasound of the retroareolar left breast demonstrates a lobulated circumscribed hypoechoic mass with associated calcifications. Subsequent biopsy revealed infiltrating duct carcinoma, nuclear grade 2, and DCIS

recommendation for women with a greater than or equal to 20% lifetime risk for breast cancer based on family history is to have annual mammography starting by age 30 (but not before age 25) or 10 years earlier than the age of diagnosis of the youngest affected relative, whichever is later. Women with a history of chest irradiation (usually as treatment for Hodgkin's disease) between the ages of 10 and 30 should have annual mammography starting 8 years after treatment (again mammography is not recommended before age 25). Those women who have had a biopsy showing lobular carcinoma in situ (LCIS), atypical lobular hyperplasia (ALH), atypical ductal hyperplasia (ADH), or DCIS should have annual mammograms from the time of diagnosis, regardless of age [27].

Although mammography has been the mainstay in breast cancer screening, other modalities, such as magnetic resonance imaging (MRI) and US, have established their relevance for women at increased risk for breast cancer.

Supplemental screening MRI has been recommended for women with a high risk of breast cancer according to a risk assessment tool that is based mainly on family history [26–28]. Several studies demonstrated significantly improved sensitivity in cancer detection in women with familial breast cancer when MRI screening was added to mammographic screening [29–31]. The cancer detection rate increased by 7–20 per 1000 by adding screening MRI in high-risk patients [32]. The screening regimen for *BRCA mutation*-positive patients, untested first-degree relatives of proven *BRCA* mutation carriers, and women with a >20% lifetime risk for breast cancer should include annual MRI at age 25–29 (or mammogram if MRI is unavailable)

and annual MRI at age 30–75 in addition to annual mammography, which could be performed concurrently or alternating with mammography every 6 months [26, 27]. Women with a history of chest radiation (cumulative dose of > or = 10 Gy) before age 30 are recommended to have screening MRI annually beginning 8 years after the radiation therapy [27]. High-risk women who are unable to undergo MRI may benefit from screening ultrasound in addition to mammography [31].

Ultrasound

Ultrasound (US) plays a pivotal role in the work-up and management of breast disease. US is a noninvasive imaging modality that uses high-frequency sound waves to produce images based on reflection from tissue interfaces. US is readily available, is relatively low cost, has no associated radiation exposure, and does not require the administration of intravenous contrast. Continued technological advancements in conjunction with improved imaging skills and operator experience have made US a critical modality in breast imaging and an indispensable adjunct to mammography. US is essential in imaging abnormalities not visible on mammography and is valuable in guiding needle biopsies of suspicious lesions and aspirating fluid collections. US can also be used to follow low-suspicion lesions and to evaluate response to neoadjuvant chemotherapy. In addition to grayscale imaging, elastography and Doppler sonography are tools that allow further characterization to help the radiologist appropriately categorize a lesion with regard to its stiffness and vascularity.

Diagnostic Breast Ultrasound

A diagnostic work-up is performed when a patient has been recalled from a screening mammogram for a definite or suspected mammographic abnormality or for a symptomatic patient, with palpable abnormality being the most common symptom. Generally, mammography with additional views (i.e., spot compression, magnification, tangential view) or tomosynthesis is initially obtained in patients over 30 years of age. If additional views alone are not able to determine if a finding is benign or due to a summation artifact from overlapping glandular tissue, US is almost invariably the next imaging modality employed to better characterize the finding. All palpable abnormalities are worked up with physical exam and US. Although calcifications are typically evaluated mammographically with magnified views and biopsied stereotactically, US may be helpful in identifying a mammographically occult mass associated with the calcifications that could be biopsied with US guidance.

US is particularly useful in young patients (under 30 years of age) and in pregnant patients with breast complaints because of the lack of ionizing radiation and the decreased sensitivity of mammography due to dense breast tissue, which is more common in young patients. In these patients US is the first (and typically the only) modality used to evaluate a breast concern. Patients under 30 years of age with focal breast signs or symptoms (most commonly a palpable lump) have an incidence of malignancy less than 1%, and the sensitivity and negative predictive value of US is extremely high [33]. Common benign findings in this age group include cysts, fibroadenomas, and normal breast tissue. US is also the first modality used in patients with a suspected breast abscess because these patients often have too much pain to tolerate the compression required for mammography (Fig. 5.13). Color Doppler ultrasound showing blood flow at the periphery rather than in the center of the lesion suggests an inflammatory process rather than a neoplasm.

Ultrasound is useful in differentiating solid versus cystic breast lesions [34]. A simple cyst should be anechoic due to its homogeneous fluid content and lack of reflective interfaces. It should exhibit acoustic enhancement (hyperechogenic appearance of the tissue posterior to the structure) due to greater sound transmission through fluid than the surrounding breast tissue (Fig. 5.14). A solid mass contains internal echoes but is almost always hypoechoic relative to breast tissue. US features of benign lesions include well-defined margins, few gentle lobulations, and a horizontal axis parallel to the chest wall (Figs. 5.3c and 5.15).

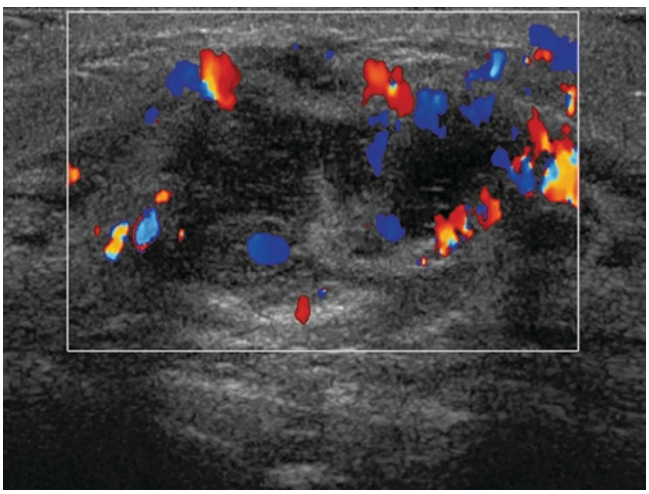

Fig. 5.13 A 46-year-old female with a newly palpable mass in the periareolar right breast at 9 o'clock. Doppler color flow ultrasound image demonstrates increased peripheral flow surrounding a heterogeneous hypoechoic mass, which had mobile debris on real-time imaging. Purulent material was aspirated from this abscess

US findings of a suspicious mass include irregular or indistinct margins, a shape that is taller than wide, marked hypoechogenicity, and acoustic shadowing (loss of reflective interfaces posterior to the lesion due to diminished sound transmission through the lesion) (Figs. 5.16 and 5.2d, and 5.6c). When a suspicious mass is identified, US evaluation of the remainder of the breast and the axilla may be performed to identify additional suspicious masses and abnormal lymph nodes (Fig. 5.17). A lymph node is considered to be abnormal when it exhibits increased cortical thickness (>3 mm), eccentric cortical thickening, absence of a visible hilus, or loss of reniform shape, among other findings. However these features can be seen with benign reactive or malignant lymphadenopathy.

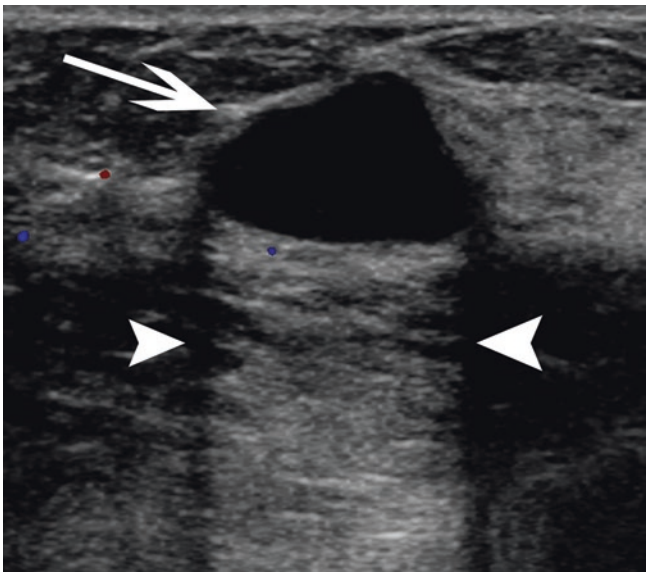

Fig. 5.14 A 49-year-old female with right breast tenderness. Doppler color flow image of the right breast demonstrates the sonographic characteristics of a simple cyst (*arrow*), including anechoic contents, imperceptible wall, and enhanced posterior through transmission of sound (*arrow heads*)

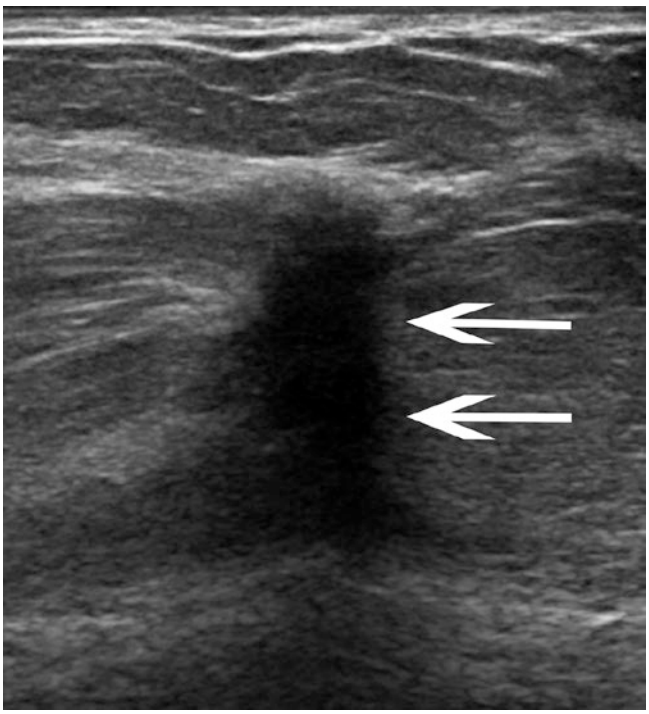

Fig. 5.16 A 41-year-old asymptomatic female recalled from screening mammogram. Ultrasound of the left breast demonstrates an indistinct irregular hypoechoic mass with angulated margins that is taller than wide and demonstrates posterior acoustic shadowing (*arrows*); all of these findings are highly suspicious for malignancy. Ultrasound-guided core biopsy revealed intermediate-grade invasive duct carcinoma

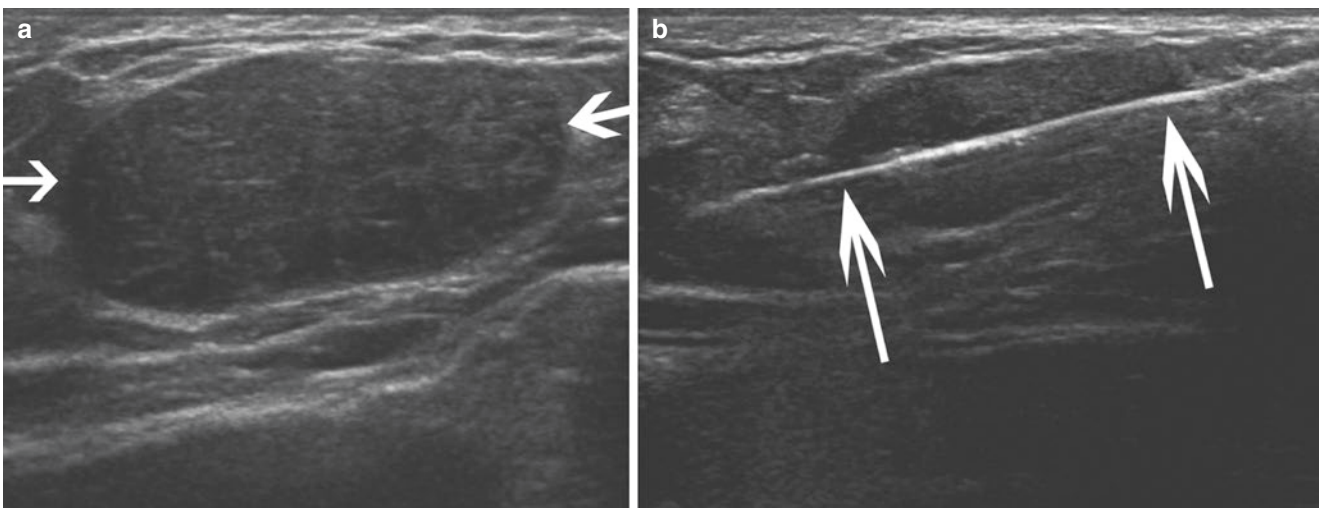

Fig. 5.15 A 55-year-old female with a palpable lump in the left upper outer quadrant. (**a**) A well-defined mass is seen on sonography. (**b**) Ultrasound-guided core biopsy of the mass revealed a fibroadenoma. The hyperechoic line traversing the lesion is the biopsy needle (*arrows*)

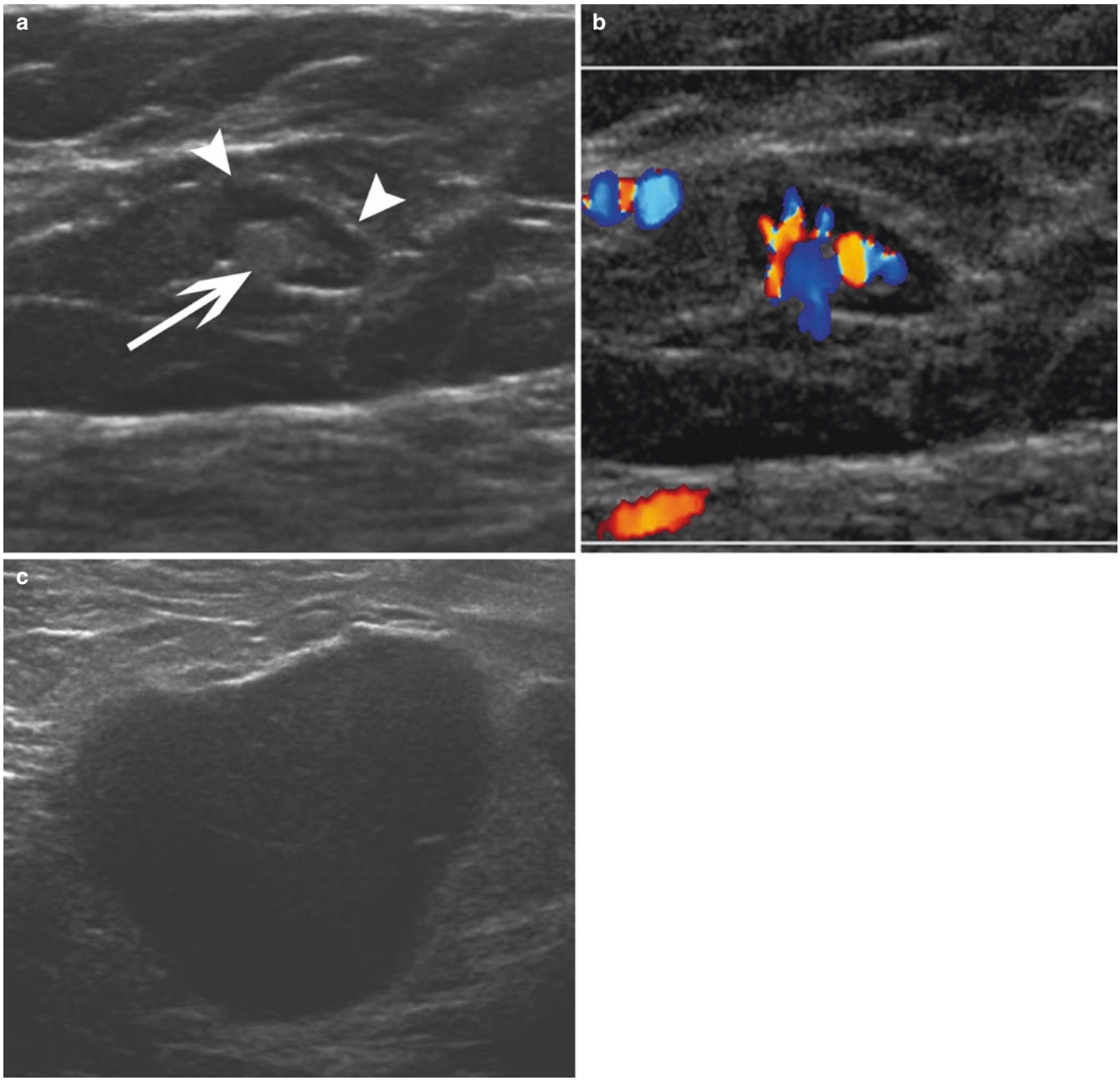

Fig. 5.17 (**a**) A normal-appearing lymph node with thin cortex (*arrowheads*) and fatty hilum (*arrow*). (**b**) Blood flow at the hilum further characterizes this mass as a lymph node. (**c**) A 56-year-old female with history of breast cancer presenting with an axillary mass. US demonstrates a markedly enlarged lymph node with a thick cortex, lack of a visible hilum, and loss of reniform shape. This mass was proven to be metastatic invasive breast cancer

Screening Breast Ultrasound

Mammography continues to be the mainstay of breast screening, although there has been growth in the use of US as a screening tool in women with dense breast tissue, for whom mammography is less effective [6]. A large multicenter trial concluded that in high-risk women, the use of handheld screening US in addition to screening mammography increases the detection of cancer by 3–4 per 1000 over mammography alone [35]. This increased detection rate does come at the cost of increased false positives. An average of 4.4% of women underwent biopsy due to screening ultrasound findings, with a positive predictive value (PPV) of 9.4% compared to a PPV for mammography of 22.6% [35, 36]. However, subsequent incident screening of the same women improved the PPV [37]. Over 90% of cancers detected by screening ultrasound are invasive, with a median size of 10 mm, and over 85% are node negative [36].

Screening whole-breast US has been increasingly incorporated into breast imaging practice, particularly as states implement legislation requiring that women be informed if they have dense breast tissue (>50% glandular). Some states require that the mammography report sent to the patient includes a statement that they may benefit from additional screening imaging, such as US or MRI [38].

Screening US is performed on an asymptomatic individual in conjunction with a screening mammogram in selected patient groups. The entire breast is scanned either using an automated system or by an US technologist using a handheld US transducer. Static or video images are recorded and subsequently are reviewed by the radiologist. If suspicious lesions are found after reviewing the screening mammogram and ultrasound, the patient is evaluated with a diagnostic US to determine if percutaneous biopsy should be performed. Automated whole-breast ultrasound systems do not require a highly trained technologist to perform the exam, and their use in screening was demonstrated in several studies to increase cancer detection compared to mammography in patients with dense breasts [39, 40, 41, 42].

Magnetic Resonance Imaging of the Breast

MRI plays an important role in breast imaging. The American College of Radiology recommends annual screening breast MRI as an adjunct to mammography for patients with a known BRCA gene mutation, untested patients with a first-degree relative who is a BRCA mutation carrier, patients who are considered high risk with a calculated lifetime risk of 20% or greater, those with a history of chest irradiation before age 30, and patients with Li-Fraumeni, Cowden, and Bannayan-Riley-Ruvalcaba syndromes and their first-degree relatives [26] and has recently recommended supplemental screening MRI in patients with personal history of breast cancer and dense breast tissue or those diagnosed before age 50. Although mammography is not recommended before age 25, annual MRI is recommended to be performed annually beginning at age 25–30 in the aforementioned groups of high-risk patients [43]. MRI has a sensitivity of 81% in screening high-risk patients and 92% when combined with mammography [44]. Despite its high sensitivity, MRI is not a replacement for mammography. MR images should be correlated with mammograms, US, and clinical findings for optimal interpretation. The sensitivity of MRI for detection of DCIS is less than for invasive cancers [45]. Suspicious calcifications on mammography require biopsy because ductal carcinoma in situ lacked enhancement in 16% of cases in a multicenter series [46]. However, not all DCIS manifests as calcifications on mammography; the sensitivity of MRI is higher than that of mammography for high-grade DCIS [47]. Although their sensitivity is high, MRI examinations suffer from a relatively lower specificity (reported at 67.4% by Bluemke et al.), leading to the potential for an increase in follow-up studies and biopsies with negative results [48].

In addition, breast MRI is performed to evaluate the extent of disease in patients with known breast carcinoma, identify sites of residual malignancy status post-lumpectomy with positive margins, monitor the response to neoadjuvant chemotherapy, evaluate breasts for occult cancer in the presence of metastatic axillary lymphadenopathy with an unknown primary, and evaluate silicone implant integrity [49]. MRI may also be used to clarify imaging and physical findings.

The examination is performed in the prone position with simultaneous imaging of both breasts, usually in the axial plane, following the intravenous administration of gadolinium contrast. An MRI unit with a magnetic field strength of at least 1.5 T and a dedicated breast surface coil is required to obtain quality images. When MRI is performed solely to evaluate silicone implant integrity, gadolinium is not necessary.

MRI incorporates an evaluation of contrast enhancement kinetics and morphology to detect and assess breast lesions. Enhancing lesions are described as a focus (an enhancing structure measuring ≤4 mm, too small to characterize), mass, or non-mass enhancement. Additional descriptors are used to explain the shape, margin, distribution, and internal enhancement pattern. However, normal fibroglandular tissue also exhibits physiological enhancement, which can make detection of malignancy more difficult and increase the chance of false positives. Because the amount of background parenchymal enhancement is affected by hormonal status, elective MRI is performed early in the menstrual cycle, approximately days 7–10 after menses, to minimize this effect. Background parenchymal enhancement is reported as minimal, mild, moderate, or marked.

The contrast enhancement kinetics of a lesion are a reflection of the degree and type of vascularity of the lesion and are determined by obtaining 2–4 sequential scans of the breasts in 60–120 s per acquisition following the intravenous administration of gadolinium. Most MRI systems for breast imaging utilize computer software to evaluate how quickly the contrast enters and exits the mass to acquire the enhancement kinetic pattern. The percentage of increase in signal intensity as a function of time is plotted, and the program applies a designated color to the image depending on the enhancement patterns. The three categories of enhancement are persistent, plateau, and washout kinetics. Persistent kinetics (type I) indicate a continued increase in enhancement over time after the initial uptake of contrast and are suggestive of a benign lesion. Lesions with plateau kinetics (type II) have a steady enhancement pattern that does not change over time after the initial uptake phase. Washout kinetics (type III) occur when there is rapid washout of contrast from the lesion and are suggestive of malignancy. Lack

of enhancement is strongly suggestive of benignity but does not necessarily exclude malignancy. Subtraction sequences are obtained during postprocessing of the images and aid the detection of enhancing lesions. These sequences subtract the pre-contrast images from the post-contrast images and are also used to generate maximum intensity projection (MIP) images that provide a 3D overview of enhancing structures.

Invasive cancers will typically appear as a mass with an irregular shape with indistinct, irregular, or spiculated margins, as on mammography or US. The additional feature of internal enhancement, provided by MRI, is often heterogeneous or rim-like and usually rapid and strong with rapid washout (Fig. 5.18). Benign masses are typically oval or round in shape, with circumscribed margins and homogenous internal enhancement that progressively increases over time (persistent kinetic pattern) (Fig. 5.19).

Non-mass enhancement is described in terms of its distribution (focal, regional, linear, segmental, or diffuse) and internal features (homogenous, heterogeneous, clumped, or clustered ring) and can be due to hormonal,

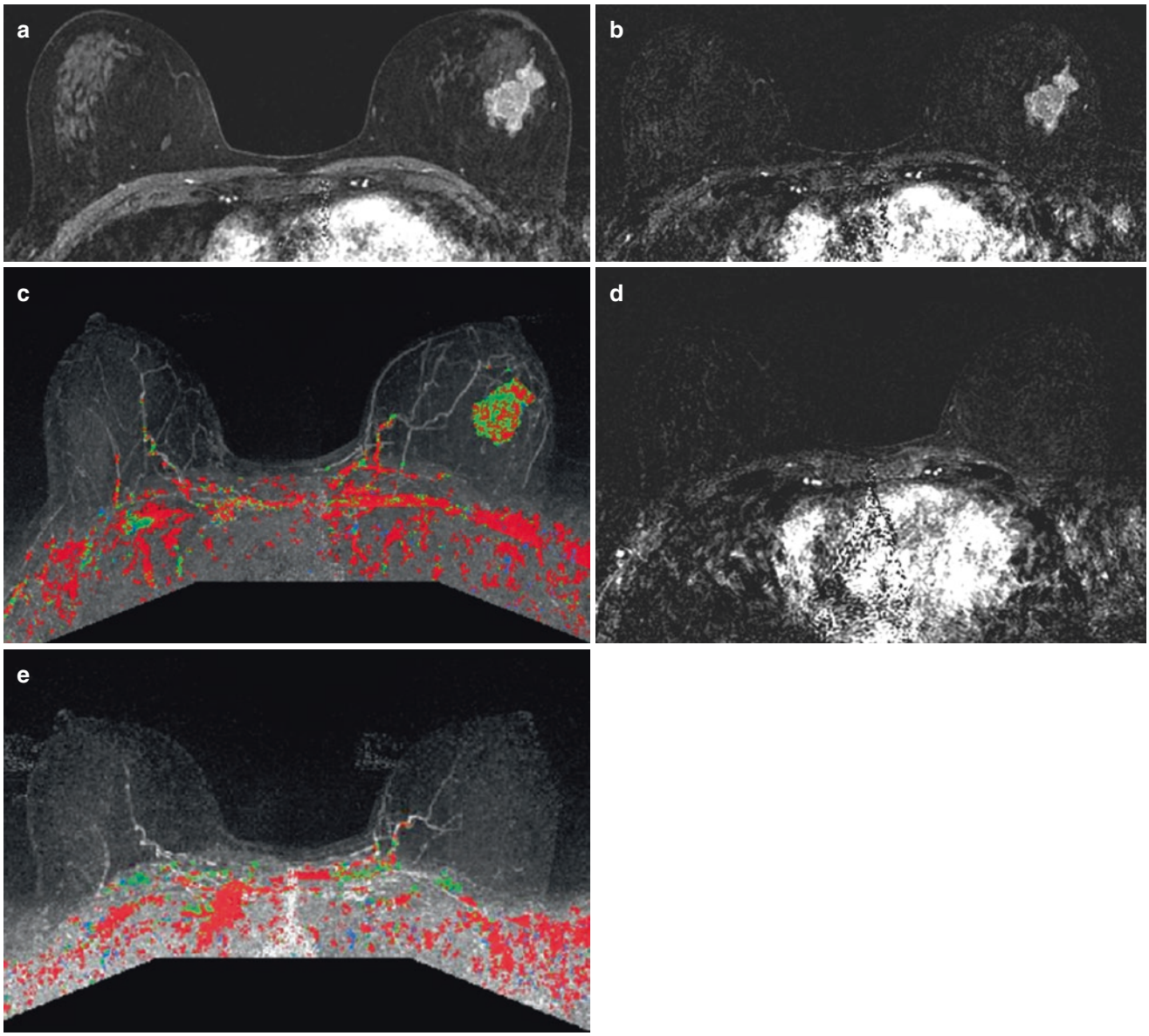

Fig. 5.18 A 56-year-old female with recently diagnosed high-grade infiltrating ductal carcinoma. (**a**) Axial contrast-enhanced, (**b**) axial subtraction MR images, and (**c**) color MIP demonstrate an irregular mass in the left breast that exhibits enhancement with plateau and washout kinetics (color coded *green* and *red*, respectively). No satellite lesions were identified, and the contralateral breast is unremarkable. Post-neoadjuvant chemotherapy (**d**) axial subtraction MR image and (**e**) color MIP image demonstrate complete response with no evidence of an enhancing mass within the breast. Final pathology of the segmental mastectomy demonstrated no residual invasive carcinoma

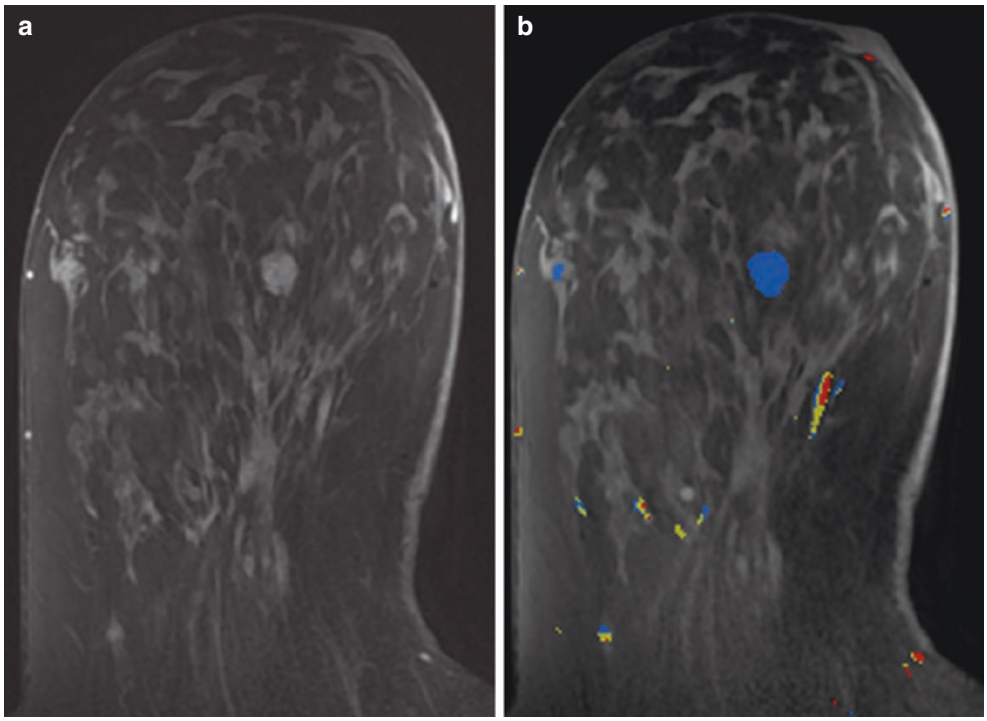

Fig. 5.19 A 60-year-old female with MR evaluation of the extent of disease for biopsy-proven carcinoma (not shown). (**a**) An axial contrast-enhanced MR of the right breast shows an oval circumscribed homogeneously enhancing mass with (**b**) persistent kinetics, indicated by the *blue color* coding, consistent with benign disease. The known IDC/DCIS is not shown

inflammatory, or fibrocystic changes. However, it can also be a manifestation of invasive lobular cancer or DCIS. Linear or segmental clumped enhancement is suggestive of malignancy (Fig. 5.20).

MRI is valuable in the detection of breast cancer in patients presenting with carcinoma of unknown primary [50, 51], particularly if the patient presents with axillary adenopathy and a negative mammogram and US. MRI detects the primary breast cancer in two-thirds of these patients, facilitating breast conservation instead of mastectomy (Fig. 5.21). When the suspected primary lesion is detected on MRI, a second-look US and US-guided biopsy will be performed. If the lesion cannot be found using US, MRI biopsy is performed. Targeted US performed to search for lesions detected on MRI is successful in finding a correlate between 50 and 60% of cases; mass lesions seen on MRI are more than two times likely to be sonographically recognized than non-mass enhancement, and malignant masses are more likely than benign masses to be found with US [52, 53].

MRI is commonly used to assess the extent of disease preoperatively in patients newly diagnosed with breast cancer to aid surgical planning. MRI detects unsuspected cancer in the contralateral breast in 3% of these patients [54]. The size of invasive carcinoma is most accurately assessed by MRI [55]. MRI can detect additional sites of malignancy in the ipsilateral breast and identify chest wall and nipple-areolar involvement. MRI detects additional disease [56–58] in 27–34% of patients, resulting in wider surgical excision or mastectomy (Fig. 5.22). However, the use of preoperative MRI has been criticized due to its potential for overtreatment without clinical benefit. A recent meta-analysis [59] showed no reduction in local or distant recurrence rates in patients who had MRI preoperative staging compared with those who had not, although an earlier study [60] did show a reduction in local recurrence. The reason for this discrepancy is not clear but has been hypothesized to be due to the effectiveness of radiation and chemotherapy in treating additional sites of disease that were not removed at surgery. In addition, the COMICE

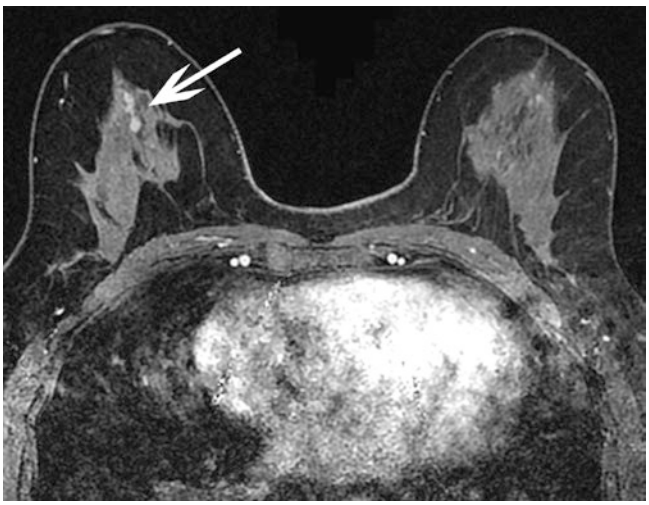

Fig. 5.20 A 50-year-old asymptomatic female for high-risk screening MR. A contrast-enhanced axial T1-weighted image demonstrates clumped linear enhancement (*arrow*) in the central upper breast. MR-guided biopsy revealed intermediate-grade invasive duct carcinoma and intermediate-grade DCIS

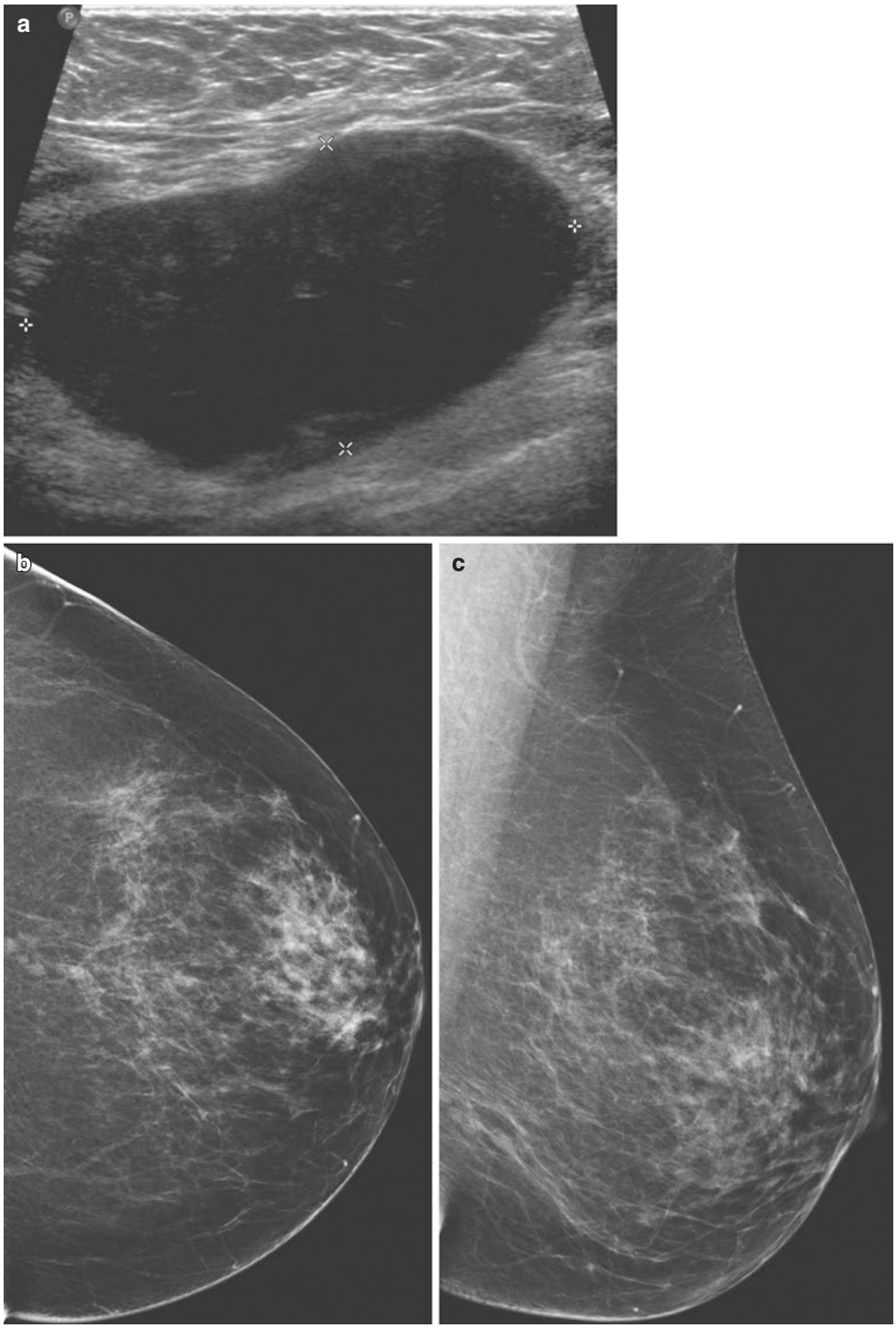

Fig. 5.21 A 54-year-old female presenting with a new lump in the left axilla. (**a**) Ultrasound of the left axilla demonstrates a grossly enlarged left axillary lymph node highly suspicious for metastatic disease. US-guided core biopsy of the node revealed high-grade infiltrating duct carcinoma. (**b**) Left CC and (**c**) left MLO mammogram performed 6 weeks prior demonstrated no evidence of malignancy. MR was subsequently performed to evaluate for an unknown primary tumor. (**d**) Contrast-enhanced axial T1-weighted images and (**e**) color MIP images demonstrate a clumped linear area of enhancement (*arrow*) with washout kinetics (color coded *red*) in the posterior left breast. (**f**) This lesion was observed on a second-look ultrasound and was biopsied with ultrasound guidance revealing infiltrating duct carcinoma

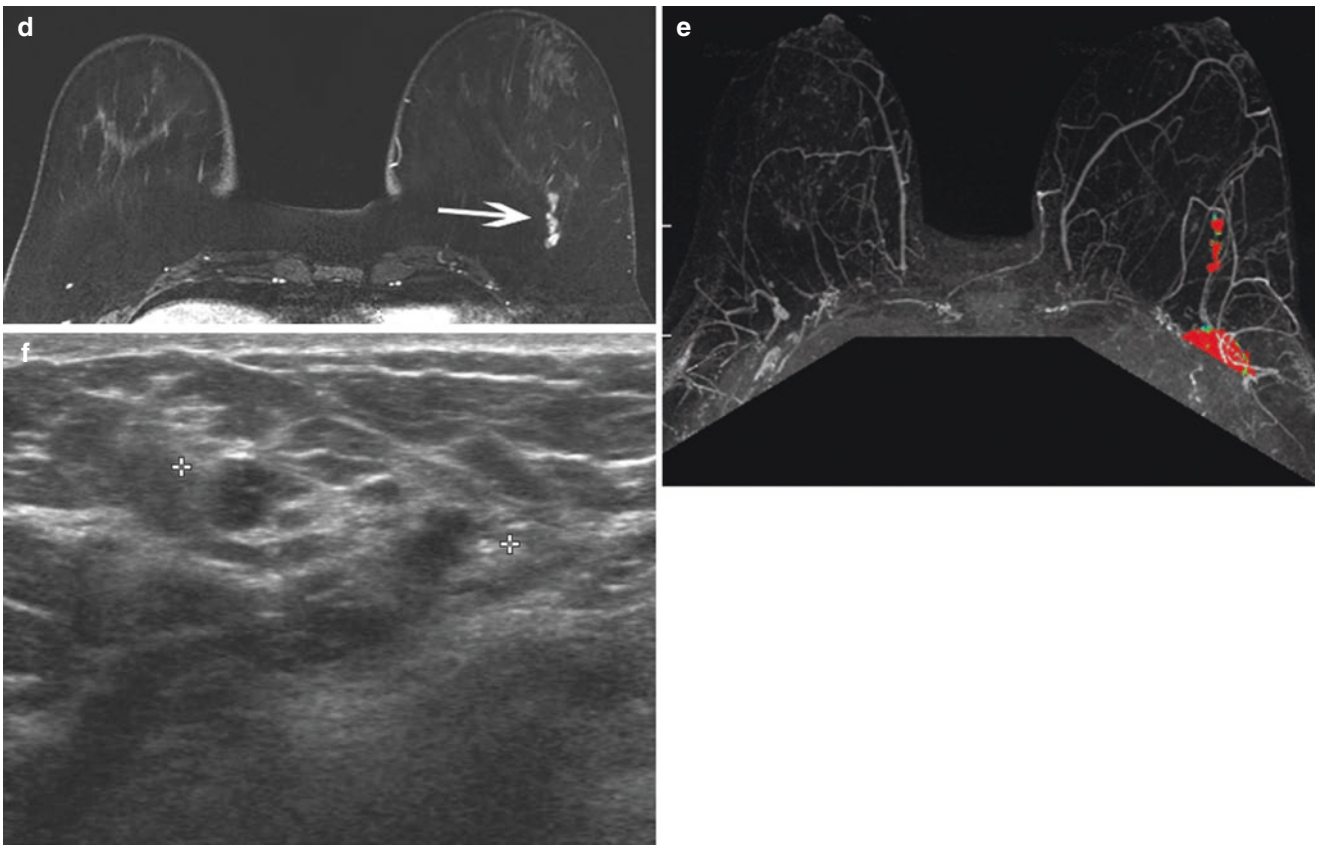

Fig. 5.21 (continued)

(Comparative Effectiveness of MRI in Breast Cancer) trial found similar reoperation rates when comparing patients with and without preoperative MRI staging [61]. If a staging MRI for extent of disease identifies additional sites of suspected malignancy, percutaneous biopsy is performed for histological proof, either with US if found on second-look or with MRI-guided biopsy, due to overlap in the appearance of benign and malignant lesions. False-positive lesions are common; the PPV of biopsy of additional lesions found on the extent of disease observed by MRI at Magee-Womens Hospital at the University of Pittsburgh Medical Center was 30% after a biopsy recommendation rate of 35% [62].

To assess the response to neoadjuvant chemotherapy (Fig. 5.18), MRI can be used to monitor early response to treatment by revealing a change in tumor kinetics before a change in tumor size or to identify residual disease prior to surgery. MRI shows better correlation with pathological response than clinical or mammographic evaluation but still may underestimate or overestimate residual disease [63–65]. A negative MRI after neoadjuvant therapy does not exclude residual disease.

MRI can be helpful in depicting residual disease after segmental mastectomy when positive surgical margins are identified pathologically, but postoperative reactive enhancement can confound the findings. Lee et al. reported a sensitivity, specificity, and accuracy of 61.2, 69.7, and 64.6% for MRI in identifying residual disease after excisional biopsy for breast cancer [66].

Contraindications to MRI include ferromagnetic medical devices such as intracranial aneurysm clips, cardiac pacemakers, and insulin pumps in addition to orbital metallic foreign bodies. Other contraindications include pregnancy (if gadolinium is required), claustrophobia that is not controlled by premedication, patient weight exceeding the limit of the MRI table, and an uncooperative patient or a patient who is unable to consent to the exam. Renal insufficiency is also a relative contraindication because there is an association with intravenous gadolinium and nephrogenic systemic fibrosis [67].

Ductography

Ductography is performed to investigate the cause of unilateral clear or bloody nipple discharge and may aid the surgeon in successfully removing the lesion by delineating its location and extent. A 30-gauge sialogram or ductogram cannula is inserted into the discharging duct, which is then gently injected with radiopaque contrast material. Magnified mammography views performed with the cannula in place should reveal a filling defect in the duct if a mass is present and if the duct is adequately opacified with contrast. A papilloma usually

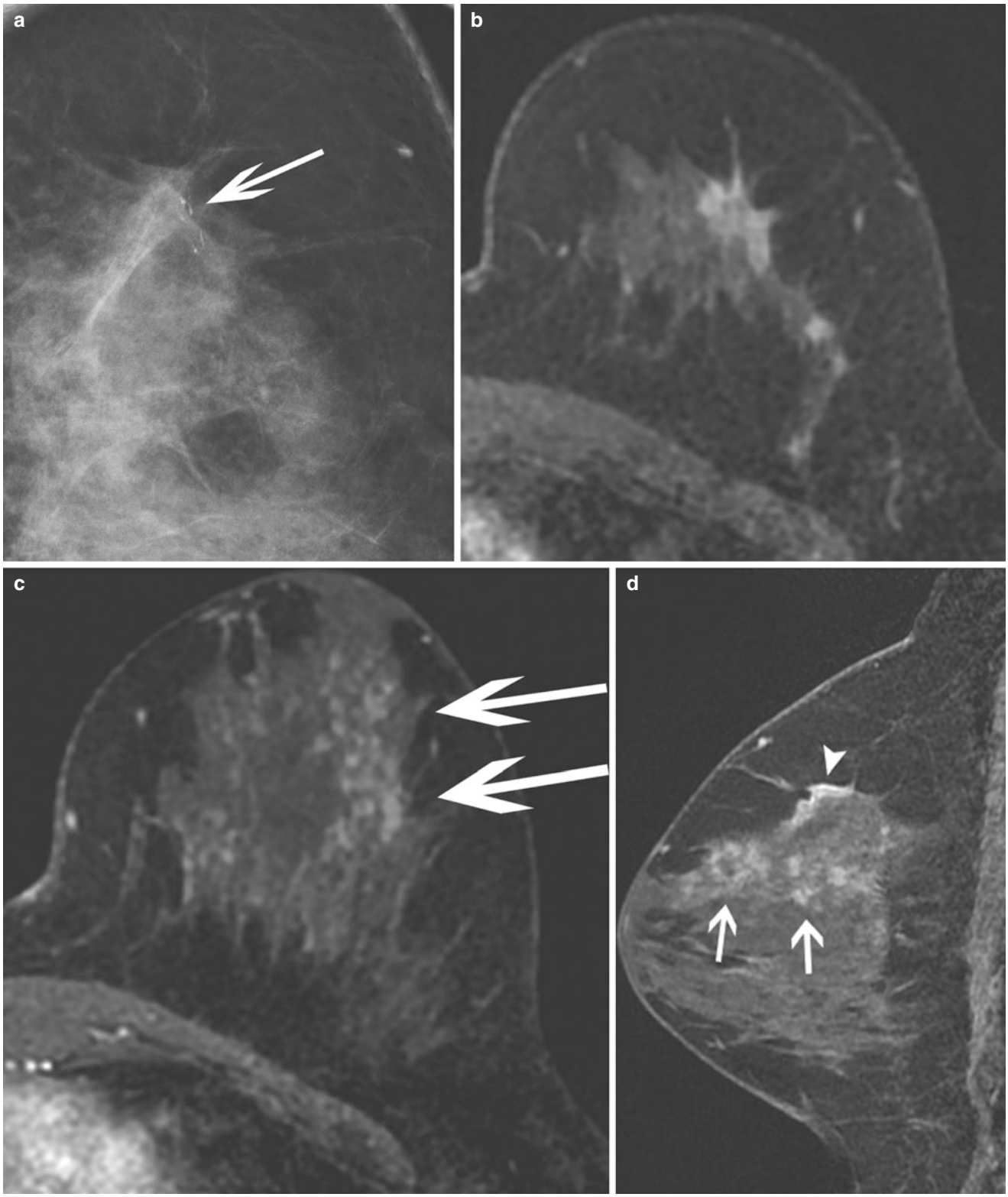

Fig. 5.22 A 57-year-old female recalled from screening mammogram for calcifications. (**a**) Spot compression magnification mediolateral view of the upper left breast demonstrates a small cluster of pleomorphic calcifications (*arrow*). Subsequent stereotactic biopsy revealed invasive duct carcinoma and DCIS. (**b**) An axial contrast-enhanced T1-weighted MR image demonstrates a spiculated enhancing mass in the upper left breast. (**c**) An axial image just inferior to this spiculated mass shows an area of segmental clumped non-mass enhancement (*arrows*) suspicious for additional disease. (**d**) A sagittal contrast-enhanced T1-weighted MR image demonstrates the segmental clumped non-mass enhancement (*arrows*), suggesting a much greater extent of disease than expected based on the calcifications observed on the mammogram; the known primary carcinoma is partially visualized superior to this enhancement (*arrow head*). The segmental enhancement was biopsied with MRI guidance, revealing invasive duct carcinoma and DCIS. The identification of additional disease resulted in mastectomy

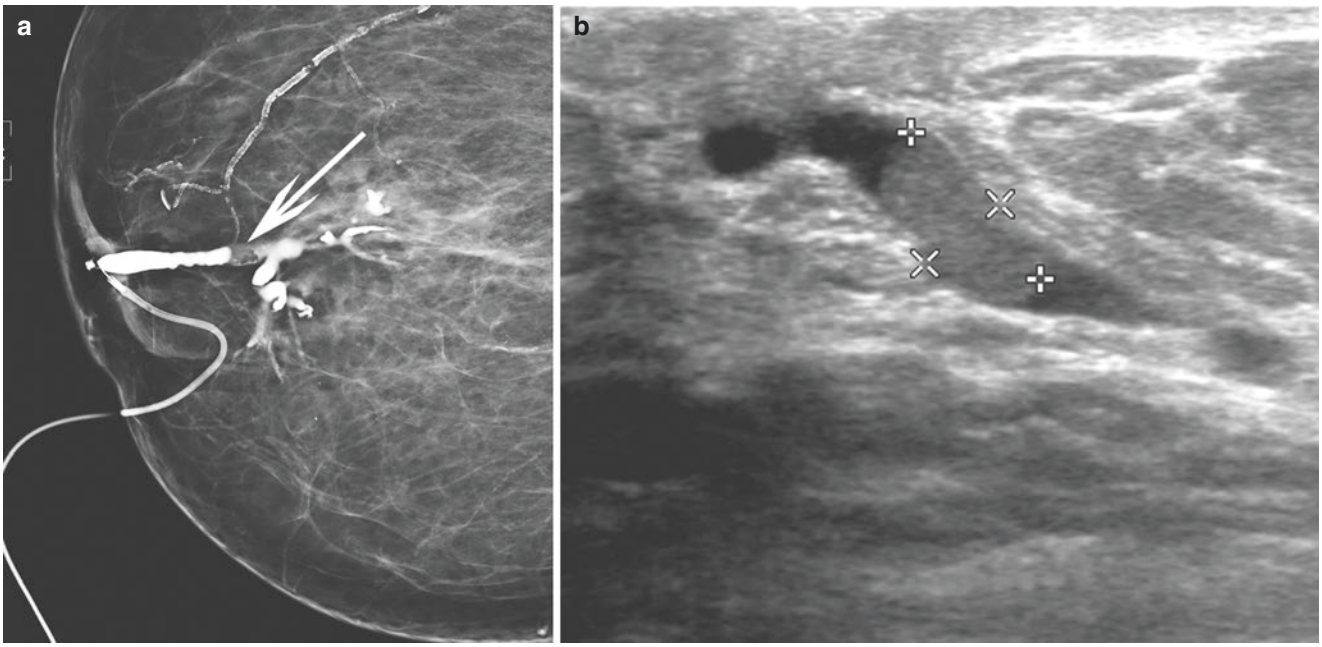

Fig. 5.23 A 72-year-old female complaining of multiple episodes of right nipple discharge. (**a**) Right CC mammogram after injection of contrast material demonstrates a circumscribed lobulated filling defect in the duct (*arrow*). (**b**) Ultrasound of the same region demonstrates an isoechoic circumscribed intraductal mass (calipers) within a dilated duct. Subsequent biopsy revealed an intraductal papilloma

appears as a small isolated lobulated retroareolar mass, but surgical excision is performed for histological diagnosis (Fig. 5.23). Irregularity and narrowing of the duct system suggest ductal carcinoma. Duct cannulation is not always successful and is not possible if the discharge cannot be expressed.

Newer and Investigational Imaging Modalities

Although mammography, US, and MRI are effective in identifying breast malignancy, there is a role for other imaging techniques that may reduce the number of diagnostic workups and biopsies of benign lesions.

Digital Breast Tomosynthesis

Digital breast tomosynthesis (DBT), also known as 3D mammography, involves the acquisition of 10–20 digital images of a compressed breast over a 15–50° angle using lower-dose X-rays than those used in standard digital mammography. These angled images are reconstructed as 1-mm thin sections through the breast [8]. This reconstruction allows the radiologist to scroll through the breast tissue in slices, removing the summation artifact caused by overlapping tissue. This results in improved differentiation between true lesions and glandular tissue and improved visualization of lesion margins while adding information about lesion location (Fig. 5.5a and b). This process reduces recall rates from screening mammography and increases cancer detection [68]. A comparative study of 12,631 patients by Skaane et al. showed an improvement in the cancer detection rate from 6.1 per 1000 to 8 per 1000 with the addition of tomosynthesis to standard screening mammography [69].

The radiation dose of a single DBT study is approximately equivalent to a two-view mammogram. Because DBT is typically performed in conjunction with a mammogram, this method doubles the radiation dose [8]. A method to synthesize a 2D mammogram from the tomosynthesis images is now FDA approved and reduces the radiation dose to that of a standard mammogram if the synthetic 2D image in conjunction with the tomosynthesis images replaces the standard 2D acquisition.

Contrast-Enhanced Mammography

Contrast-enhanced spectral mammography (CESM) uses a dual-energy weighted logarithmic subtraction technique where a low-energy mammogram image is subtracted from a high-energy mammogram image approximately 2 min after intravenous injection of iodinated contrast material, resulting in a mammogram image with suppressed visualization of normal breast tissue, thus allowing visualization of tumors that take up contrast material [70] (Fig. 5.24). Because there are two image acquisitions per view, the radiation dose is greater than for a 2D digital mammogram [71]. Potential indications for CESM

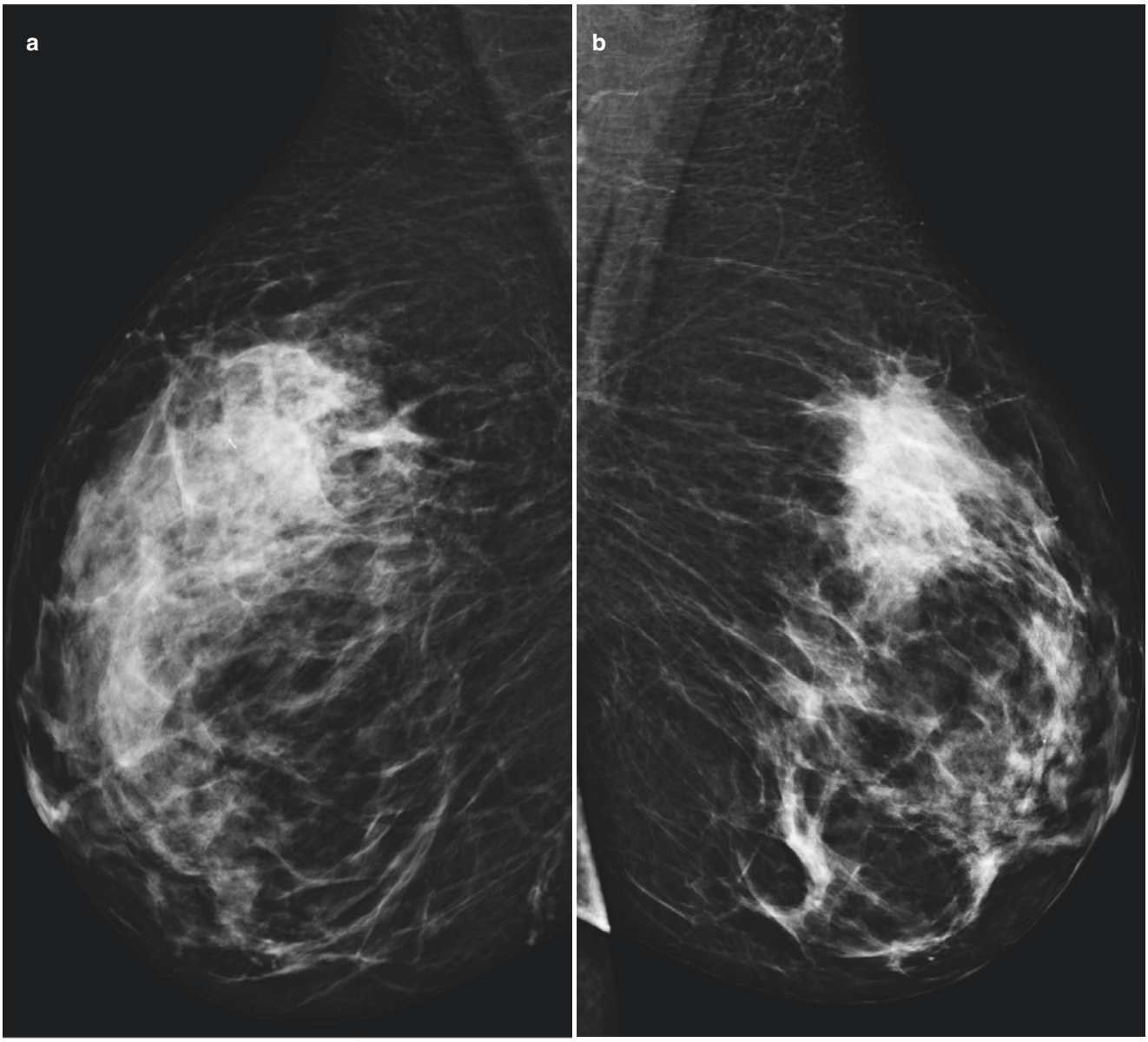

Fig. 5.24 Right (**a**) and left (**b**) MLO standard digital mammogram views show heterogeneously dense breast tissue. The carcinoma in the right breast is not well seen on the standard view due to obscuration by the glandular tissue, but is well seen on the subtracted view (**c**) due to uptake of contrast material by the tumor and suppressed visualization of glandular tissue. The subtracted view of the left breast (**d**) shows no contrast enhancement

include assessment of disease extent and response to neoadjuvant chemotherapy in patients with breast cancer and screening of high-risk and dense-breasted women [70]. Small-scale studies have shown increased sensitivity of CESM compared to digital mammography in women with dense breasts [72, 73]. Lewin et al. reported equivalent sensitivity and improved specificity of CESM compared to MRI in diagnostic patients, but limited ability to visualize the chest wall and axilla [74]. Compared to MRI, CESM is less costly and may be more accessible in the future. Disadvantages of CESM include the need for intravenous contrast and attendant concern for those with impaired renal function and history of contrast reaction.

Elastography

Multiple techniques and technological approaches are used in elastography, some enabling only qualitative and others both qualitative and quantitative assessment of lesions. The technology is incorporated with US and allows assessment of the stiffness of a lesion. Breast cancers tend to be stiffer than normal breast tissue; therefore, elastography may improve the characterization of indeterminate lesions on US and help determine which lesions should be biopsied and which can be safely followed [75, 76].

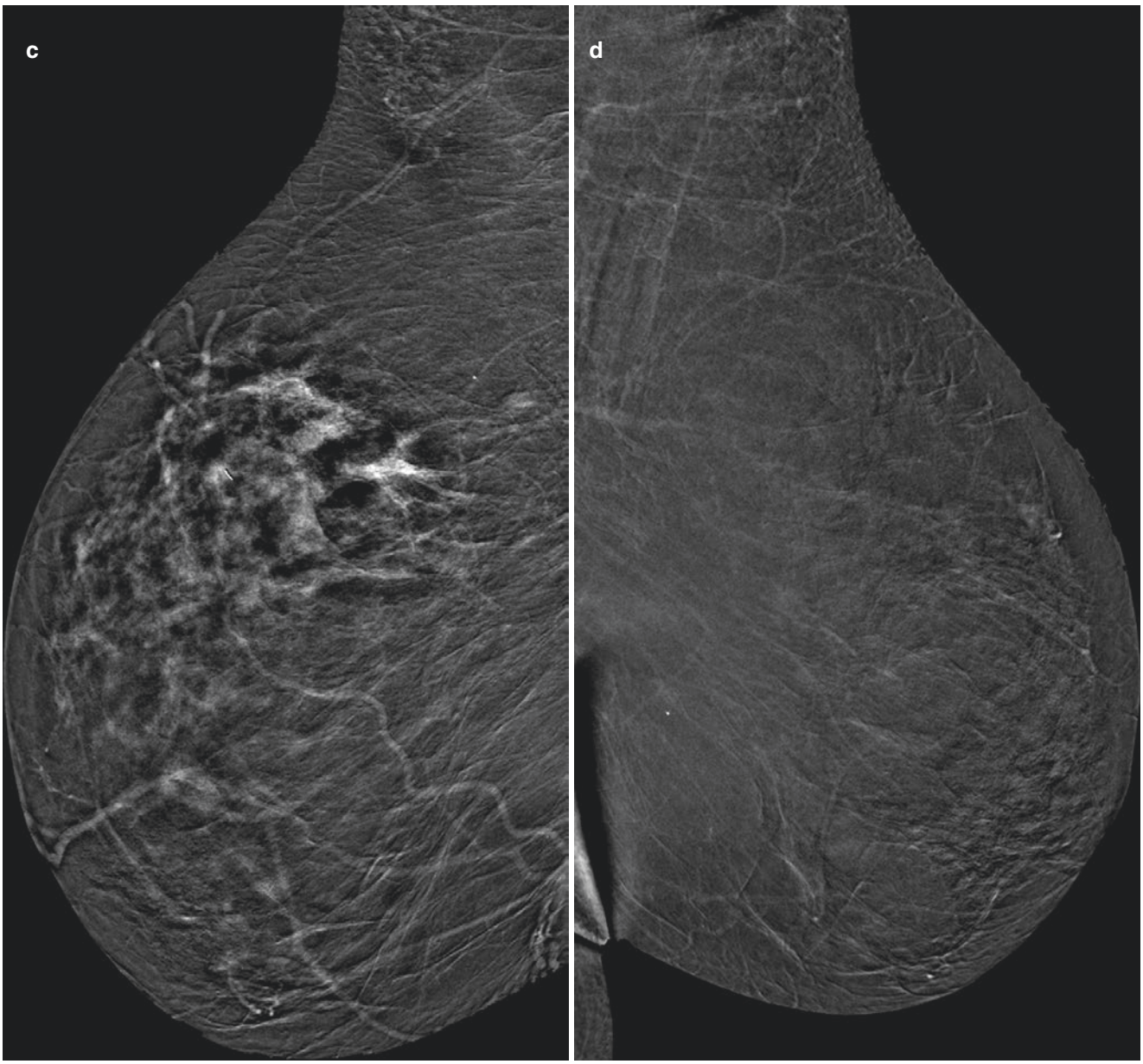

Fig. 5.24 (continued)

Nuclear Medicine Breast Imaging

Nuclear medicine breast imaging has been used for screening high-risk women with dense breasts, to evaluate the extent of disease and response to treatment in patients diagnosed with breast cancer, and problem-solving. It began as scintimammography with the use of a sodium iodide nuclear camera but had limited ability to detect small lesions. Breast-specific gamma imaging (BSGI) uses a single detector with a sodium iodide camera, and molecular breast imaging (MBI) uses two opposing cadmium zinc telluride (CZT) digital detectors, which allows the detection of smaller lesions and the administration of lower doses of the technetium-99 m sestamibi radionuclide.

The breast is placed in mild compression to immobilize the breast and is imaged after the intravenous injection of technetium-99m sestamibi, which accumulates in the mitochondria of the cells. The amount of cellular mitochondria is a marker of cellular proliferation. Rapidly dividing cells such as cancer cells have more mitochondria and therefore have a higher uptake of the radiotracer than normal cells [77].

The detectors create a 2D image of the breast, usually in the craniocaudal and mediolateral oblique positions, although other projections may be obtained. Each image takes approximately 10 min to acquire; therefore, standard four-view exams take 40 min (Fig. 5.25). Because this method is a physiological examination, adjunct imaging techniques must be used for lesion characterization and to guide biopsy.

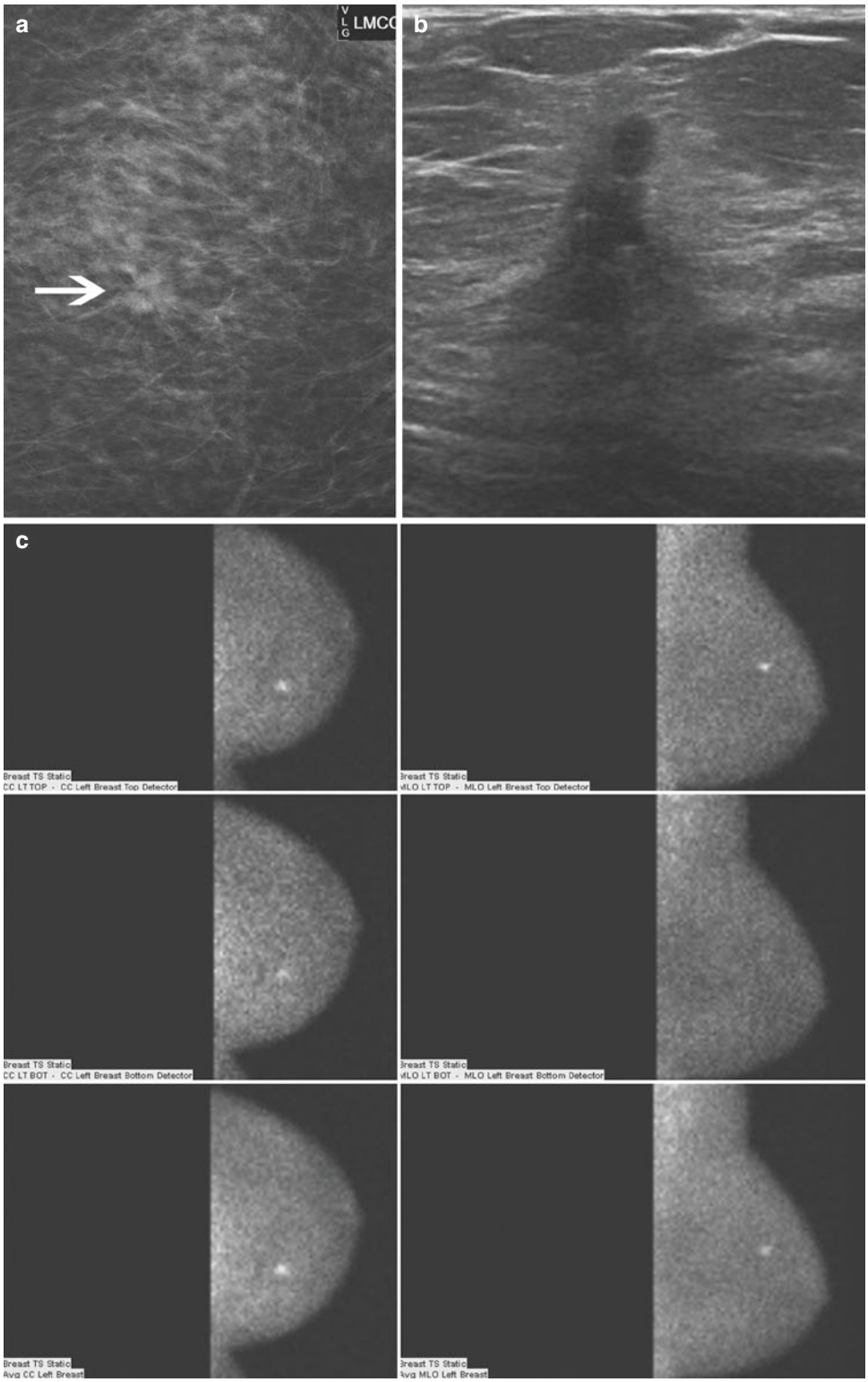

Fig. 5.25 A 62-year-old female recalled from a screening mammogram. (**a**) A left spot compression magnified CC view demonstrates a spiculated mass in the inner left breast (*arrow*). (**b**) Ultrasound demonstrates a hypoechoic mass with indistinct margins and posterior shadowing. (**c**) Tc 99m sestamibi left breast CC and MLO images and (**d**) *color* MBI CC images of the left breast demonstrate uptake in the inner left breast. Subsequent ultrasound-guided biopsy revealed high-grade invasive duct carcinoma

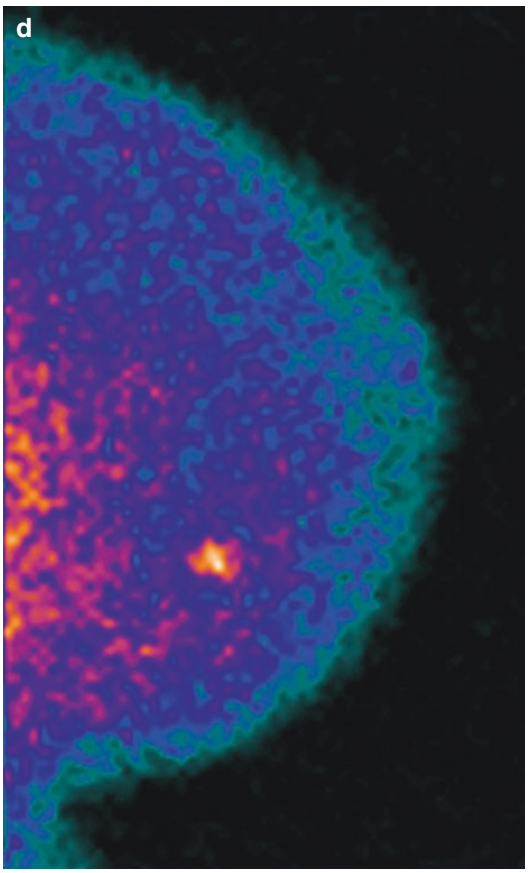

Fig. 5.25 (continued)

Technology is being developed that would incorporate guidance for biopsies.

MBI has a high degree of sensitivity, particularly for invasive cancers [78]. Studies have indicated a sensitivity of MBI of 85–100% and a specificity of 60–90% [77]. Several studies have reported MBI to have similar sensitivity and greater specificity compared to MRI [79]. A disadvantage of MBI is the higher whole-body radiation dose compared to mammography. A BSGI examination provides the highest organ doses to the large intestine wall, kidneys, bladder wall, and gallbladder wall [8]. The sensitivity and specificity of imaging with smaller doses of the radionuclide has been reported to be preserved. The breast dose is approximately 0.53 mGy from an 8 mCi injection of technetium-99m sestamibi [80].

Positron Emission Mammography

Positron emission mammography (PEM) was developed to overcome the limitations of whole-body PET (positron emission tomography) in detecting breast cancer and uses two opposing dedicated gamma radiation detectors placed above and below the breast while the breast is in mild compression, resulting in improved sensitivity and better spatial resolution and allowing the detection of lesions <1 cm [81]. The images are acquired after the intravenous administration of fluorine^{18} fluorodeoxyglucose (FDG), a radionuclide that is similar in structure to glucose and is taken up by metabolically active tumor cells. The images are displayed as 12 tomographic slices in 2 projections of each breast, facilitating 3D localization of abnormal FDG uptake. A disadvantage of this technology is the required fasting for 4–6 h prior to injection and the 10-min craniocaudal and mediolateral oblique view imaging time [82]. Another disadvantage is the higher radiation dose to organs other than the breast compared with mammography. The highest organ doses associated with PEM are to the bladder, uterus, and ovaries. The breast dose is approximately 2.5 mGy [8]. A meta-analysis of 8 studies of 873 women with suspected breast malignancy reported that the pooled sensitivity and specificity values of PEM were 85% and 79%, respectively [83]. In comparing PEM and MRI, Berg et al. found MRI to be more sensitive (14/15 vs. 3/15) for detecting contralateral cancer in women who were newly diagnosed with breast cancer. Although in a study of 388 women, Berg et al. observed similar sensitivity of the two modalities in disease detection, MRI was more sensitive in identifying women who needed additional surgery to excise all disease in the ipsilateral breast. PEM was more specific than MRI and less likely to prompt unnecessary biopsies. PEM is an alternative for women who are unable to tolerate MRI to evaluate the extent of disease [84, 85].

Image-Guided Biopsy and Localization of Non-palpable Breast Lesions

Stereotactic-Guided Biopsy

Stereotactic guidance is used for percutaneous biopsy of mammographically identified lesions, including microcalcifications, masses, asymmetries, and architectural distortions that cannot be located using US. The patient is positioned either prone or seated, and the breast is compressed between the image receptor and the compression plate. The lesion is targeted by the radiologist with either two 15-degree angled X-ray images or tomosynthesis imaging to produce computer-generated coordinates that are transferred to the stereotactic biopsy device. After local anesthetic, the biopsy needle is advanced into the breast, and additional images are obtained to confirm

appropriate positioning. Tissue samples are then obtained, typically utilizing an 8- to 11-gauge vacuum-assisted biopsy device (Fig. 5.9).

When the biopsy is performed for calcifications, magnification X-rays of the tissue specimen are obtained to verify that the calcifications of interest were sampled. Following biopsy, a tissue marker ("biopsy clip") is placed at the biopsy site. The tissue marker indicates the site that was biopsied and facilitates localization if surgical excision needs to be performed. Radiological pathological correlation should be performed to ensure that adequate sampling was performed.

Ultrasound-Guided Needle Aspiration and Fine-Needle Aspiration Biopsy

Aspiration of cysts or fluid collections may be performed under US guidance for greatest accuracy because the needle can be visualized within the fluid; the fluid will diminish as it is aspirated. Simple cysts do not require treatment; however, some patients desire aspiration for symptomatic relief. If the cyst contains a solid nodule, the nodule should undergo core biopsy prior to aspiration of the fluid. Complicated cysts contain homogenous echogenicity or mobile internal echoes on US. If the radiologist is not sure if a lesion is a complicated cyst or a solid mass, aspiration may be performed initially (Fig. 5.26). If the fluid is cloudy yellow, green, gray, or clear yellow, it is usually discarded. If the fluid is bloody, it should be sent for cytology, and if infection is suspected, the material is sent for culture and sensitivity. If no fluid can be aspirated, it can be assumed that the mass is solid, and a core biopsy may be performed.

Due to the increased rates of insufficient sampling and higher false-negative rates with fine-needle aspiration biopsy, core biopsy is preferred for breast masses [86, 87]. Fine-needle aspiration may still be performed in cases in which the lesion may be difficult to safely biopsy due to an adjacent vessel or breast implant. Typically, a 21- or 25-gauge needle is rapidly and manually oscillated throughout the lesion under US guidance, while suction is applied with a syringe connected to the needle with flexible tubing until there is blood or material within the needle hub. The material is immediately applied to slides, smeared, and sent for cytology.

Ultrasound-Guided Core Biopsy

US is the most common method to guide biopsy of an indeterminate or suspicious lesion and allows visualization of the biopsy needle within the lesion in real time. US-guided core biopsy is usually performed with an 11- to 18-gauge biopsy device. Although there are a variety of devices, the most widely used are spring loaded, in which the needle is rapidly "fired" through the lesion (Fig. 5.15). Vacuum-assisted core biopsy devices are also available and use vacuum suction to pull the tissue into the needle trough prior to cutting. A review of 7 series including 884 cancers revealed that 840 (95%) of the cancers were diagnosed at initial 14-gauge core biopsy [88]. In a review of 2420 cases with long-term follow-up by Youk et al., US-guided 14-gauge core needle biopsy had a 96% sensitivity rate and a false-negative rate of 2.4% for malignancy (1.9% with high-risk lesions on core were upgraded to malignancy). The malignant upgrade rate for high-risk lesions was 27%, and the upgrade rate for DCIS at core to invasive disease was 29% [89]. Inadequate sampling is particularly a concern with smaller lesions. The needle may appear to be within the lesion when it is actually adjacent to it. Therefore, turning the US probe perpendicular to the lesion after a pass to visualize the needle within the lesion and acquiring more passes can be helpful in ensuring

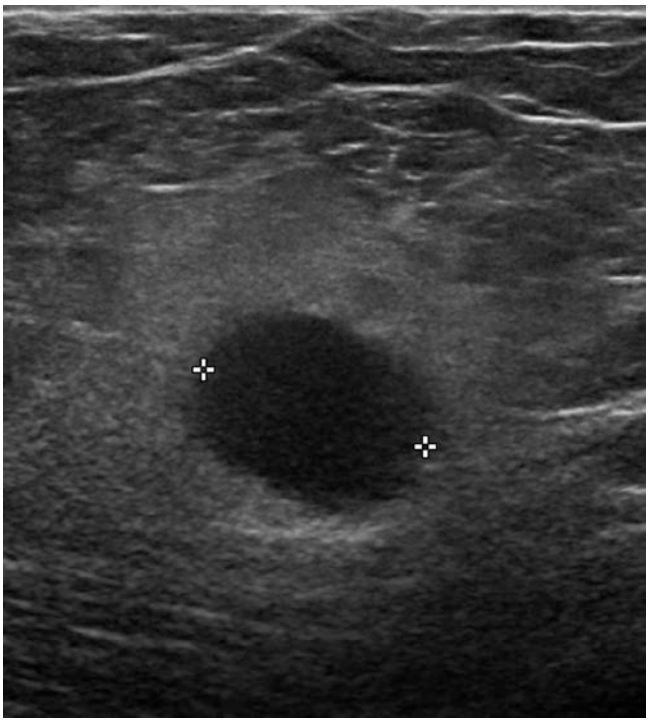

Fig. 5.26 A 61-year-old female with a palpable area in the upper outer right breast. Ultrasound demonstrates an oval hypoechoic mass in the upper outer right breast with internal echoes. Aspiration was performed, yielding turbid white fluid consistent with proteinaceous debris, which was discarded. The lesion resolved upon aspiration, indicating that it was a complicated cyst

adequate sampling. If the pathology results are not concordant with the imaging finding, surgical excision or additional tissue sampling is recommended.

A biopsy marker clip should be placed into the lesion following core biopsy to facilitate removal of the lesion in cases of atypia or malignancy. A post-procedure mammogram is obtained to ensure that the clip is in the expected location.

US-guided biopsies are typically better tolerated by patients than stereotactic or MRI-guided procedures because the patient is supine and the breast is not in compression during a US-guided procedure. Therefore, a "second-look" US will often be performed to identify a sonographic correlate to a concerning MRI finding to facilitate a US-guided biopsy. Common post-procedure symptoms include bruising and pain, while hematomas are uncommon. Color Doppler should be used to ensure that blood vessels are avoided.

MRI-Guided Biopsy

MRI-guided biopsies are performed when a lesion can only be identified on the MR examination. The computer system used for evaluation of enhancement patterns typically also has an adjunct system to aid in performing percutaneous biopsy. However, MRI biopsy is challenging because the lesion identified for sampling may be more difficult to identify when the patient's breast is compressed by the grid required for immobilization and localization. The patient must remain in this position after the initial post-contrast images, during imaging to visualize the position of the introducer relative to the lesion, and during subsequent sampling with a vacuum-assisted biopsy device, clip placement, and post-biopsy imaging. After the procedure is completed, the patient is transferred from the MRI suite to the mammography suite to confirm clip placement.

Preoperative Lesion Localization

Wire localization or radioactive seed localization is performed preoperatively under mammographic or US guidance to direct the surgeon to the tissue that needs to be excised and is required to ensure that non-palpable lesions are removed.

When the procedure is performed using mammographic guidance, the breast is placed into compression, and a grid is projected over the skin and used to target the area to be removed. After obtaining local anesthesia, a hollow needle containing a fine wire is placed into the breast. An orthogonal mammogram is then performed to evaluate the needle depth. Once the needle is in the appropriate position, the needle is removed, and the wire is left in place. The wire has a hooked or curved end that is designed to prevent dislodgement from the breast (Fig. 5.27). When the procedure is performed using US guidance, the needle containing the wire is placed through the lesion, and the needle is removed, leaving the wire in place.

A radioactive seed may also be placed through the hollow needle instead of a wire to localize the lesion to be excised. The seed contains a small amount of I-125 within a titanium shell. The surgeon uses a gamma probe to detect the seed and guide the excision in addition to the mammogram images depicting the location of the seed. The benefits of using radioactive seeds instead of wires include the markedly reduced chance of dislodgement, lack of interference with the surgical approach, and the ability to perform the localization up to 5 days prior to surgery, facilitating scheduling. Other localization methods under investigation include magnetic seed localization [90] and localization with reflector seeds detected with infrared electromagnetic wave technology [91].

If the lesion to be excised is large (typically a large area of malignant calcifications), the area will be localized by placing two or more wires or seeds at opposite margins of the lesion, thus "bracketing" the boundaries of the lesion.

In all cases, craniocaudal and mediolateral mammogram views are obtained and annotated by the radiologist to delineate the lesion to be removed. A specimen radiograph should be obtained at the time of surgery to confirm that the localized lesion has been removed, along with biopsy clips, wires, or radioactive seeds. The radiologist should call the operating room to inform the surgeon that the lesion or biopsy marker and wire or seed is present in the specimen and to notify the surgeon if the excised lesion is close to the margin of the specimen so that additional tissue can be obtained immediately if deemed necessary by the surgeon. It may be difficult to determine which margin is close; surgically placed markers at the margins, orthogonal specimen views and careful viewing of the lesion and its relationship to the localizing markers will aid this assessment.

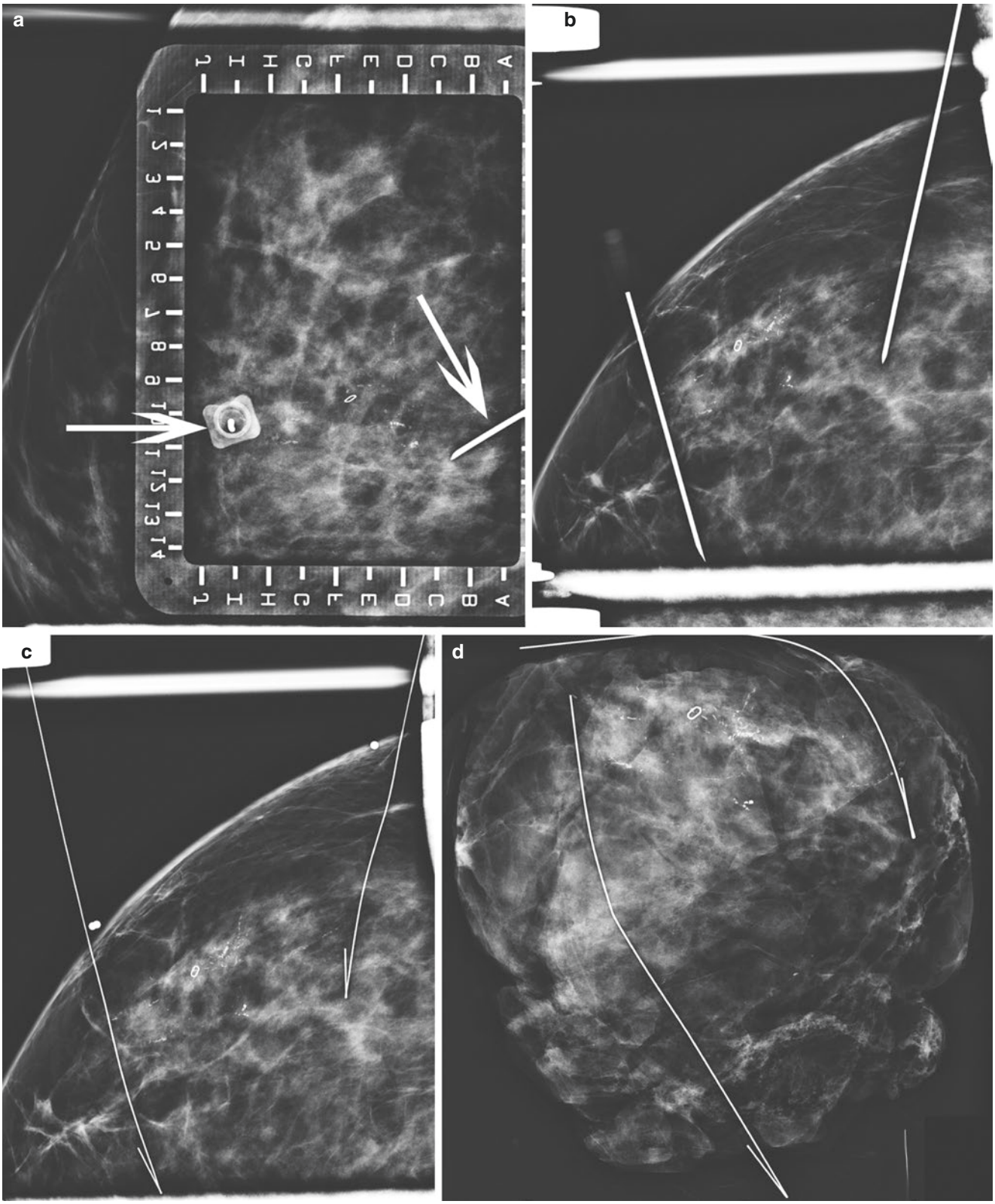

Fig. 5.27 (**a**) A compression plate with a grid is placed on the breast overlying the calcifications to be localized for surgical excision. Needles (*arrows*) with coaxial wires are placed into position from a lateral approach. (**b**) An orthogonal view shows the position of the needles, which can be adjusted. (**c**) The wires are deployed, bracketing the anterior and posterior extent of the calcifications. (**d**) A specimen radiograph shows the wires, the excised calcifications, and the stereotactic biopsy clip. Stereotactic biopsy and surgical excision led to a diagnosis of high-grade DCIS

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Nuclear Medicine in the Diagnosis and Treatment of Breast Cancer

6

Cuneyt Turkmen and Zeynep Gozde Ozkan

Introduction

In nuclear medicine, radioactive substances (radiopharmaceuticals) are used for the diagnosis and treatment of diseases. A radiopharmaceutical has two parts: a chemical part for targeting and a radioactive part for either imaging or therapy. Nuclear medicine imaging systems that convert gamma rays emitted from the patient as a result of a previously administered radiopharmaceutical to diagnostic images are mainly designed for whole-body imaging. In radionuclide therapies, radiopharmaceuticals that have either beta or alpha ray-emitting radioactive parts are given to patients. The chemical parts of the radiopharmaceuticals enable localization to and internal radiotherapy in diseased tissues.

Nuclear medicine imaging systems capable of single photon emission computed tomography (SPECT) and positron emission tomography (PET) as a special function of the device are able to measure the in vivo cellular, molecular, and biochemical properties of neoplasms and normal tissues. Hybrid imaging systems, such as PET/CT, PET/MR, and SPECT/CT devices, combine the functional information provided by the use of a radiopharmaceutical with anatomical information provided either by the computerized tomography (CT) or magnetic resonance imaging (MRI) unit of the same machine in a single acquisition.

SPECT imaging devices mostly use radiopharmaceuticals with technetium-99 m (Tc-99m), among other radionuclides, which decays with a single gamma ray at a time. The energy of the gamma ray differs for different radionuclides, such as Tc-99m, iodine-123 (I-123), iodine-131 (I-131), indium-111 (In-111), and gallium-67 (Ga-67). In contrast to SPECT agents, PET agents use pharmaceuticals labeled with positron-emitting radionuclides, such as fluorine-18 (F-18), carbon-11 (C-11), nitrogen-13 (N-13), oxygen-15 (O-15), and gallium-68 (Ga-68), which emit two gamma photons per each decay with an energy of 511 keV.

In PET/CT devices, radiopharmaceuticals are most commonly used to target cancer cells. In current oncology practice, imaging with PET/CT is an essential component of staging and monitoring treatment for numerous types of cancer. In recent years, there has been technological advancement of PET equipment through the development of new detectors and equipment designed specifically for breast imaging, such as positron emission mammography (PEM) devices. In addition, the development of more specific PET radiopharmaceuticals that target different biological processes of breast cancer will enable personalized therapy for patients with breast cancer. Although molecular imaging with PET is a rapidly emerging approach in breast cancer, conventional single photon nuclear medicine imaging, including bone scintigraphy and sentinel lymph node scintigraphy, still has an important role in the management of breast cancer. For several decades, systemic radionuclide treatment of painful bone metastases has been performed in breast cancer patients. New radiopharmaceuticals not only palliate pain but also prolong survival in patients with bone and liver metastases.

In this chapter, we will review diagnostic and therapeutic applications of nuclear medicine for breast cancer, starting from conventional single photon nuclear medicine techniques and then moving to PET applications and radionuclide treatment options for breast cancer patients.

Scintimammography

Scintimammography is a functional imaging method that enables differentiation of malignant from benign processes when mainstay anatomic modalities, such as mammography, ultrasound, and MRI, are limited [1]. In recent years, SPECT and hybrid SPECT/CT imaging have enhanced conventional planar scintimammography along with dedicated small field-of-view (FOV) breast-specific gamma imaging (BSGI)

C. Turkmen (✉) · Z. G. Ozkan
Department of Nuclear Medicine, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

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devices. Tc-99 m methoxyisobutylisonitrile (MIBI) is the radiopharmaceutical of choice for SPECT studies in breast imaging [2]. Tc-99m MIBI is localized in mitochondria, which are abundant in malignant cells. The uptake of Tc-99m MIBI depends on regional blood flow, tumor angiogenesis, and increased metabolism and is driven by plasma and mitochondrial membrane potentials [3, 4]. Studies have shown that the early uptake of Tc-99m MIBI reflects mitochondrial status, which is affected by both apoptosis and proliferation, but the clearance of the tracer reflects the activity of drug transporters, such as P-glycoprotein [5, 6]. Both proliferative activity and the apoptotic index have been shown to be directly correlated with Tc-99m MIBI uptake [7, 8].

A recent meta-analysis that evaluated the diagnostic value of BSGI and MRI in the same patient cohort with breast cancer showed that BSGI had similar sensitivity as MRI (84% vs 89%) but higher specificity (82% vs 39%) and diagnostic efficacy (AUC 0.93 vs 0.72), indicating excellent diagnostic performance [9]. The high specificity of scintimammography allows a positive scintigraphic finding to be supported by an invasive evaluation. Tumor types, such as poorly differentiated DCIS and lobular and tubulolobular carcinomas, and tumors with a size <1 cm and diminished cellularity, blood supply, and cell viability can cause a false-negative result on scintimammography [10, 11]. Benign hyperplasia lesions, such as fibrocystic changes and fibroadenomas, can also cause false-positive results in scintimammography.

The inability to detect axillary lymph nodes and delineate adjacent lesions are other limitations of scintimammography. SPECT/CT hybrid imaging, which combines functional and morphological information, enables an increase in the noninvasive diagnosis of axillary lymph node invasion by breast cancer. In a study of 60 patients, the addition of SPECT/CT evaluation increased sensitivity by 1.4 times (from 55% to 75%) compared with that of CT, with excellent specificity (97% and 89%) and comparable overall accuracy (82% and 84%) [12]. An effective radiation dose was estimated to be 5.9–9.4 mSv compared to 0.44 mSv for digital mammography [13].

Tc-99m MIBI scintimammography can also be used to monitor the treatment response to neoadjuvant chemotherapy. In a recent meta-analysis that include 14 studies, pooled sensitivity was 86% (95% CI, 0.78–0.92), and pooled specificity was 69% (95% CI, 0.64–0.74) for Tc-99m MIBI scintimammography in the prediction of neoadjuvant chemotherapy response in breast cancer [14]. This analysis suggested that negative scintimammography could not fully exclude the presence of a residual tumor, especially remaining ductal carcinoma in situ or a residual tumor of less than 1 cm in size. Subgroup analysis also showed that performing early mid-treatment Tc-99m MIBI scintimammography (using the reduction rate of one or two cycles or within the first half-course of chemotherapy compared with the baseline) was superior to later treatment (after three courses or more) or posttreatment scintimammography in the prediction of neoadjuvant chemotherapy response. In a study by Lee et al., although the direct comparison between MRI and scintimammography was statistically insignificant, MRI added value to scintimammography in the detection of residual tumor after neoadjuvant chemotherapy, and scintimammography also helped to locate tumors after therapy that were false negative on MRI. Thus, the authors concluded that a combination of scintimammography and MRI would be more accurate in the prediction of treatment response [15].

Sentinel Lymph Node Scintigraphy

Axillary lymph node status is a major prognostic factor in early-stage breast cancer. Sentinel lymph node (SLN) biopsy is the standard surgical procedure for staging clinically tumor-free regional nodes in patients with early-stage breast cancer. In this patient group, axillary lymph node dissection is no longer recommended, as it only adds to limb morbidity without providing any prognostic or staging benefit [16].

Tumors drain in an orderly manner through the lymphatic system. The SLN is the first to be affected by metastasis if the tumor has spread. A tumor-free SLN makes it highly unlikely for other nodes to be affected. SLN scintigraphy (lymphoscintigraphy) using radiolabeled colloids can accurately localize the sentinel nodes and can show atypical drainage patterns preoperatively (Fig. 6.1). Although lymphoscintigraphy and SLN biopsy (SLNB) have been used to stage many solid cancers, these procedures are most commonly performed in patients with breast cancer and melanoma. In the SLNB procedure, lymphoscintigraphy can improve accuracy, especially in extra-axillary lymph nodes, and can also reduce surgical morbidity [17]. The SPECT/CT procedure may improve the localization of SLNs during the acquisition of lymphoscintigraphy images. Intraoperative detection of SLNs is managed by a gamma probe. Recently, several portable gamma cameras have been developed to provide real-time image guidance for the detection of SLNs during the operation. The most recent developments include the combination of conventional gamma probes with position- and orientation-tracking systems, which permits virtual reconstruction in a three-dimensional environment.

Currently, the radioactive SLNB technique is combined with a dye technique to improve the detection rate. Recently, near-infrared fluorescence imaging using indocyanine green (ICG) has been applied to SLN procedures, and experience is growing in breast cancer [18–20]. Investigations have shown comparable results for radioactive and fluorescence techniques and that ICG fluorescence imaging can be a helpful tool for institutions without radioactive equipment. ICG

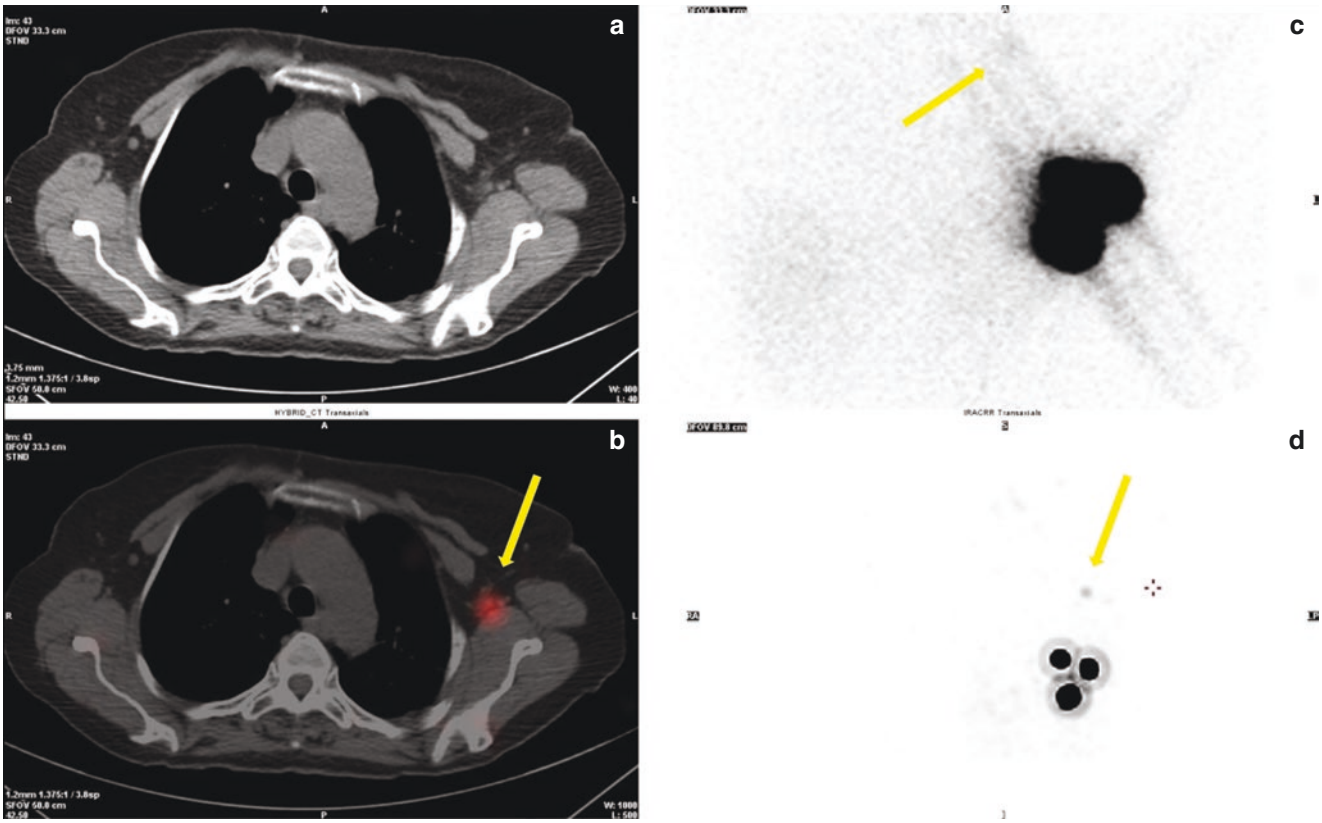

Fig. 6.1 A 52-year-old woman with a newly diagnosed left breast cancer was scanned for preoperative sentinel lymph node evaluation with Tc-99m nanocolloid lymphoscintigraphy. The Tc-99m nanocolloid was injected intramammary in the region of the tumor and periareolar subcutaneously. Dynamic, planar, and SPECT/CT images were recorded after the injections. Planar (**c**) and SPECT/CT images (**a**, CT image; **b**, fusion image; **d**, SPECT image) showed increased radiotracer uptake in the left axillary lymph node suggestive of the sentinel lymph node. The patient underwent a left mastectomy and left axillary sentinel lymph node biopsy. The surgical pathology report of the left axilla was negative for lymph node metastasis

fluorescence guidance has also been investigated for the excision of nonpalpable breast cancer lesions, and the first results are encouraging [21]. Clinical trials that are underway for ICG fluorescence guidance both for SLN procedures and for nonpalpable lesions in breast cancer will give more solid results (NCT02875626 and NCT01796041).

Identification of the SLN is crucial to the success of SLNB, and with a detection rate between 94% and 100%, preoperative SLN imaging is ideally suited for this purpose [22–25]. Recent multi-institutional studies have revealed SLNB false-negative rates ranging from 5.5% to 16.7%, higher than the target set by the 2005 ASCO guidelines (<5%) [26, 27]. Unfortunately, SLNB remains an unstandardized procedure with many unresolved controversies concerning the technique itself. The radiopharmaceuticals that are routinely used for SLNB are Tc-99m sulfur colloid (particle size, 15–5000 nm), Tc-99m nanocolloid (5–100 nm), and Tc-99m antimony trisulfide (3–30 nm). The radiocolloid measuring 100–200 nm is considered the best compromise between fast lymphatic drainage and optimal retention in SLNs [28]. The use of small volumes (0.3–0.4 ml) with high specific activity improves SLN detection. The standard procedure for SLN detection is based on the use of radiocolloid alone or in combination with blue dye, especially when the SLN is suspected to be diffusely metastatic [29]. Currently, no clinical consensus exists on the optimal site of injection of the radiocolloid or blue dye. Superficial (periareolar, subareolar, intradermal, subdermal) and deep (peritumoral, intratumoral) injections within the breast have been reported widely for radiocolloid administration [26, 30]. A recent meta-analysis comparing superficial and deep injections of radiocolloid demonstrated no significant difference in the SLN detection rate on lymphoscintigraphy or during intraoperative SLNB [31]. The rate of extra-axillary SLN identification was significantly greater when deep rather than superficial injection was used (OR: 3.00; 1.92–4.67).

Primary contraindications for SLNB include grossly palpable lymph nodes and inflammatory breast cancer. Healthy lymphatic tissue is necessary for the localization and retention of radiocolloids in lymph nodes. A metastatic lymph node that is enlarged with no healthy lymphatic tissue can lead to a false-negative SLNB procedure. Investigations of inflammatory breast cancer have also reported an SLN identification rate of only 80–85% with a relatively high

false-negative rate (6.18%) [32]. Since the updated ASCO guidelines were published in 2017, no new data have become available to support the benefit of SLNB in women with large or locally advanced invasive breast cancers (T3/T4) and inflammatory breast cancer [33]. SLNB is also not recommended for women who have DCIS and for whom breast-conserving surgery is planned. SLNB is instead recommended for smaller tumors (T1 and T2), multiple tumors, and DCIS when mastectomy is planned, for older or obese patients, in male patients with breast cancer, and in patients with prior breast or axillary surgery. SLNB may be offered before or after neoadjuvant systemic therapy, but the procedure appears to be less accurate after neoadjuvant systemic therapy.

Today, the prognostic relevance of isolated tumor cells and micrometastases is negligible. Two multi-institutional randomized studies demonstrated an SLNB detection rate of 98% in cN0 stage I/II breast cancer patients [34, 35]. Thus, SLNB could prevent axillary lymph node dissection for SLN-negative women. In the ACOSOG Z0010 trial, occult metastases were detected in 9% of cases, but no difference was observed in disease-free survival and overall survival [36]. The 10-year follow-up data of the NSABP B-32 trial, which reported a prevalence of occult metastases of 15.9% of patients, revealed small differences in disease-free survival and overall survival that were statistically but not clinically significant. Therefore, complete axillary lymph node dissection in cases of SLN micrometastases is no longer recommended [37].

Bone Scintigraphy

The skeleton is the most common site for metastases from breast cancer. In approximately 50–70% of recurrent patients, skeleton metastases are detected, and it is the only metastatic site of disease in 28–44% of patients [38]. It is important to detect bone metastases at an early stage to minimize skeleton-related events. In patients who are receiving treatments, it is also important to determine the response to therapy as early as possible to limit toxicity and accelerate the therapeutic transition in nonresponding patients. Imaging has always played a key role in the diagnosis of bone metastases in breast cancer, and planar Tc-99m diphosphonate bone scanning remains widely used. The sensitivity of bone scintigraphy is high, and its lack of specificity has been improved with the addition of SPECT and SPECT/CT imaging to the acquisitions (Fig. 6.2). Despite improved accuracy in staging of the skeleton, effective monitoring of the treatment response is lacking. Although radiographs have been used historically to determine a response by lesion resolution or

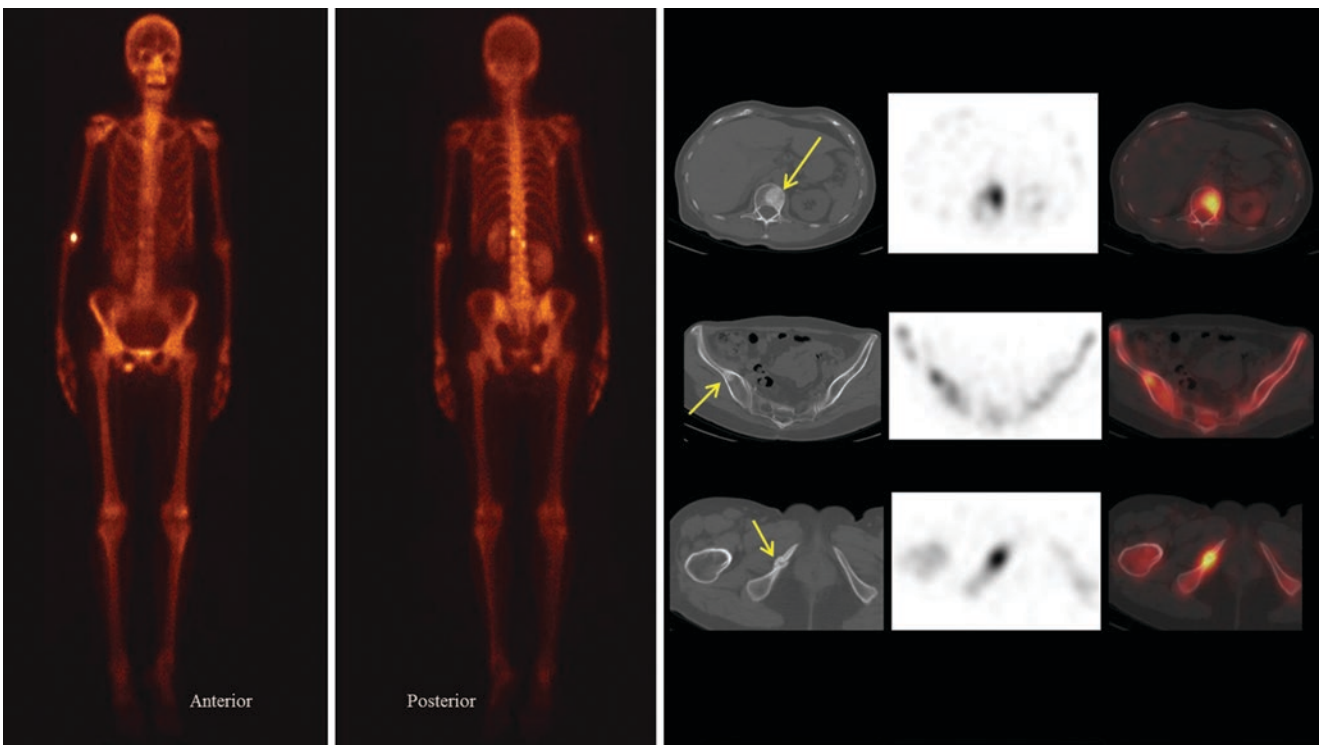

Fig. 6.2 A 67-year-old female patient with breast cancer had a mastectomy and received chemoradiotherapy. Due to new onset of back pain, she underwent bone scintigraphy. On whole-body images, pathologic Tc-99m MDP uptake in the vertebrae and pelvis was seen. On SPECT/CT images of the lumbar and pelvic regions, sclerotic metastatic lesions, indicated by arrows, on lumbar 1 and 2 vertebral bodies (upper row) and right iliac bone (middle row) were observed with pathologic Tc-99m MDP uptake. In addition, a pathologic fracture on the right ischium with increased Tc-99m MDP uptake (lower row) was detected

sclerosis, this method has been recognized as insensitive and may take at least 6 months to yield a confident assessment of response. Abnormal accumulation of Tc-99m diphosphonates is related to changes in local blood flow and osteoblastic activity. The mechanism of accumulation indicates that the uptake of Tc-99m diphosphonate is not specific for metastatic disease. Increased reparative osteoblastic activity resembles unresponsive progressive disease. The problem of the flare phenomenon (a temporary osteoblastic response to successful therapy), which makes the differentiation of progression from healing difficult for 3–6 months, has been described after chemotherapy and endocrine therapy in breast cancer [39]. Limitations of bone scintigraphy are reported when evaluating treatment response, with only 52% of responders showing scintigraphic improvement and 62% of nonresponders showing scintigraphic deterioration at 6–8 months in breast cancer [40].

Positron Emission Tomography/Computed Tomography

PET/CT with F-18 fluoro-2-deoxy-D-glucose (FDG) has been established as an effective modality for different stages of evaluation of various types of cancer: making the diagnosis, determining the stage, evaluating the response to therapy, and follow-up.

Currently, FDG PET/CT is not used in breast cancer screening or diagnosing primary breast cancer mainly due to the high prevalence of false-negative results, particularly for tumors with a diameter smaller than 1 cm and tumors with low metabolic activity. The sensitivity of FDG PET/CT in primary breast cancer detection has been reported to be worse than that of ultrasonography, MRI, or mammography [41]. The metabolic activity of breast tumors is variable. For example, invasive lobular breast cancer has a considerably lower FDG uptake than invasive ductal cancer. Relatively high physiological glucose uptake in the surrounding mammary tissue is also another difficulty for the detection of tumors with low metabolic activity. The highest FDG uptake is observed for high-grade tumors, triple-negative tumors (ER-, PR-, HER2-), and inflammatory breast cancer [42, 43].

In early-stage breast cancer with clinically negative axilla, FDG PET/CT is not recommended due to its limited role in initial staging and treatment planning in most patients. In regional staging of these patients, FDG PET/CT is less sensitive than SLNB in assessing axillary lymph node involvement. In addition, the low prevalence of distant metastases in these patients and the probability of false-positive findings prevent the use of FDG PET/CT for distant staging [44]. By contrast, in patients with clinically positive axilla, especially in those with locally advanced breast cancer, FDG PET/CT can be useful prior to surgery or neoadjuvant chemotherapy, based on the high rate of detection of distant metastases, which ranges from 6% to 26% [45]. Extra-axillary lymph node involvement is detected by FDG PET/CT in 10–29% of patients with locally advanced breast cancer [46, 47]. FDG PET/CT changes the initial treatment in 1–8% of patients with early-stage breast cancer, in 7–13% of those with locally advanced breast cancer, and in up to 52% of those with more aggressive tumors, such as inflammatory breast cancer [48–50].

The level of FDG uptake by a primary tumor also has a prognostic value in many types of cancer. The prognostic impact of the glycolytic activity (SUVmax) of the primary breast tumor is controversial. Whereas some authors have found no correlation between FDG uptake by the tumor and the prognosis, others have reported that patients with high tumor uptake had worse outcomes [51–54]. Furthermore, the cutoff values for the SUVmax value ranged from 3 to 6. The evidence for the prognostic value of SUVmax in axillary lymph nodes is also limited, although higher values have been associated with higher recurrence rates [55, 56].

Changes in tumor metabolic activity have been shown to be an early indicator of effective treatment of breast cancer, mainly in the neoadjuvant setting. A decrease in tumor metabolic activity enables both assessment of the treatment response after the completion of therapy and early prediction of therapeutic effectiveness after the first or second cycle of chemotherapy. Identifying nonresponding patients on the basis of changes in tumor metabolic activity early during treatment can facilitate a change from an ineffective to a more effective treatment approach. In a study of 64 stage II and III breast cancer patients, Rousseau et al. observed a marked decrease in FDG uptake at multiple cycles during neoadjuvant chemotherapy in nearly all patients who had a therapeutic effect of more than 50% [57]. They determined that FDG PET after the second cycle of treatment potentially provided a more accurate prediction of treatment response. Using a 40% decrease of SUV as a cutoff value, Rousseau et al. found a negative predictive value of 68% for identifying nonresponders to therapy after the first cycle; this value increased to 85% after the second cycle. Schwarz-Dose et al. confirmed, in 104 patients, that the greater the reduction in tumor metabolic activity early during neoadjuvant treatment, the more likely that the patients would achieve a pathologic response [58]. In their study, they found that after the first cycle of chemotherapy, tumor metabolic activity decreased by 50% ± 18% in pathologic responders; by comparison, the decrease in pathologic nonresponders was 36% ± 20%. Of note, all breast carcinomas (23%) with a baseline SUV of less than 3.0 did not respond to chemotherapy. A recent meta-analysis of 19 studies with more than 900 patients found that the best cutoff value for decrease in FDG uptake for predicting response to therapy was 55–65% [59]. Although the sensitivity and specificity for identifying patients responding to treatment were limited (84% and 66%, respectively), the negative predictive value for identifying nonresponders was high (91%).

Changes in the sizes of bone metastases are particularly difficult to evaluate with conventional imaging as sclerotic lesions do not disappear and lytic lesions can show sclerotic changes as an indication of a treatment response. Two studies demonstrated a high sensitivity of FDG PET/CT for the detection of osseous metastases in patients with newly diagnosed metastatic breast cancer, and the metabolic activity of osseous breast cancer metastases provided prognostic information [60, 61]. In a retrospective analysis, bone metastases in 102 patients were assessed with FDG PET/CT before and after treatment, and a decrease in FDG uptake was a significant predictor of the response duration in univariate and multivariate analyses [62].

The early detection and accurate restaging of recurrent breast cancer are of significant importance for selecting the best therapeutic option for better prognosis and lower mortality. For breast cancer with suspicious recurrence, however, there is no standard follow-up protocol to date, and further examination of radiologic imaging, such as CT, bone scintigraphy, MRI, and PET/CT, may be needed. FDG PET/CT is a valuable technique that can show functional information for early detection of whole-body multifocal malignant lesions, thus enabling a correct diagnosis of recurrence that might be missed by conventional imaging modalities. Because it allows better discrimination between posttreatment scarring or fibrosis and viable tumor tissue, FDG PET/CT is efficient for detecting locoregional recurrence, especially in the chest wall, axilla, and extra-axillary lymph node basins, with better performance than CT or MRI (Fig. 6.3). A meta-analysis systematically summarized the overall diagnostic value of FDG PET/CT for the diagnosis of recurrence in breast cancer patients. The pooled sensitivity was 0.90 (95% CI, 0.88–0.92), indicating a high capacity for FDG PET/CT analysis in the early detection of recurrent breast cancer [63]. In addition, the pooled specificity was 0.81 (95% CI, 0.78–0.84), which showed a relatively higher ability to exclude recurrence compared with that of the other imaging modalities, such as CT or MRI. In other words, a negative test of FDG PET/CT can indicate the absence of recurrent breast cancer, with 81% probability.

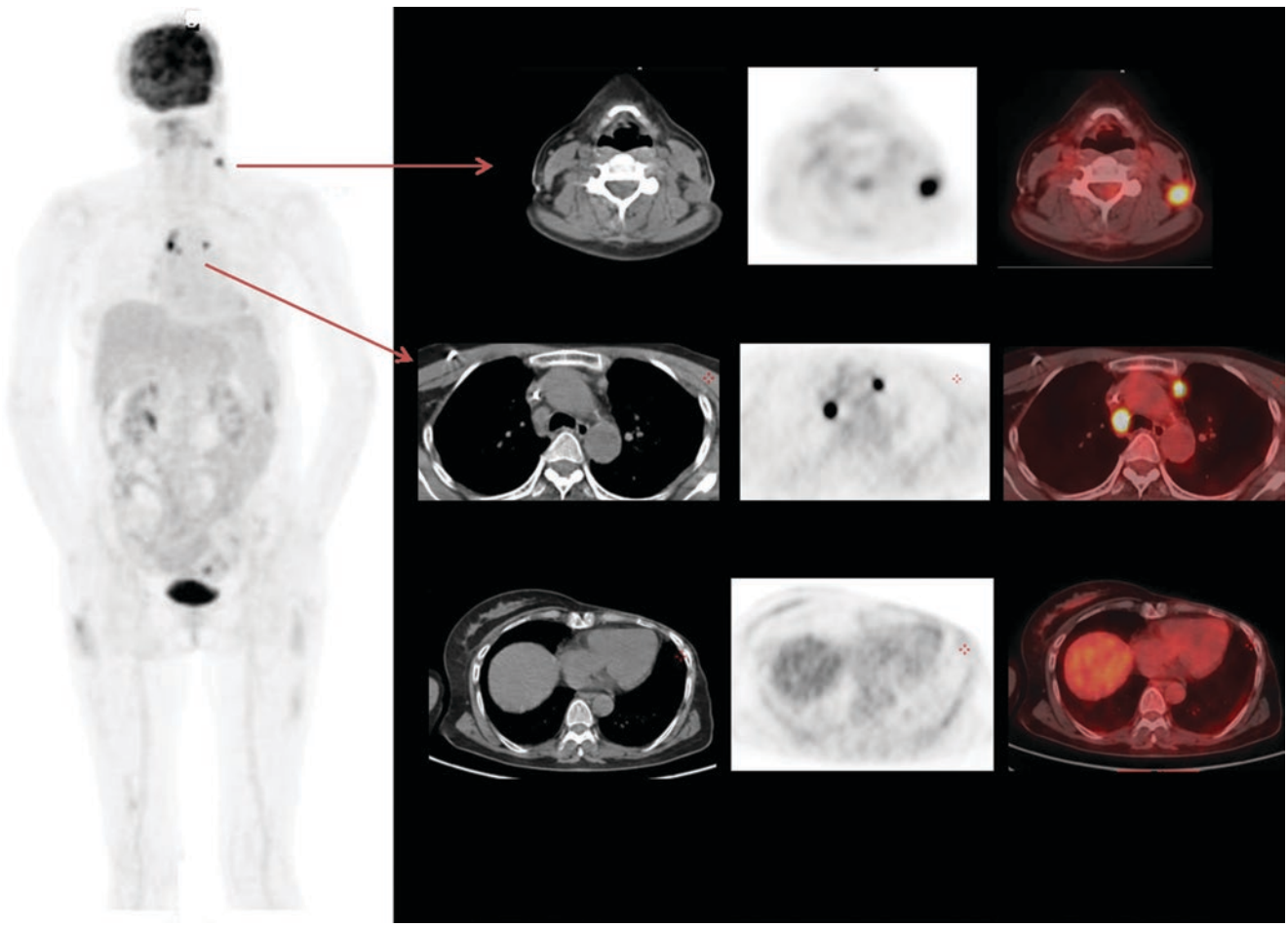

Fig. 6.3 A 58-year-old female patient underwent mastectomy due to breast cancer. During follow-up, her tumor marker levels started to increase. On her control mammography and breast USG, there was no sign of local recurrence, but on her FDG PET/CT images, there were metastatic lymph nodes with increased FDG uptake in the left posterior cervical region (upper row) and in the mediastinum at the right lower paratracheal and para-aortic regions (middle row). In the mastectomy region (lower row), there was no pathologic FDG uptake

F-18 sodium fluoride (NaF) is a positron emitter that is used for bone imaging in PET/CT machines. Its mechanism of uptake is quite similar to that of Tc-99m diphosphonate, which is the SPECT radiopharmaceutical for bone scintigraphy. Studies comparing the utility of NaF PET/CT with Tc-99m diphosphonate whole-body bone scintigraphy have shown that NaF PET/CT generally has higher sensitivity and specificity than bone scanning. The higher uptake of NaF than Tc-99m diphosphonate in the skeleton and the faster blood clearance yield a better target/background ratio in a shorter time period. Factors that contribute to the success of NaF PET/CT include NaF uptake in both lytic and blastic metastases, sectional imaging along with the advantage of whole-body scanning, easy detection of small lesions with improved resolution of PET technology, and better visualization of bone marrow lesions [64]. Recently, the frequent use of SPECT/CT utility along with planar whole-body scintigraphy has augmented the specificity of Tc-99m diphosphonate bone scintigraphy and reduced the demand for NaF PET/CT.

In addition to FDG and NaF, other PET radiopharmaceuticals have been used in breast cancer in both preclinical and clinical settings. Radiolabeled hypoxia-avid compounds, such as F-18-labeled fluoromisonidazole (FMISO), can be used to evaluate oxygenation status in experimental or human tumors. This PET radiotracer has affinity for hypoxic cells with functional nitroreductase enzymes; therefore, it accumulates in hypoxic cells but not in necrotic cells. F-18-labeled fluorothymidine (FLT) has been proposed as an early molecular imaging biomarker to evaluate treatment response with taxanes [65]. Uptake of FLT is correlated with the Ki-67 labeling index, another proliferation parameter, in breast cancer. Some studies have reported a strong correlation of FLT uptake with cell proliferation in untreated patients with breast cancer, enabling detection of response as early as 1 week after chemotherapy. Pio et al. compared FDG and FLT imaging in 14 patients with newly diagnosed primary or metastatic breast cancer to monitor and predict tumor response to chemotherapy [66]. The group concluded that FLT may be more accurate than FDG 2 weeks after the end of the first course of chemotherapy for predicting longer-term efficacy of chemotherapy for women with breast cancer. F-18-labeled fluoroestradiol (FES) is a novel radiopharmaceutical that noninvasively measures ER expression in tumors and has emerged as a valuable method to predict response to hormone therapy in recurrent or metastatic breast cancer patients [67, 68]. Level of FES uptake predicted the likelihood of response to tamoxifen and aromatase inhibitor treatment, and some studies support its use in treatment response assessment in some groups with recurrent or metastatic breast cancer [69].

Positron Emission Mammography

To overcome the limited resolution of PET equipment as well as space limitations of current CT acquisition protocols, which cause false-negative evaluations by FDG PET/CT protocols in small breast tumors, a new imaging modality, PEM, has emerged. PEM, which is a high-resolution tomographic molecular imaging device, has a pair of dedicated gamma radiation detectors that are placed above and below the breast. Mild breast compression, similar to conventional mammography, is necessary both to attain higher spatial resolution (1–2 mm for PEM vs 4–6 mm for PET) and to reduce the radiation dose by reducing breast thickness [70, 71]. The crystal detectors, which are constructed to provide improved spatial resolution and count rate efficiency, collect gamma rays emitted from the breast tissue due to previous injection of FDG. The result is a set of 12 slices each in the craniocaudal and mediolateral oblique positions, similar to conventional mammography.

The advantage of PEM is its ability to detect small hypermetabolic lesions. PEM can detect lesions <2 cm due to its higher spatial resolution (up to 2.4 mm) compared to that of whole-body PET [70]. Even small tumors <1 cm can be detected by PEM with a sensitivity of 60–70% [72]. Studies that compared PEM with MRI and whole-body PET/CT showed similar high sensitivities for PEM (93% for known index lesions, 85% for unsuspected additional lesions) and MR but low sensitivity for whole-body PET/CT (67.9%) [73, 74]. As both MRI and PEM have similar sensitivities, the indications for both of the exams are quite similar: in preoperative surgical planning or pre-chemotherapy evaluation to detect and characterize primary breast lesions [70]. PEM can be an alternative for patients who cannot tolerate MRI or have a contraindication to MRI, but in this context, the radiation exposure in PEM is a disadvantage.

PEM also suffers from the same specificity issues as breast MRI. Nonmalignant lesions, such as fibroadenomas, fibrocystic changes, and fat necrosis, can also accumulate FDG, mimicking a malignant lesion [70]. The specificity for detecting carcinoma ranges from 92% to 97% for PEM and 85% to 92% for MRI [75]. There are commercially available vacuum-assisted biopsy systems that can be used with PEM devices. The positive predictive values of these biopsies are similar to those of MRI-guided biopsies and higher than those of mammography-guided biopsies [70].

Positron Emission Tomography/Magnetic Resonance Imaging

PET/MR imaging is particularly interesting as a possible improvement over PET/CT oncologic whole-body imaging because MRI provides improved lesion detection in the brain, breast, liver, kidneys, and bones compared with lesion detection via CT. For breast malignancies, PET/MR can bring metabolic, anatomic, spectroscopic, and diffusion- and perfusion-based data together in a single examination. In whole-body imaging for breast cancer, PET/MR has been shown to provide improved sensitivity over PET/CT, particularly for breast lesions and liver and bone metastases [76, 77]. In local staging, PET imaging, which provides greater sensitivity for axillary nodes, appears to be complementary with MRI,

which provides greater accuracy for satellite lesions. PET/MR has been shown to be more likely to determine the correct maximum diameter of the tumor (T stage) than PET/CT, which may be useful in surgical and oncological planning [78].

When separated out by sequence, dynamic contrast-enhanced (DCE) MRI has been shown to be most useful for breast and brain lesions, diffusion-weighted imaging (DWI) has been shown to be most useful for liver and bone metastases, and PET has been shown to be most useful for lymph node metastases [77]. These variable strengths highlight the advantage of multimodality imaging. In particular, combining PET and DWI may be important because PET has been shown to greatly improve the specificity of DWI in whole-body imaging [79]. In addition, omitting whole-body CT from the PET examination can decrease the radiation dose by half [77]. These data suggest a wider role for PET/MR imaging in breast cancer staging and surveillance, particularly in young patients and in patients undergoing serial examinations.

Radionuclide Therapies in Breast Cancer

Palliative Treatment of Painful Osteoblastic Skeletal Metastases

Postmortem studies indicate that 75% of breast carcinoma patients develop bone metastases [80]. The majority of patients with bone metastases develop severe pain that reduces their quality of life. A multidisciplinary approach to palliating pain is usually necessary. In patients with pain with multifocal, osteoblastic metastatic lesions, low-energy beta-emitting radionuclides, such as samarium-153-ethylene diaminetetramethylenephosphonate (Sm-153 EDTMP) and strontium-89, can be used to deliver high radiation to metastases but only a negligible dose to the hematopoietic marrow. Radionuclide therapy is indicated in patients with failure of conventional analgesics and to palliate recurrent pain in a previously irradiated site. The uptake of radiopharmaceuticals in radionuclide therapy depends on the osteoblastic activity and the calcification of the tumor tissue. The response rate is approximately 75%, and 25% of the patients may even become pain-free [81]. The majority of patients are able to reduce or withdraw opioid analgesics and continue using nonsteroidal anti-inflammatory medication. The therapy can be repeated if the cell counts are appropriate. Patients should have reasonable bone marrow reserve and must be monitored after treatment for probable temporary bone marrow suppression. Concomitant treatment with bisphosphonates does not interfere with the radionuclide treatment [81].

Baczyk et al. reported the results of a randomized controlled trial comparing Sm-13 EDTMP and Sr-89 in metastatic prostate cancer (n = 60) and breast cancer (n = 40) patients [82]. Although there was no difference in pain relief between the two radionuclides, patients with purely blastic metastatic lesions experienced more pain relief than patients with a mixed blastic/lytic pattern of metastases.

Radium-223 (Ra-223) is a bone-seeking alpha particle emitter radionuclide that delivers higher absorbed radiation to the bone surface, thus sparing the bone marrow due to its limited range. A double-blind, randomized, placebo-controlled phase III trial (ALSYMPCA) in prostate cancer patients showed a survival advantage (14 vs 11.2 months) in the Ra-223 arm with a low toxicity profile [83]. The median time of new skeletal events was also longer in the Ra-223 arm (13.6 vs 8.4 months). With respect to its tumoricidal effect in skeletal metastases, Ra-223 promises more than pain palliation in metastatic breast cancer patients.

Radioembolization for Liver Metastases

Radioembolization is a liver-directed therapy that involves injection of micron-sized embolic particles loaded with a radionuclide via percutaneous hepatic artery catheterization under fluoroscopic guidance. Because cancer cells are supplied by the hepatic artery and normal hepatocytes by portal venous blood, radioembolization targets tumor cells with a high dose of lethal radiation while sparing healthy hepatocytes. The antitumor effect is mainly from radiation rather than embolization. Because the hepatic artery is not embolized totally during radioembolization, portal vein thrombosis, which is a contraindication for other transarterial techniques, such as chemoembolization, is not a contraindication for radioembolization.

Yttrium-90 (Y-90) is the most commonly used radionuclide in radioembolization. Y-90 is embedded in either glass- or resin-based microspheres. Holmium-166 (Ho-166) microspheres have also been used recently. The procedure is performed on an outpatient basis. The probable complications are less commonly seen than in other locoregional therapies and may include nausea, fatigue, abdominal pain, hepatic dysfunction, biliary injury, and fibrosis. The complications that may be caused by the spread of radioactive microspheres to extrahepatic locations, such as gastrointestinal ulcers, cholecystitis, and radiation pneumonitis, can be avoided by meticulous pretreatment angiographic assessment and dosimetric calculations.

Radioembolization is an effective treatment for both primary and secondary liver tumors. ECOG performance status $\leq$2, adequate hematological parameters, and pulmonary, renal, and liver function tests are mandatory. Significant extrahepatic tumor burden, which diminishes expected survival, is also an exclusion criterion. When there is a bilobar, multicentric tumor load in the liver, instead of treating the whole liver in one session, sequential treatments are administered 6–8 weeks apart.

Liver metastases in breast cancer patients have been treated by radioembolization, and accumulating experience is encouraging (Fig. 6.4). Bangash et al. investigated Y-90

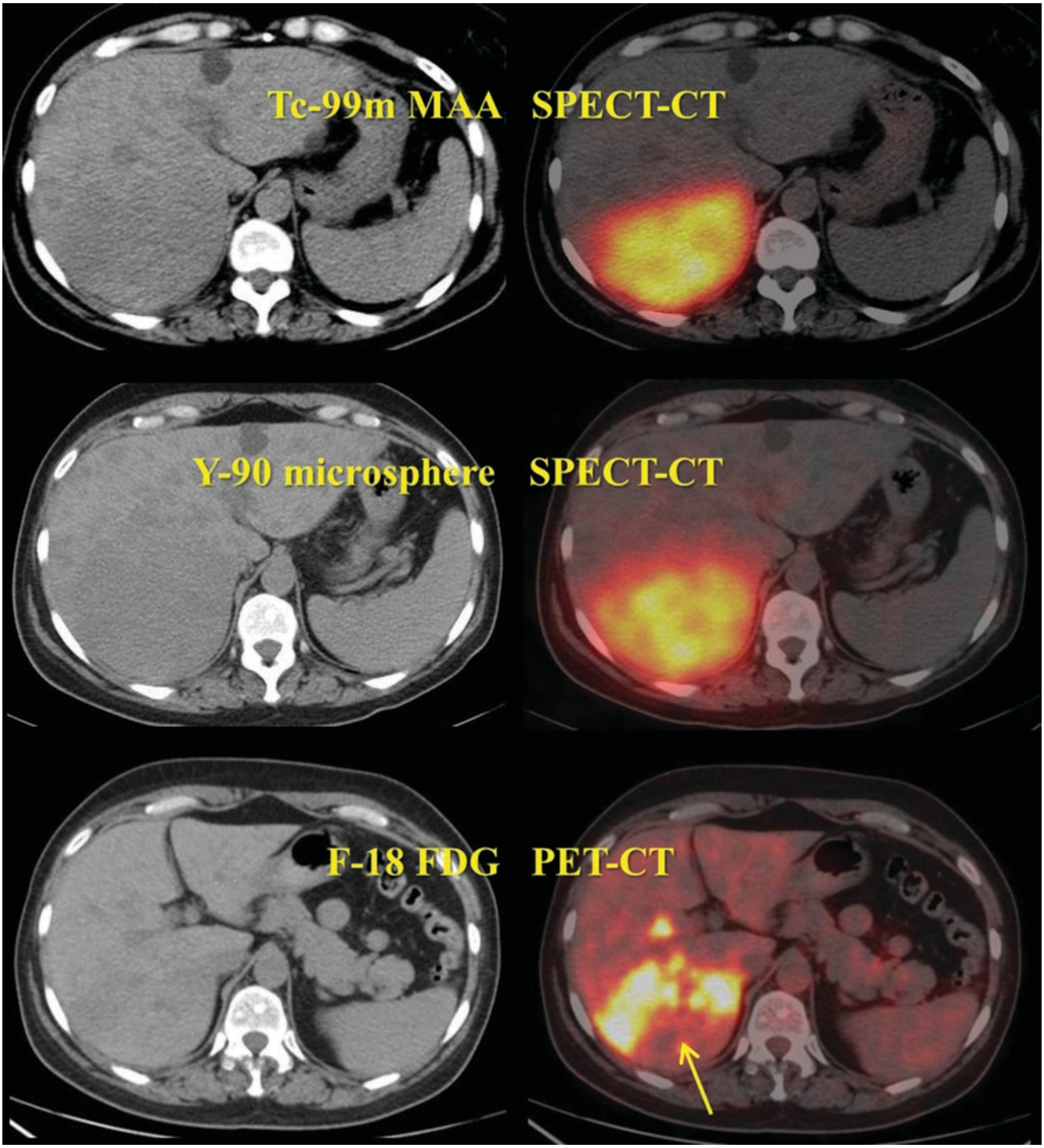

Fig. 6.4 In a 49-year-old female breast cancer patient, multiple metastases were detected in the lungs, liver, and bones 1 year after completion of adjuvant chemotherapy. Although the metastases responded well to second-line chemotherapy, a large metastatic lesion located in the posterior section of the right lobe of the liver did not decrease much in size. Therefore, before continuing with the chemotherapy regimen, radioembolization was planned for this lesion. In pretherapy angiographic evaluation, Tc-99m MAA was given in the posterior branch of the right hepatic artery, and SPECT/CT images (upper row) taken afterward showed homogeneous distribution of the radiopharmaceutical. After dosimetric calculations, 150 Gy of Y-90 microspheres was given via the same vascular route, and images (middle row) taken afterward showed a homogeneous distribution of Y-90 microspheres in the lesion. The control FDG PET/CT imaging (lower row) showed the response to radioembolization as necrosis (shown with an arrow)

radioembolization in 27 breast cancer patients with progressing liver metastases on standard polychemotherapy [84]. The response rate was 39.1%, and stable and progressive disease was observed in 52.1% and 8.8%, respectively. Median survival was 6.8 and 2.6 months in patients with ECOG 0 vs 1, 2, and 3. In a multi-institutional study of 44 breast cancer patients with chemorefractory liver metastases, the response to Y-90 radioembolization was 95% when evaluated by PET and 47% when evaluated by CT [85]. Even patients without a PET or CT response had a median survival of 3.6 months. Median survival for the whole patient group was not reached at a follow-up of 14 months. Pieper et al. reported a disease control rate (response+stable disease) of 71.1% and an objective response rate (complete+partial response) of 28.9% in their single-center experience of 44 liver-dominant metastatic breast cancer patients with Y-90 radioembolization [86]. The median time to progression of the treated liver lobe was 101 days, and the median overall survival was 184 days. The authors stated that radioembolization can successfully delay progression of therapy-refractory liver-dominant metastatic breast cancer patients with a low complication rate.

There are many ongoing prospective trials examining the role for radioembolization in unresectable liver tumors, one of which includes breast cancer patients (SIRMITOC). The results of these trials will further clarify the efficacy and position of radioembolization.

Conclusion

The general advantage of nuclear medicine imaging is its ability to show deteriorations in a functional level, such as changes in a molecular structure or physiological processes, which makes it very different from radiological techniques that image on the basis of morphological alterations. Scintimammography is indicated for the study of breast lesions in patients in whom mammography or MRI is nondiagnostic or difficult to interpret; it may also be useful for assessing and even predicting the response to chemotherapy. Similar notions are also true for PEM imaging, which is a fairly new technique. Although whole-body FDG PET/CT imaging does not have sufficient utility in the detection of primary disease and is not optimized to replace the SLN procedure for initial axillary staging, FDG PET/CT scanning has efficacy superior to that of conventional imaging for the detection of locoregional and metastatic spread in the appropriate patient population and has a better diagnostic performance for the detection of skeletal metastasis compared with that of routine bone scanning. The major roles for PET/CT in breast cancer are detecting and localizing metastasis, monitoring the response to treatment, and early detection of recurrence. With PET/MR imaging, several drawbacks of PET/CT imaging, such as an inferior image quality in brain and liver lesions, can be improved. On the basis of the abovementioned evidence, the integration of nuclear medicine techniques with radiological techniques offers an interesting opportunity to improve the diagnostic imaging yield in breast cancer, which will eventually lead to better patient management. Another aspect of nuclear medicine, radionuclide treatments, also serves breast cancer patients. Radionuclide treatment for metastatic bone pain palliation is a safe and effective option for patients with multifocal osteoblastic metastases that has been used in breast cancer patients for years. Radioembolization, which is a fairly new radionuclide treatment option, is a novel transarterial locoregional therapy that is gaining recognition as a treatment option for primary and metastatic liver cancers and for which promising experience is also increasing in breast cancer patients.

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Lobular Carcinoma In Situ

7

Priscilla McAuliffe

History

Lobular carcinoma in situ (LCIS) was first described in the 1940s [1]. LCIS was first treated similarly to invasive carcinoma—with radical mastectomy—because it was often diagnosed concurrently with invasive lobular carcinoma (ILC). It was subsequently recognized that LCIS is a marker of risk for breast cancer that does not itself progress to malignancy, and treatment has thus evolved to close observation with early detection of subsequent malignancy. This management change was based in part on a 1978 review of 211 cases of women with LCIS treated by observation alone (without surgery). There was a 17% incidence of subsequent invasive carcinoma, with equivalent risk in both breasts, and only six (3%) patients died of breast cancer [2]. Close observation was associated with early breast cancer detection and high associated cure rates. However, more recently, as mammography and image-guided needle biopsies have become more widespread, the biological heterogeneity of LCIS has become more apparent, and now certain subtypes of LCIS, including the pleomorphic variant, are recognized as indolent precursors of ILC for which surgical resection with negative margins and often radiation therapy is indicated.

Epidemiology

The incidence of LCIS is difficult to estimate because it lacks specific clinical abnormalities and is always identified incidentally [3]. LCIS is generally not detectable by palpation on physical exam, by mammogram, or by gross pathological examination [4]. LCIS is identified in 0.5–3.9% of breast biopsy specimens [5, 6].

The mean age at diagnosis of LCIS is 10–15 years younger than that for invasive breast cancer. It has been described as being more common in premenopausal than in postmenopausal women [2, 7]. However, while LCIS is more often diagnosed in women between age 40 and 50, a review of the Surveillance, Epidemiology and End Results (SEER) program database from 1978 to 1998 revealed that LCIS increased during that time period in all age groups [5]. Interestingly, in women older than age 50, the incidence of LCIS increased concurrently with the incidence of ILC, whereas in women younger than 50, an increase in ILC was not observed as LCIS increased. In women aged 40–49 years old, rising LCIS diagnoses leveled off at approximately 1989, whereas the increase of LCIS in women aged 50–79 years old was the most profound and sustained. The reason for this increase in LCIS in postmenopausal women is likely multifactorial, including the increased availability of screening mammography, the implementation of MRI in breast cancer patient management, the use of hormone replacement therapy in postmenopausal women, and more accurate molecular diagnosis, to be discussed in the "Pathology" section.

Risk Assessment

Patients with LCIS have an 8- to 12-fold greater lifetime risk than the general population for developing invasive breast cancer in either breast [8, 9]. Numerous studies have documented that after the diagnosis of LCIS, if diligently sought, LCIS can be found elsewhere in the index breast and also in the contralateral breast. Approximately 50% of LCIS is multifocal, and in 30% of patients, LCIS is found within the contralateral breast [2, 9]. However, despite the bilateral risk, cancer development is skewed toward the ipsilateral breast. Furthermore, although subsequent invasive breast cancer can be either of ductal or lobular origin, 70–89% of invasive

P. McAuliffe (✉)
Department of Surgery, Division of Surgical Oncology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Magee-Women's Surgical Associates, Pittsburgh, PA, USA

Women's Cancer Research Center of UPMC Hillman Cancer Center, Pittsburgh, PA, USA
e-mail: mcauliffepf@upmc.edu

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carcinoma after LCIS is ILC [9, 10]. The time between LCIS and invasive cancer development is approximately 15–30 years [9]. LCIS is associated with approximately 90% of ILC cases [11].

Pathology

The hallmark of LCIS is the proliferation of the epithelial cells of the terminal ductal-lobular unit, with no penetration of the basement membrane. Compared to the cells that normally line the lobular acini, LCIS cells are larger and monomorphic. There are also a loss of cellular cohesion and the presence of intracytoplasmic vacuoles. Mitoses and necrosis are infrequent, and nucleoli are inconspicuous, without prominent chromatin. The difference between LCIS and the high-risk lesion atypical lobular hyperplasia (ALH) is quantitative, with fewer abnormal cells and the preservation of residual lumen in the lobules with ALH compared to complete replacement of the lobular unit with LCIS. Many utilize the term "lobular neoplasia" to encompass both ALH and LCIS because they may represent early and later points on a spectrum of abnormal lobular proliferation [12]. LCIS is distinguished from ILC because it is contained by the basement membrane on hematoxylin- and eosin-stained sections. Cases of mixed lobular and ductal in situ lesions have also been described, with genetic aberrations of a hybrid phenotype [12].

The pleomorphic variant of LCIS (PLCIS) is architecturally similar to LCIS. However, PLCIS has substantially larger nuclei and greater nuclear polymorphisms. In contrast to classic LCIS, PLCIS has prominent nucleoli, central necrosis, and large, clustered calcifications. In some cases, PLCIS cells have eosinophilic cytoplasm, imparting an "apocrine appearance," or intracytoplasmic vacuoles, imparting a "signet ring cell appearance" [12]. Her2/neu overexpression and gene amplification have been reported in PLCIS with apocrine differentiation [13]. The combination of calcifications, necrosis, and cellular features can complicate the distinction of PLCIS from high-grade DCIS. Whereas classic LCIS is generally not associated with direct clonal progression to ILC, the pleomorphic variant lesions are. These data suggest that pleomorphic LCIS may not only be a marker for increased risk of invasive breast cancer but also a direct precursor of ILC. Classic and pleomorphic LCIS can coexist in the same lesion [14].

Molecular analyses of LCIS (as well as ALH and ILC) have revealed decreased expression or the loss of the cell surface adhesion molecule E-cadherin [15]. The loss of E-cadherin is the defining molecular event of lobular breast pathology. This contrasts with ductal lesions, in which E-cadherin expression is generally maintained. Immunohistochemistry using anti-E-cadherin antibodies can be used to distinguish ductal and lobular lesions.

E-cadherin is the protein product of the *CDH1* gene (16q22.1) and is expressed on epithelial cells [12]. The cadherins are a family of adhesion proteins that span the cell membrane and, through a calcium-dependent mechanism, form dimers with cadherins on other cells and interact with the actin cytoskeleton [12]. The portion of E-cadherin that is intracytoplasmic binds to p120-catenin [16]. In normal mammary cells, p120-catenin is present at the cell membrane. However, if the E-cadherin protein is nonfunctional or lost, p120 accumulates in the cytoplasm, where it activates cytoplasmic Rho-GTPases, resulting in increased cell motility [17]. The loss of E-cadherin and the cytoplasmic accumulation of p120-catenin are pathognomonic for lobular breast pathologies [12]. This feature can be critically important when LCIS is diagnosed concurrently with lesions, such as sclerosing adenosis or radial scars, as these together can produce patterns that mimic ILC. The lack of E-cadherin staining and cytoplasmic p120-catenin in the areas of question can differentiate LCIS and ILC [12]. Furthermore, some high-grade triple-negative DCIS may display diminished E-cadherin expression, suggesting PLCIS [12]. In addition to the loss of E-cadherin, the loss of high-molecular-weight keratins (cytokeratins 5/6, 14, and 17), which are generally present in high-grade DCIS, suggests PLCIS [12].

Some LCIS may display aberrant E-cadherin membrane expression that is not completely absent from the cell membranes, but it is fragmented, focal, or beaded. In these cases, double staining for E-cadherin and immunostaining for beta-catenin can be helpful to establish the diagnosis. The loss of beta-catenin also indicates that the E-cadherin is dysfunctional and not associated with other molecules in the cadherin-catenin complex [18, 19].

CDH1 gene mutations, deletions, and methylation have been identified in LCIS, as well as abnormal transcriptional regulation of E-cadherin [12]. Furthermore, LCIS also exhibits a loss of heterozygosity [20]. Other target genes that have been associated with the development of LCIS include fibroblast growth factor receptor 1 (*FGFR1*) and cyclin D1 (*CCND1*) [21, 22]. Pleomorphic LCIS has also been associated with *CCND1* and the oncogenes *MYC* and *HER2* [13, 23].

Diagnosis

Clinical Presentation

The clinical presentation of patients with LCIS is highly variable. LCIS is usually not detectable by physical examination and does not have pathognomonic features on mammogra-

phy. In the era of widespread mammographic screening and the shift to percutaneous breast biopsy, LCIS is most commonly diagnosed as an incidental finding on image-guided core-needle biopsy. It can also be found incidentally on surgical lumpectomy specimens removed for another indication.

Radiographically, classical LCIS is associated with small punctate calcifications in 42% of cases, whereas the pleomorphic variant of LCIS is more likely to have large and clustered calcifications related to the presence of comedo-type necrosis [4]. Pathological diagnosis is described above. Occasionally, even in the presence of E-cadherin, p120-catenin, beta-catenin, and cytokeratin staining, the diagnosis of LCIS is ambiguous and difficult to distinguish from DCIS. In this case, diagnosis should employ a multidisciplinary approach. However, when a definitive diagnosis cannot be rendered even after a multidisciplinary discussion or in the case of mixed LCIS and DCIS, the lesion should be managed as DCIS.

Treatment

Surgery

After an incidental diagnosis of LCIS by percutaneous image-guided core-needle biopsy, surgical excisional biopsy should be performed to rule out synchronous invasive cancer and DCIS. Percutaneous biopsy is limited by sampling error, and it can present difficulty in making a definitive histological diagnosis [24]. Upgrading to invasive cancer when the biopsy site is surgically excised can occur [25]. The goal of surgical excisional biopsy is to remove the biopsy site and any residual imaging abnormalities.

Excisional biopsy demonstrates a 0–10% risk of synchronous invasive breast cancer and a 0–50% risk of synchronous DCIS [6, 26, 27]. Surgical excisional biopsy is most commonly performed using a technique to localize a titanium marker clip placed radiographically during percutaneous biopsy. Two such localization techniques are wire or radioactive seed localization. To document the removal of the LCIS on excisional biopsy, mammography of the surgical specimen after excision should reveal the presence of the clip. Furthermore, the surgical pathology report should describe residual biopsy site changes due to the percutaneous core-needle biopsy. Contralateral mirror-image breast biopsy, a procedure described in the past for patients with LCIS, is no longer performed. Instead, close observation of all remaining breast tissue is recommended.

The management of microscopic margin status in LCIS is guided by the results of several studies described below. In a study of 180 patients who underwent observation alone after margin-negative surgical excision of LCIS, the overall ipsilateral and contralateral breast cancer event rates at 12 years of follow-up were 14.4% and 7.8%, respectively [10]. The rate of invasive breast cancer was 5.6%. This rate was similar whether ipsilateral or contralateral, although contralateral cancers occurred later. Nearly 85% of subsequent ipsilateral breast tumors were detected mammographically. More than 96% of all ipsilateral tumors occurred in the same quadrant as the original LCIS. Breast cancer-specific mortality was 1.1% at 12 years [10]. In another study of 100 patients with LCIS in which margin status was not documented, the ipsilateral and overall breast cancer event rates were 13% and 16%, respectively [28]. Finally, in a retrospective analysis of 2894 patients who underwent breast-conserving surgery for DCIS or early breast cancer between 1980 and 2007, 10% had LCIS within the lumpectomy specimen, and of those, approximately one-third had LCIS at the margin [29]. The difference in crude local recurrence rate between the patients with LCIS within the specimen (4.5%) and in those with no LCIS (3.8%) was not statistically significant [29]. Furthermore, there was also no significant difference in actuarial 5- and 10-year local recurrence rates if LCIS was present at the margin (6% and 6%), if LCIS was present but not at the margin (1% and 15%), or if no LCIS was present at all (2% and 6%). The results of these studies suggest that re-excision to achieve negative margins for classical LCIS is not warranted. However, for the pleomorphic PLCIS subtype, re-excision to achieve negative margins is indicated. In addition, identification of LCIS in a lumpectomy specimen resected for the diagnosis of DCIS or invasive cancer should not alter surgical management of the primary breast because the presence of LCIS does not increase the rate of in-breast recurrence in patients undergoing breast conservation [29].

Once a diagnosis of LCIS has been rendered and concurrent malignancy excluded, patients with LCIS should be counseled regarding their increased lifetime risk of breast cancer development. The surgical management of LCIS is generally conservative, and only a small minority pursue bilateral risk-reducing mastectomy, although this number has recently been increasing [30]. This approach is usually reserved for patients who have additional risk factors for breast cancer development or who experience significant anxiety regarding observation and/or chemoprevention options. It is important that patients considering this option are aware that bilateral mastectomy does not completely eliminate the risk of breast cancer development [31]. Because LCIS poses no risk of regional metastasis, sentinel lymph node biopsy or axillary node dissection is not required. Immediate breast reconstruction should be offered for patients who undergo risk-reducing mastectomy for LCIS. Women should be informed about the impact of this treatment approach on quality of life, particularly body image and sexual function [32]. Nipple-areola complex-sparing mastectomy may be a viable option in carefully selected women pursuing surgical risk reduction [33].

Risk-Reducing Endocrine Therapy

Risk-reducing therapy, often called "chemoprevention," is an important treatment option for patients with LCIS. In the NSABP P-1 breast cancer prevention trial, the incidence of invasive breast cancers was reduced by 56% in women with LCIS who received tamoxifen compared to observation alone [34]. Women with LCIS represented 6.2% of the patients in that trial. The annual hazard rate of invasive cancer was 5.69 per 1000 women who received tamoxifen compared with 12.99 per 1000 women who did not. In the NSABP P-2 trial, postmenopausal women with LCIS were randomized to tamoxifen or raloxifene [35]. Women with LCIS comprised 9.2% of the patients on the trial. There was no difference in risk reduction for invasive breast cancer between the two agents (incidence 4.30 per 1000 vs. 4.41 per 1000 for tamoxifen and raloxifene, respectively). Patients receiving raloxifene had a lower incidence of thromboembolic events and cataracts. There was no significant difference in the risk of other cancers, fractures, ischemic heart disease, or stroke for the two drugs. At 81 months of median follow-up, raloxifene was 78% as effective as tamoxifen at preventing invasive disease but had fewer toxicities, with significantly fewer endometrial cancers [36]. Raloxifene may be of particular benefit to postmenopausal women with an intact uterus and a risk of osteoporosis; tamoxifen would be an appropriate choice for high-risk postmenopausal women.

Radiation Therapy

Adjuvant radiation therapy is not recommended for the treatment of LCIS. If synchronous DCIS or invasive breast cancer is found in an excised LCIS specimen, the patient should be treated according to the guidelines for DCIS or invasive breast cancer and may benefit from radiation.

Surveillance

Following excisional biopsy demonstrating LCIS, patients should undergo annual bilateral breast physical examinations and diagnostic mammography. Screening ultrasound in patients with high breast cancer risk, including LCIS, is associated with high false-positive results [37]. A recent single-institution analysis revealed that with either annual mammograms or MRI, the cancer detection rate was 13% [38]. MRI was not associated with diagnosing breast cancer at earlier stage, smaller size, or node negativity. For this reason, the routine use of MRI for screening patients with a diagnosis of LCIS is not recommended. Patients with LCIS who undergo a bilateral mastectomy with or without reconstruction should also undergo an annual physical examination, but routine imaging is not indicated. Any suspicious lesions should be evaluated with ultrasound and biopsy analysis.

Conclusion

LCIS is a histological finding characterized by an intact basement membrane with a loss of E-cadherin leading to a dysfunctional E-cadherin/catenin complex. LCIS confers increased long-term risk of breast cancer that may affect either breast. The pleomorphic subtype is also a non-obligate precursor to invasive cancer. Patients found to have LCIS on core-needle biopsy are evaluated with bilateral diagnostic imaging, and additional suspicious lesions are further evaluated. Marker clips should routinely be placed at the time of percutaneous image-guided biopsy. Patients diagnosed with LCIS should undergo surgical excisional biopsy with localization of the percutaneous biopsy cavity to increase accuracy. If synchronous DCIS or invasive breast cancer is diagnosed, subsequent treatment is administered according to the guidelines for these tumors. Re-excision to attain negative margins is not performed in patients with classical LCIS unless pleomorphic LCIS is identified, in which case negative margins should be achieved. Bilateral risk-reducing mastectomy is generally reserved for patients with additional risk factors for breast cancer or with extreme anxiety regarding observation and/or chemoprevention options but does not completely eradicate the risk of subsequent breast cancer development. Patients with LCIS should receive systemic risk reduction with antiestrogen therapy, namely, tamoxifen or raloxifene. Follow-up includes clinical and imaging surveillance. All patients with LCIS should be considered for clinical trials.

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Ductal Carcinoma In Situ

8

Priscilla McAuliffe

History

Before the introduction of screening mammography, most cases of ductal carcinoma in situ (DCIS) remained undetected until a palpable mass formed. However, widespread use of screening mammography has resulted in a tenfold increase in the reported incidence of DCIS since the 1980s [1]. The Surveillance, Epidemiology, and End Results (SEER) program database indicates that in 1975, 5.8 per 100,000 women were diagnosed with in situ breast cancer in the USA, whereas in 2007, 34.9 per 100,000 women received the same diagnosis [2].

Epidemiology

DCIS accounts for more than 25% of all new cases of breast cancer [1]. Approximately 64,640 new diagnoses of in situ breast cancer are expected among US women in 2013; more than 85% of these will be DCIS [1]. The median age of diagnosis of DCIS ranges from 47 to 63 years, but more than 75% of patients will receive this diagnosis over age 50 [1]. In the USA, in women between the ages of 50 and 69, one case of DCIS is detected per 1000 screening mammograms [3]. Diagnosis of DCIS peaks between ages 60 and 74, which is earlier than for invasive ductal carcinoma (IDC), in which diagnosis peaks between ages 75 and 79 [4]. Many risk factors for the development of DCIS are similar to those for IDC, including female sex, older age, family history of breast cancer, BRCA 1/2 gene carriers, increased breast density, history of previous breast biopsy, and nulliparity or older age at the time of first full-term pregnancy [4, 5]. However, studies have been inconsistent with regard to risk of DCIS after exposure, for more than 5 years, of estrogen hormone replacement therapy and related to body mass index, both of which are known risk factors for development of IDC [4]. Oral contraceptive pills do not appear to increase the risk of DCIS [5].

Natural History of DCIS

Several strong pieces of evidence suggest that not all DCIS lesions are clinically significant. The prevalence of DCIS on autopsy studies ranges from 1% to 14.3% [6, 7]. Furthermore, in studies in which DCIS was initially misdiagnosed as benign and treated by biopsy alone, 14–53% of DCIS progressed to IDC over a period of 10 or more years [6]. This suggests that there is a cohort of patients for whom DCIS would not have had a clinical impact on the patient's life. This underscores the critical need for research to identify markers that can aid in selection of patients for personalized treatment [5]. Development of molecular risk profiles is of particular interest [8]. Furthermore, this has also led to a discussion about the best terminology for DCIS and consideration for omission of the word "carcinoma" [9]. Several ongoing clinical trials are investigating de-escalation of treatment for DCIS. Two important examples are the LORIS (low-risk DCIS) trial and the COMET (comparison of operative to monitoring and endocrine therapy) for low-risk DCIS trial, both of which are comparing surgery to active monitoring [PMID: 26296293][PMID: 28925613].

Pathology

DCIS is a proliferation of malignant cells arising from ductal epithelium in the terminal ductal-lobular unit that has not breached the ductal basement membrane. Malignant cells proliferate until the ductal lumen is obliterated. DCIS has traditionally been considered one

P. McAuliffe (✉)
Department of Surgery, Division of Surgical Oncology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Magee-Women's Surgical Associates, Pittsburgh, PA, USA

Women's Cancer Research Center of UPMC Hillman Cancer Center, Pittsburgh, PA, USA
e-mail: mcauliffepf@upmc.edu

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stage in the continuum of progression from atypical ductal hyperplasia (ADH) to IDC [10]. The lesions can be heterogeneous, with variable histologic architecture, molecular and cellular characteristics, and clinical behavior. DCIS has also been associated with changes in surrounding stroma, resulting in fibroblast proliferation, lymphocyte infiltration, and angiogenesis [10]. Although the process is poorly understood, most IDCs are believed to arise from DCIS and poorly differentiated DCIS to evolve from well-differentiated DCIS [11]. Genetic defects that lead to progression appear to be randomly acquired and accelerated by p53 mutation which results in genetic instability [11]. Furthermore, genes involved in cell adhesion and signaling, motility, angiogenesis, and extracellular matrix formation have also been identified that may lead to progression from DCIS to IDC [12, 13]. DCIS may represent a later stage of molecular progression, as many gene mutations occur prior to invasion [14, 15].

Classification

No single classification system for DCIS has been universally accepted. The most common subtypes based on the architectural pattern of the proliferating cells—comedo, solid, cribriform, micropapillary, and papillary—can coexist. Nuclear grade has also been used to classify lesions. DCIS with high nuclear grade and comedo necrosis is predictive of local recurrence [16]. At 8 years of follow-up, patients whose tumors had a high nuclear grade and comedo necrosis had a 20% local recurrence rate after breast-conserving surgery and irradiation, compared with 5% for those patients whose tumors did not have necrosis and had a lower nuclear grade [16]. A classification system devised by Silverstein called the Van Nuys classification also utilized high nuclear grade and comedo-type necrosis. Their early studies suggested that this classification was strongly predictive of disease-free survival, but this could not be prospectively validated [17].

Multifocality

Multifocal DCIS is defined as the presence of DCIS in two or more foci in the same breast quadrant, separated by 5 mm. Careful serial pathologic subsectioning of multifocal lesions suggests that these actually represent intraductal spread from a single focus of DCIS. In 81 of 82 mastectomy specimens, multifocal lesions which appeared to be separate using conventional pathologic techniques were found to originate from the same focus [18].

Multicentricity

Multicentric DCIS presents as separate, discontinuous foci of DCIS outside of the index breast quadrant. The incidence of multicentricity varies in the literature, and it likely depends on the extent of the imaging and the pathological review. Most local recurrences after treatment of DCIS occur in the same quadrant as the index lesion, implicating residual untreated disease rather than multicentricity [19].

Microinvasion

According to the American Joint Committee on Cancer (AJCC), microinvasion is defined as invasion of breast cancer cells through the basement membrane at one or more foci, none of which exceeds a dimension of 1 mm. In the AJCC staging system, DCIS with microinvasion (DCISM) is classified as a "T1mic" tumor, whereas DCIS is classified as "T0." Microinvasion upstages the AJCC cancer stage from 0 to 1. DCISM is found in 5–10% of cases of DCIS [20]. By definition, DCIS does not metastasize to axillary lymph nodes or distant sites, whereas DCISM can. Axillary metastasis has been reported in 0–28% of patients with DCISM [21, 22].

Microinvasion in DCIS varies according to the size and extent of the index lesion. When DCIS less than 25 mm in diameter was compared to those 25 mm and larger, the incidence of microinvasion was 2% and 29%, respectively [16]. The incidence of microinvasion is also higher with high-grade or comedo-type DCIS and when DCIS presents as a palpable mass or with nipple discharge [20].

Disease-specific survival is worse for DCISM than DCIS [23]. In a retrospective study of 1248 cases of DCIS, the 10-year distant metastasis-free survival rate was significantly better in patients with DCIS compared to DCISM (98% and 91%, respectively). The overall survival rate was also better (96.5% vs. 88.4%) [22]. On the other hand, when compared to IDC, metastasis-free and overall survival rates were better in patients with DCISM. These results suggest that DCISM should be characterized as an invasive tumor with a good outcome, and the therapeutic approach for these patients should be similar to that for patients with IDC. Further study is needed to investigate the biology of microinvasion.

Diagnosis

Clinical Presentation

Prior to routine screening mammography, patients with DCIS presented most commonly with a palpable mass, nipple thickening or discharge, or Paget's disease of the nipple.

Occasionally, DCIS was found incidentally in an otherwise benign breast biopsy specimen. With the advent of screening mammography, DCIS is more likely to be diagnosed when the tumor is still clinically occult. Patients with mammographically detected DCIS should always undergo contralateral breast imaging because patients may have synchronous occult abnormalities or cancers in the contralateral breast. To establish interval changes, current images should be compared with previous mammograms, if available. Magnification, spot compression, and other mammographic views are routinely used to further delineate the abnormality, especially calcifications, in the index breast. Ultrasound can also be used to assess tumor size and multicentricity.

Imaging

Mammographic and Ultrasonographic Features

On a mammogram, DCIS can present as microcalcifications, a soft tissue density/asymmetry, or both. Microcalcifications are the most common mammographic manifestation of DCIS. Microcalcifications can be divided into two classes that are suggestive of the architectural type of DCIS: (1) linear branching type, which are more often associated with high-nuclear-grade comedo-type lesions and (2) fine, granular-type, which are generally associated with micropapillary or cribriform lesions of lower nuclear grade without necrosis [18]. Mammographic findings can significantly underestimate the pathologic extent of disease, particularly in cases of micropapillary DCIS [18]. Lesions were more than 2 cm larger by histologic examination than by mammographic estimation in 44% of cases of micropapillary lesions, compared with only 12% of cases of the pure comedo subtype. Magnification views on mammography more accurately predict the extent of disease, which was underestimated in only 14% of cases of micropapillary tumors. Hence, magnification views increase image resolution and are better able to discern the shape, number, and extent of calcifications when compared with screening mammographic films, and they should be used routinely in the evaluation of suspicious mammographic findings, especially microcalcifications.

Magnetic Resonance Imaging (MRI)

MRI is not routinely employed in the preoperative evaluation of patients with DCIS. Instead, mammogram remains the gold standard for radiographic evaluation of DCIS. The cost and accessibility of MRI make it less feasible as a screening method. However, it may have utility in patients at high risk for breast cancer or with extremely dense breasts. Contrast-enhanced MRI is more sensitive than mammography in detecting both DCIS and invasive cancer, but because DCIS can mimic fibrocystic change and other benign findings on MRI, it can also lead to false-positive and unnecessary biopsies [24–27]. MRI is sometimes used after initial diagnosis to identify multicentric and contralateral lesions, because the presence of either of these may change the surgical treatment strategy [28]. MRI-detected multicentric disease was found in 4.3% of 149 patients who presented with DCIS [29]. MRI can also detect contralateral breast cancer in patients presenting with DCIS [28]. Of 196 patients with DCIS, MRI prompted biopsy in 18 patients. Contralateral breast cancer was detected in five patients (28% of those biopsied and 2.6% of those with DCIS). The sensitivity and specificity of detecting contralateral breast cancer are 71% and 90%, respectively. However, no benefit of MRI in reducing local recurrence has been observed. This is likely due to the fact that MRI cannot detect all clinically occult cancer in the breast, and, in addition, local failure after breast conservation is uncommon in contemporary studies [30, 31]. Finally, acting on the findings of MRI leads to exclusion of patients from breast-conserving therapy and an increase in performance of mastectomies [26, 32]. Therefore, breast MRI likely leads to overtreatment [30].

Diagnostic Biopsy

The preferred method for diagnosis of DCIS is percutaneous biopsy, either with ultrasound-guided or vacuum-assisted stereotactic core-needle biopsy technique. Patients who cannot lie prone, exceed the weight limit for the stereotactic system, or cannot cooperate during the procedure are not good candidates for stereotactic biopsy. Bleeding disorders and the concomitant use of anticoagulation are relative contraindications. Patients who are not candidates for image-guided biopsy should undergo excisional biopsy. Biopsy tissue cores are radiographed after the procedure to document sampling of suspicious microcalcifications. Marking the biopsy site with a metallic clip is standard of care.

Because percutaneous image-guided breast biopsy specimens represent only a sample of an abnormality observed on mammography, the results are subject to sampling error. Invasive carcinoma is found on lumpectomy in 10–20% of patients in whom DCIS was diagnosed by a stereotactic core-needle biopsy, and DCIS is diagnosed on excisional biopsy in 10–30% of patients with atypical ductal hyperplasia or radial scar on stereotactic biopsy [33–35]. If core-needle biopsy results are discordant with the findings on imaging, repeat image-guided percutaneous biopsy or an excisional biopsy should be performed to clarify the diagnosis.

Excisional biopsy is performed with the assistance of preoperative image-guided localization of the mammographic abnormality or of the previously placed metallic clip marking the biopsy site. Two localization techniques utilize either wire or radioactive seed. Specimen

radiography is essential to confirm the removal of microcalcifications of interest. The surgical pathology report should document the presence of biopsy site changes to confirm appropriate localization.

Treatment

The treatment of DCIS is multidisciplinary. Surgical treatment can be either breast conservation (also referred to as segmental mastectomy, lumpectomy, or wide local excision) or mastectomy. Most patients who undergo breast-conserving surgery receive postoperative radiation therapy to improve local control. Postoperative systemic endocrine therapy, most often with tamoxifen, is also utilized for those patients whose tumors are estrogen receptor positive.

Surgery

Mastectomy

Traditionally, DCIS was treated with total mastectomy. The rationale for mastectomy for DCIS was based on the high incidence of multifocality and multicentricity, as well as on the risk of occult invasion associated with the disease. Retrospective reviews show a mortality rate of 0–8% after mastectomy for DCIS [36–38]. Local recurrence rates for DCIS after mastectomy were 1–3% [36, 39, 40]. More recently immediate reconstruction is offered to patients with utilization of skin-sparing or nipple-areola mastectomy in carefully chosen patients [41]. Occasionally, even after mastectomy, close or positive margins are seen on the pathologic specimen, and these have been identified as an independent risk factor for locoregional recurrence. However, this rate is so low (10-year locoregional recurrence rate of 1%); therefore postmastectomy radiation therapy is not warranted, except for patients with multiple positive margins that cannot be surgically excised [42].

Breast-Conserving Surgery

The goal of breast-conserving surgery in the treatment of DCIS is to remove all suspicious calcifications and obtain negative surgical margins. Because DCIS is usually nonpalpable, breast-conserving surgery is performed with preoperative image-guided localization, utilizing either a wire or a radioactive seed. In patients with extensive calcifications, bracketing of the calcifications with two or more wires or seeds may assist in the excision of all suspicious calcifications. Orientation of the specimen intraoperatively with two or more marking sutures is critical for margin analysis. In addition, specimen radiography is essential to confirm removal of both the marking clip and the microcalcifications. After mammography of the surgical specimen, it should be inked and then serially sectioned for pathological examination to evaluate the margin status and extent of disease.

The goal of breast-conserving surgery is to obtain tumor-free margins. Negative margins reduce by half the risk of ipsilateral breast tumor recurrence compared with positive DCIS margins. Residual tumor was found on re-excision in 41% of patients with DCIS with 0- to 1-mm margins, 31% of patients with 1- to 2-mm margins, and 0% of patients with greater than 2-mm margins [43]. Consensus guidelines released jointly by the Society of Surgical Oncology, American Society for Radiation Oncology, and American Society of Clinical Oncology advocate a 2-mm margins for breast-conserving surgery with whole-breast irradiation for ductal carcinoma in situ (PMID: 27538810). The recommended "no ink on tumor," which is the standard for an adequate margin in *invasive* cancer, should *not* be extrapolated to DCIS [44]. Furthermore, while utilization of endocrine therapy in estrogen receptor-positive DCIS is associated with reduced in-breast tumor recurrence, it is not associated with negative margin width (PMID: 27538810). However, margin widths narrower than 2 mm alone should not always be an indication for mastectomy. Consideration should also be given to the presence or absence of unfavorable factors such as multifocality, increasing number of closed or involved margins, comedo necrosis, high grade, large size of DCIS, young patient age, and negative ER status [45] [PMIDD:27538810].

Role of Sentinel Lymph Node Biopsy and Axillary Staging

Because DCIS is a noninvasive disease, lymph node involvement is not expected. Thus, axillary lymph node dissection or sentinel lymph node biopsy should not be performed for DCIS treated with breast conservation [46]. If microinvasion or frank invasion is identified on final pathology after breast-conserving surgery, the patient should return to the operating room for sentinel lymph node biopsy. Sentinel lymph node biopsy was positive for metastasis among 12% of patients with DCIS who were considered to be at high risk for invasion due to the presence of a large palpable mass and among 10% of patients who had DCIS with microinvasion [47]. Patients who undergo mastectomy for DCIS should routinely undergo sentinel lymph node dissection because it is not possible to perform lymphatic mapping after a mastectomy if invasive cancer is incidentally found in the mastectomy specimen. These are reflected in the most recently issued American Society of Clinical Oncology clinical practice guidelines [48]. Sentinel node dissection is associated with a small risk of lymphedema of approximately 5% [49–52].

Radiation Therapy

Most patients with DCIS who undergo breast-conserving surgery receive postoperative radiation therapy. Three prospective randomized studies have evaluated the role of radiation therapy following breast-conserving surgery for DCIS. In the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-17 trial, 818 women with DCIS were randomized, after margin-negative resection, to observation or radiation therapy [53]. At 12 years of follow-up, the cumulative incidence of ipsilateral breast tumors decreased, with addition of radiation therapy, from 14.6% to 8% for noninvasive disease and from 16.8% to 7.7% for invasive disease [54]. There was no difference in the 12-year overall survival rate with the addition of radiation: 86% and 87% of women were alive in the observation versus radiation therapy group, respectively. However, 58% of all deaths occurred before any breast cancer event. The death of 12 patients (3%) in the observation group and 15 patients (3.6%) in the radiation therapy group was attributed to invasive breast cancer [54].

The benefit of radiation therapy for DCIS was also observed in the European Organisation for Research and Treatment of Cancer (EORTC) 10,853 trial [55]. In this trial, 1010 women with DCIS were randomized to breast-conserving surgery or breast-conserving surgery plus radiation therapy. At a median follow-up time of 4.25 years, radiation therapy was associated with a reduction in the incidence of noninvasive ipsilateral breast tumors from 8.8% to 5.8% and with a reduction in the incidence of invasive ipsilateral breast tumors from 8.0% to 4.8%. The 15-year invasive locoregional recurrence-free rate was 84% in the local excision-only group and 90% in the local excision and radiation group [56].

A third trial conducted by the UK Coordinating Committee on Cancer Research also confirmed the benefits of radiation therapy for local control [57]. At a median follow-up of 4.4 years, the incidence of ipsilateral breast tumors decreased from 7% to 3% for noninvasive lesions and from 6% to 3% for invasive lesions if radiation was administered. Taken together, these three trials demonstrate that the addition of radiation therapy following breast-conserving therapy for DCIS results in approximately a 50% relative reduction in breast cancer recurrence.

Whole-breast radiation is standard for patients undergoing breast-conserving surgery and is generally tolerated well. The most common morbidity is radiation-induced skin changes including discoloration, fibrosis, and telangiectasias. Rare, severe side effects include damage to the heart and lungs, rib fractures, and radiation-induced secondary malignancy, angiosarcoma.

Partial Breast Irradiation

Local recurrences in the breast after a diagnosis of DCIS tend to occur in the immediate vicinity of the surgical resection cavity. Therefore, the impact of whole-breast irradiation in reducing local recurrence is most critical in the area immediately surrounding the original tumor bed. Based on this knowledge, the theory of partial breast irradiation is that equivalent local control may be achieved by focusing the treatment on tissue surrounding the surgical resection cavity. Accelerated partial breast irradiation is a technique where high-dose radiation is delivered over a shorter period of time to a limited region of the breast surrounding the primary tumor site. The treatment is completed over 4–5 days, whereas conventional whole-breast external beam radiation therapy typically requires 5–6 weeks. Several methods of partial breast irradiation have been described, including brachytherapy via multiple catheters placed in the breast parenchyma, localized conformal external beam radiation therapy, brachytherapy via bead or seed implants, single-dose intraoperative radiation therapy, and brachytherapy via a balloon catheter inserted into the cavity after breast-conserving surgery. On review of The American Society of Breast Surgeons' registry of accelerated partial breast irradiation, of 194 patients with DCIS, 63 had at least 5 years of follow-up [58]. Of these, 92% had favorable cosmetic results. The 5-year actuarial local-regional recurrence rate was 3.39%, which is comparable to that of 7.5% reported in the NSABP B-17 trial, which used whole-breast irradiation. The NSABP B-39/RTOG 0413 trial (ClinicalTrials.gov identifier NCT00103181), which opened in 2005, will provide additional information about the potential role for accelerated partial breast irradiation in patients with DCIS and those with invasive breast cancer as well. In this trial, patients with 3 cm of DCIS or less, or with invasive stage I or II breast cancer, who undergo breast-conserving surgery with negative margins are being randomized to standard adjuvant whole-breast external beam radiation therapy or accelerated partial breast irradiation. Patients will receive systemic therapy at the discretion of their treating physician. Local tumor control is the primary endpoint, and the secondary endpoints are disease-free and overall survival, cosmetic outcome, and treatment toxicity. Study completion is expected in 2016.

Omitting Radiation Therapy

Radiation therapy use varies depending on socioeconomic status, race, and the region of the USA in which the patient lives in [59–62]. Many patients with DCIS who are candidates for breast-conserving surgery choose mastectomy because they are unable to complete 6 weeks of daily radiation therapy because of social considerations or due to concerns about postirradiation complications. An estimated 20%

of women undergoing breast-conserving surgery who would benefit from radiation therapy does not receive it as part of their treatment. Breast-conserving surgery without radiation therapy may be sufficient in selected patients with DCIS. In a study of 79 patients with DCIS who underwent margin-negative excision alone, after 124 months of follow-up, the local recurrence rate was 16% overall—33% for the subgroup of patients with high-grade lesions and comedo necrosis versus only 2% for the patients with low- or intermediate-grade lesions [16].

Margin width is an independent prognostic factor for local recurrence, but alone, it is insufficient to predict which patients can safely forgo radiation therapy. In a retrospective analysis of 469 patients with DCIS who underwent breast conservation with margins of at least 10 mm, postoperative radiation therapy was not associated with a lower recurrence rate [63]. In contrast, on reanalysis of the NSABP B-17 data, all patient cohorts benefitted from radiation therapy, regardless of the clinical or mammographic tumor characteristics [64]. Furthermore, a prospective trial from the Dana-Farber/Harvard Cancer Center, in which radiation therapy was omitted in patients with grade 1 or 2 DCIS, measuring 25 mm or less, and resected with at least a 10 mm margin, was terminated early because the number of local recurrences was higher in the no-radiation group. The 10-year cumulative incidence of local recurrence was 15.6%, and the annual local recurrence was 1.9% per patient-year [65]. A multivariable nomogram which estimates local recurrence in women with DCIS treated with breast-conserving surgery calculates an estimate of absolute risk of ipsilateral breast tumor recurrence at 5 or 10 years, and the risk can be weighed against the use of other adjuvant treatment options. The nomogram incorporates age at diagnosis, family history, type of patient presentation (radiologic or clinical), nuclear grade, necrosis, margins, number of excisions, and receipt of radiation and/or adjuvant endocrine therapy, all of which are factors that were previously shown to affect the risk of ipsilateral breast tumor recurrence [66].

Two prospective studies investigating omission of radiation therapy after breast-conserving surgery for DCIS are the Eastern Cooperative Oncology Group E-5194 and the Radiation Therapy Oncology Group (RTOG) 9804. In the E-5194 trial, all patients had breast conservation with margins measuring 3 mm or more and no radiation therapy. Patients with low- or intermediate-grade DCIS of 25 mm or less were compared to high-grade DCIS of 10 mm or less [67]. The ipsilateral breast event rate at 5 years in the 565 patients in the low−/intermediate-grade group was 6.1% and in the 105 patients in the high-grade group was 15.3%. Long-term follow-up of this cohort is ongoing. Similarly, the RTOG 9804 trial randomized patients with low- or intermediate-grade DCIS, sized 25 mm or less, and excised with margins of at least 3 mm, to postoperative radiation therapy versus observation. Tamoxifen use was permitted in both groups. The trial closed early. At the American Society of Clinical Oncology (ASCO) meeting in 2012, it was presented that addition of radiation showed a statistically significant reduction in breast cancer recurrence at 5 years to 0.4% from 3.2% in the observation group. Reports of long-term outcomes are anticipated.

Endocrine Therapy

The NSABP B-24 trial involved 1804 women with DCIS treated with breast-conserving surgery and radiation therapy [64]. Patients were randomized to either tamoxifen (20 mg/day) or placebo for 5 years. Sixteen percent of patients in the study had positive margins at resection. At follow-up of 15 years, the ipsilateral invasive breast cancer rate was lower (8.5% after tamoxifen and 10% after placebo) representing a 32% reduction in events. The 15-year cumulative incidence of contralateral invasive or noninvasive breast cancer was 7.3% and 10.8% in the tamoxifen and placebo group, respectively. Invasive ipsilateral recurrence was associated with an increased mortality risk, whereas recurrence of DCIS was not. Benefit of tamoxifen persisted even in patients with positive or unknown margin status. In a subgroup analysis of patients with estrogen receptor-positive DCIS, those who received tamoxifen had a 59% reduction in their relative risk of breast cancer events compared to those who received placebo. Patients with estrogen receptor-negative DCIS derived no benefit from tamoxifen [68].

In the UK Coordinating Committee on Cancer Research trial, patients who underwent breast-conserving surgery were randomized to no adjuvant treatment, adjuvant radiation therapy, tamoxifen treatment, or adjuvant radiation therapy plus tamoxifen. Positive margin after breast-conserving surgery was an exclusion criterion for the trial. Compared with 33% of patients in the NSABP B-24 trial, only 10% of the women were younger than 50 years of age. After 4.4 years of median follow-up, radiation therapy had the greatest impact on reducing ipsilateral breast cancer events, whereas tamoxifen added to radiation therapy did not portend additional benefit [57].

Adjuvant tamoxifen should only be used in estrogen receptor-positive DCIS. Tamoxifen is generally very well tolerated, but it has been associated with vasomotor symptoms, deep vein thrombosis, pulmonary embolism, and increased cataract formation. The risk of endometrial cancer is increased two to seven times normal but still remains low. Tamoxifen is also associated with increased risk of stroke and benign ovarian cysts.

Current National Comprehensive Cancer Network guidelines recommend tamoxifen treatment for 5 years for patients with ER+ DCIS; however, they do not specifically recom-

mend treatment with aromatase inhibitors. Aromatase inhibitors have fewer cardiovascular side effects than tamoxifen and are used in postmenopausal women. The randomized prospective clinical trial, NSABP B-35 (ClinicalTrials.gov identifier NCT00053898), "Anastrozole or Tamoxifen in Treating Postmenopausal Women With Ductal Carcinoma in Situ Who Are Undergoing Lumpectomy and Radiation Therapy," has completed patient enrollment, and results are expected in 2016 [69]. The International Breast Cancer Intervention Study (IBIS-II), which enrolled postmenopausal woman at increased risk of breast cancer, showed a reduction in the cumulative incidence of breast cancer from 5.6% in the placebo group to 2.8% in the anastrozole group, with no difference in mortality [70]. A small proportion (8%) of the 3864 women randomized had been treated within the last 6 months for ER+ DCIS with mastectomy (n = 326). A subset analysis of this group of patients has not yet been reported.

Surveillance

The National Comprehensive Cancer Network treatment guidelines recommend annual physical examination and mammogram for follow-up of patients after breast-conserving surgery with or without radiation and/or endocrine therapy. Whether this improves the detection of recurrence and outcome is not entirely clear. Both patients who undergo breast-conserving therapy and those who undergo mastectomy should be monitored for the development of new primary cancers in the contralateral breast. Annual mammogram should be done, except in the case of mastectomy, which should be evaluated with physical exam.

Local Relapse

Predictors of Local Relapse

Features of DCIS associated with a greater risk of local recurrence are larger tumor size (>3 cm), high nuclear grade, comedo-type necrosis, and positive margins. Involved margins are the most important prognostic variable for predicting local relapse, but, as mentioned above, margins alone cannot be used independently to assess risk. Age less than 50 years and a strong family history of breast cancer are also associated with an increased risk of local recurrence. However, none of the above factors are contraindications for breast-conserving therapy. Molecular markers, such as overexpression of HER-2/*neu*, nm23, heat shock protein, and metallothionein, low expression of p21 Waf1 and Bcl2, and DNA aneuploidy have been reported to be associated with high-grade comedo lesions, but their importance as independent prognostic variables in DCIS is not currently known [7].

Treatment and Outcome of Local Recurrence

Patients with DCIS have an excellent overall survival. In the NSABP B-17 trial, at a median follow-up of 12 years, only 27 deaths were attributed to breast cancer (3.3%) [64]. In the B-24 trial, after 7 years of follow-up, 0.8% died from breast cancer [64]. The management of local recurrence depends on the therapy the patient received for the primary cancer. In patients who had breast-conserving surgery without radiation therapy, re-excision to negative margins and postoperative radiation therapy are recommended. If patients had breast-conserving surgery with radiation therapy, mastectomy is the standard treatment. Rarely, for patients who had mastectomy, local recurrence should be treated with wide local excision followed by postmastectomy radiation. If tissue coverage is a concern, tissue transfer techniques such as a latissimus dorsi flap can be employed. A reconstructive surgeon should be involved preoperatively.

In both B-17 and B-24, approximately 50% of local recurrences were invasive. In one population-based study, younger age played a role in local recurrence after DCIS treatment and was associated with more invasive recurrences [71]. Prognosis after treatment of local recurrence is worse when the recurrence is invasive compared to noninvasive. Disease-specific mortality after invasive recurrence is approximately 15% [72, 73]. If a recurrence is invasive, the axilla should be staged with lymphatic mapping and sentinel lymph node dissection. Follow-up for patients with DCIS should be long term, not only to detect recurrent disease but also the development of new ipsilateral or contralateral primary tumors.

Conclusion

DCIS is most commonly diagnosed as a mammographic abnormality which is subsequently percutaneously biopsied with image guidance. The pathological evaluation includes tumor type and grade and any evidence of microinvasion. Estrogen and progesterone receptor status should also be determined, but this is usually deferred until after surgical resection because if an invasive portion is unexpectedly identified, the receptors should be measured in that tissue, given the heterogeneity of breast lesions.

Therapy for DCIS should be personalized for each patient, based on tumor size, tumor to breast size ratio, mammographic appearance, and margin width, as well as patient preference. The benefits and risks of breast-conserving surgery and mastectomy should be discussed in detail with each

patient. Most patients with DCIS are candidates for breast-conserving therapy, and this is the preferred method of local treatment because it offers equivalent overall survival compared to mastectomy. Re-excision is recommended for patients who have margins less than 2 mm on final pathological examination after breast-conserving surgery. Mastectomy is indicated in patients with persistently positive margins after attempts at breast conservation, in those with a contraindication to postoperative radiation therapy, and in patients with diffuse, malignant-appearing calcifications throughout the breast. Mastectomy may also be a better option if a patient's anxiety about possible local recurrence outweighs the impact of a mastectomy on quality of life. For all patients undergoing mastectomy, immediate breast reconstruction should be considered.

Patients who have mastectomy for DCIS should also undergo sentinel lymph node biopsy. In patients who undergo breast-conserving surgery, sentinel lymph node dissection is performed if a diagnosis of invasive breast cancer is subsequently confirmed.

In patients undergoing breast-conserving surgery, the risk of local recurrence is reduced with adjuvant radiation therapy. Omitting radiation therapy is considered in carefully selected patients with small (<1 cm in diameter), low-grade lesions that have been excised with margins of at least 5 mm and who can be observed diligently for recurrence. New genomic-based multigene assays also hold promise in determining which patients may safely omit radiation. Partial breast irradiation is offered most commonly on a research protocol. Tamoxifen is offered for 5 years to women with estrogen receptor-positive DCIS who do not have a history of venous thromboembolism or stroke.

Posttreatment surveillance for patients treated for DCIS includes annual breast and/or chest wall physical examinations and diagnostic mammograms. All patients with DCIS should be considered for clinical trials.

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Biology and Genetics of Breast Cancer

9

M. Emre Gedik and A. Lale Dogan

Genetics of Breast Cancer

Breast cancer is known as a multifactorial disease, with factors including advanced age, early menarche, obesity, sedimentary lifestyle, late pregnancy, and menopause. All of these factors can trigger the process of carcinogenesis. In addition, family history and the accumulation of genetic aberrations are considered the most prominent and major factors for increasing the risk of breast cancer [1]. Genetic aberrations include point mutations, deletions, amplifications, rearrangements, translocations, and duplications.

Breast cancer susceptibility increases significantly in those with a familial history of breast cancer compared with the general population. This situation can be clarified with susceptibility genes, which play key roles in breast cancer progression [2].

Breast cancer susceptibility can be classified into two forms: hereditary and sporadic [3]. Germline mutations account for 10% of all breast cancers.

Hereditary Mutations in Breast Cancer

Mutations in *BRCA1* and *BRCA2* are the primary hereditary genetic aberrations in breast cancer. These mutations account for approximately half of all hereditary breast cancers. The *BRCA1* and *BRCA2* genes encode large proteins with multiple functions. *BRCA1* is localized on the 17th chromosome and encodes an 1863 amino acid protein with a zinc finger domain. The BRCA1 and BRCA2 proteins are involved in many cellular functions, including the repair of double-stranded DNA breaks for protection of the genome during replication. In short, they act mainly as tumor suppressor gene products. Mutations in *BRCA1* and *BRCA2* and age of cancer onset appear to vary interdependently. *BRCA2* is localized in the 13th chromosome, and in the case of *BRCA2*, the mutation is a secondary major factor for breast cancer predisposition. The occurrence of even one of these mutations can increase the risk of breast cancer to 25% [3–5].

It was noted that the *BRCA1/BRCA2* mutation-carrying breast cancer patients' samples differed from tumors with BRCAness (*BRCAx*), such that the *BRCA* mutation-bearing samples showed a pleomorphic structure, tubular formation, and more aggressive tumor characteristics than non-BRCA tumors [6]. *BRCA-1*-related breast cancers typically occur in younger women and are described as "triple negative" because of the absence of estrogen and progesterone receptors and human epidermal growth factor receptor 2 (HER2). When the wild-type *BRCA1* or *BRCA2* allele is lost, mutated, or silenced, defective DNA repair occasionally occurs. Consequently, additional mutations accumulate during replication, and carcinogenesis is promoted. Poly(adenosine diphosphate-ribose) polymerase-1 (PARP1) is an enzyme involved in single-stranded DNA repair utilizing base excision repair. PARP1 inhibitors hold a promising therapeutic strategy in *BRCA*-defective tumor cells. When PARP inhibition is applied, BRCA-deficient tumor cells are completely devoid of repair mechanisms and undergo cell cycle arrest, genomic instability, and cell death. In the breast cancer model with *BRCA-1* deficiency, endogenous estrogen oxidative metabolites increase the amount of ROS in the tissue and cause various damages to DNA. Strategies that inhibit ROS production reduce the development of DNA lesions [7]. Current treatment approaches for *BRCA* mutant patients include oophorectomy and cisplatin therapy [8].

The *TP53* tumor suppressor gene mutation is another important mutation in breast cancer. Additionally, the *TP53* mutation is the primary symptom of Li-Fraumeni syndrome (LFS). LFS patients are particularly prone to the progression of some cancer types, notably breast cancer. The *TP53* mutation also leads to an increase in the rate of the $HER2^{+}$ breast cancer subtype because of the localization of the *TP53* and *HER2* genes on the same chromosome (17th chromosome). The loss of p53 functions because of

M. E. Gedik · A. L. Dogan (✉)
Department of Basic Oncology, Hacettepe Cancer Institute, Hacettepe University, Ankara, Turkey
e-mail: memre.gedik@hacettepe.edu.tr; laled@hacettepe.edu.tr

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mutational changes may influence HER2-related signal transduction pathways featuring sustainable activity. Because of this phenomenon, cancer cells might display drug-resistant phenotypes [9–12].

Hereditary mutations can be divided into three categories in terms of mutation risk and the frequency of mutation. The first category includes the *BRCA1/BRCA2*, *PTEN*, and *TP53* mutations, which are classified as high-penetrance, low-frequency predisposition genes. The second category includes the *CHEK2*, *ATM*, and *PALB2* genes; these are moderate-penetrance, low-frequency predisposition genes. Finally, the third category consists of the *FGFR2*, *MAP3K1*, and *TGFB1* gene mutations, which are low-penetrance, high-frequency predisposition genes [13, 14] (Fig. 9.1). In a recent multicentric study, it is suggested that the *PHIP* gene located at 6q14.1 might be a breast cancer susceptibility gene [15].

Somatic Genetic Alterations in Breast Cancer

Apart from germline mutations, some gene deletions or amplifications occur as somatic alterations in breast cancer. Among these, the *HER2* (20%), *cyclin D1* (12%), and *WIP1* (13%) gene amplifications and the *PTEN* and *p53* gene deletions frequently occur in breast cancer [13].

In some situations, single-nucleotide polymorphisms (SNPs) may correlate with cancer pathogenesis. For example, the *MDM2* gene, which encodes an important ubiquitin ligase that negatively regulates p53, has two different alleles, T-T and G-G, in the SNP390 intron region. The G-G allele leads to high binding affinity to the ER transcription factor SP-1. As a result, the G allele character on the *MDM2* gene may cause the overexpression of the MDM2 protein, the repression of p53 function, and increased ER expression [13]. Patients with *CCDN1* amplification had a significantly higher risk of recurrence than other patients [16, 17]. Single-nucleotide polymorphism (SNP) in *JAK2, ESR1, NOTCH3, MAP3K1, HCN1*, and *HIF1A* gene regions has been shown to significantly increase breast cancer risk [18, 19].

One consequence of somatic mutations is copy-number alterations (CNAs). The long arm of chromosome 1 is the main region in which amplifications are detected in breast cancer. Some gene amplifications in the long arm of chromosome 16 and some gene deletions in the short arm may be observed in genome-stable tumors. Additionally, there are some gene amplifications that occur in genome-unstable tumors, concerning the 8th chromosome and the 17th chromosome in which the *MYC* and *ERBB2* genes are localized on, respectively. Furthermore, genome-unstable tumors show a poor prognosis compared to genome-stable tumors. Because of that, in luminal A, luminal B, and normal-like breast cancer subtypes, disease-free survival (DFS) rates are higher than those in basal-like and HER2⁺ breast cancer subtypes [20] (Fig. 9.2).

Mitochondrial DNA polymorphism is implicated in the development of tumor formation and metastatic spread differences encountered in breast cancer development [21, 22]. It is also noted that metabolic pathway differences play an important role in the formation of different tumorogenic characters [23]. The increase of the metabolic enzyme phosphoglycerate dehydrogenase (PHGDH) in cancer cells leads to impaired epigenetic regulation. The expression of various methyltransferase enzymes is increased through PHGDH activity. Overexpression of methyltransferase increases invasion and migration of cancer cells [24]. In this context, it is stated that metformin used in the treatment of diabetes can be also used in the treatment of breast cancer [25, 26].

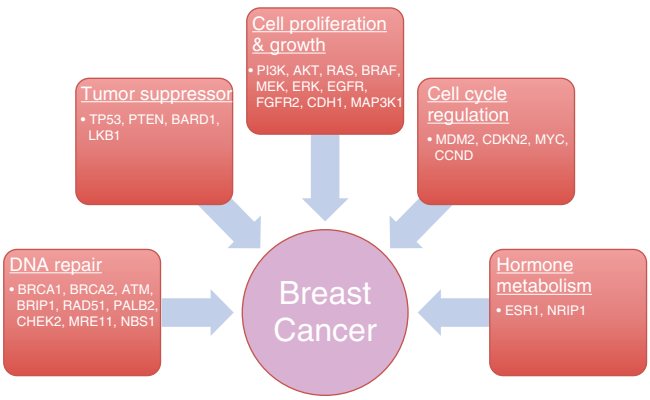

Fig. 9.1 Mechanisms and related genes involved in breast cancer susceptibility

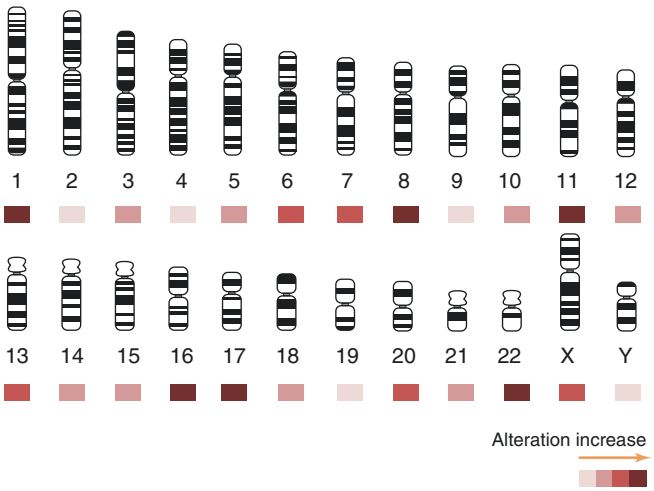

Fig. 9.2 Copy-number alterations in breast cancer

Breast Cancer Epigenetics

In higher eukaryotes, DNA methylation and chromatin modifications are essential epigenetic mechanisms that operate in association with genetic mechanisms such as replication and transcription. In many cancers, including breast cancer, tumor cells have altered patterns of methylation and histone modification, resulting in transcriptional deregulation in favor of oncogenesis. DNA methyltransferases catalyze methyl donor transfer from S-adenosylmethionine to cytosine bases, which undergo methylation. Cytosine-phosphate-guanine (CpG) dinucleotide sequences frequently localize at gene promoter regions, where transcription initiates. Thus, DNA methylation at the promoter proximal CpG sequences, called CpG islands, is associated with gene silencing. In tumor cells, oncogenes undergo hypomethylation, whereas tumor suppressor genes are hypermethylated. In active chromatin, unmethylated promoter regions can be transcribed by means of transcriptional machinery composed of transcription factors, co-activators with histone acetyltransferase (HAT) activity, and acetylated histones H3, H4, and H2A and histone H3, which is methylated mainly at lysine residue K4 (H3-lysine 4). In heterochromatin, also called silent chromatin, transcription of the methylated regions is blocked by methylcytosine-binding protein; histone H3 is methylated at the lysine residues K9, K27, and K79 along with corepressors with histone deacetylase (HDAC) activity. Furthermore, there are certain differences between epigenetic alterations and mutations; for example, mutations are irreversible changes, whereas epigenetic alterations are reversible. According to Knudson's two-hit hypothesis, the first hit is developed by epigenetic dysregulation and the second hit is the result of somatic mutations in nonhereditary tumors. Carcinogenic processes consequently evolve [27–29].

RASSF1A is a tumor suppressor gene that encodes the Ras association domain-containing protein 1. In breast cancer, the deletion or altered patterns of expression of this gene appear to be related to pathogenesis. Functional studies documented decreasing levels of histone H3-lysine 4, whereas levels of histone H3-lysine 9 increased in tumor cells, resulting in *RASSF1A* silencing via epigenetic mechanisms. Previously, it was noted that the silencing of *RASSF1A* was associated with the overactivation of the *RAS* signaling pathway. As a result, tumor cells divide and proliferate in an uncontrolled fashion [30]. *BRCA1*, *E-cadherin*, *TMS1*, *ER, RUNX3, and CHL1* are among the other genes that are hypermethylated in breast cancer [31–33].

Tumor cells in breast cancer subtypes that develop as a result of ER, PR, and androgen receptor hypermethylation have a more aggressive character and are inadequate for steroid hormone therapies, thus leading to a decrease in the rate of disease-free survival (DFS) [34] (Fig. 9.3).

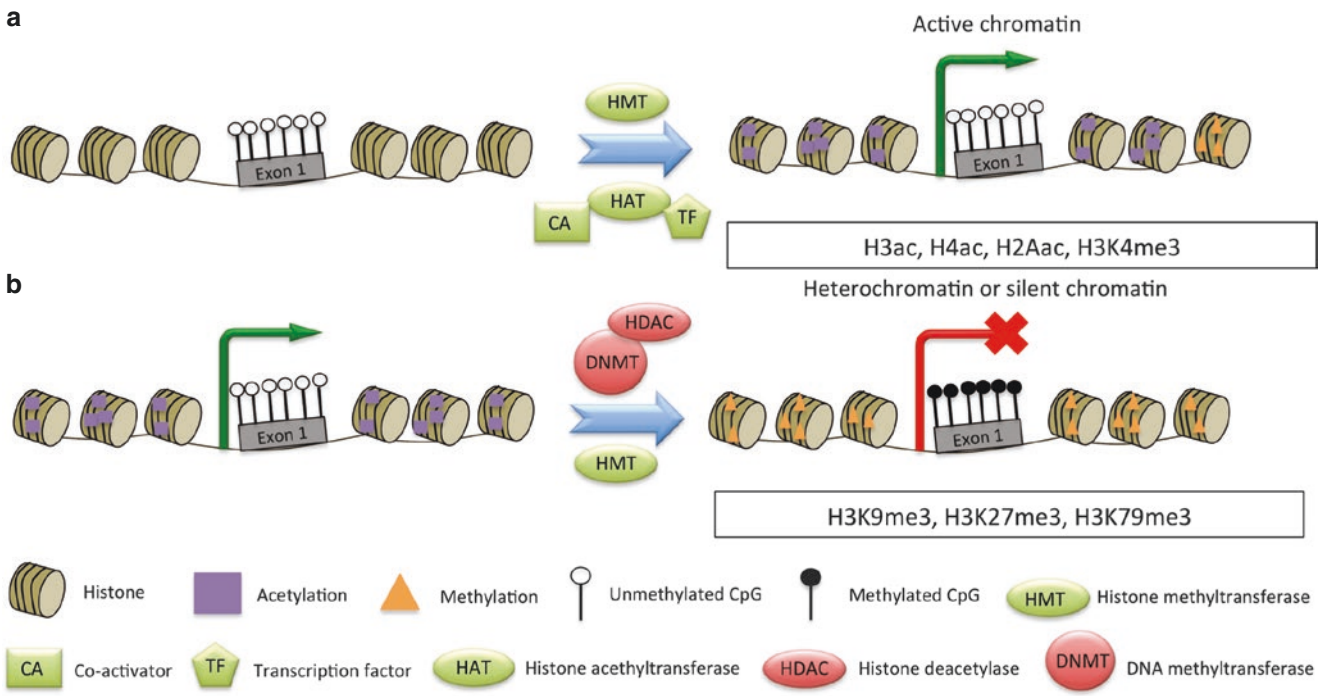

Fig. 9.3 Chromatin modification by epigenetic mechanisms. (**a**) In active chromatin, histone acetyltransferase (*HAT*) mediates the acetylation of H2A, H3, and H4, and histone methyltransferase (*HMT*) mediates the trimethylation of lysine residue K4 at histone H3 (H3K4me3), leading to the accessibility of the unmethylated promoter region to the co-activator protein (*CA*) and the transcription factor (*TF*). (**b**) Epigenetic gene inactivation by CpG island methylation by DNA methyltransferase (*DNMT*) is associated with histone modifications involving the trimethylation of lysine residue K27 (H3K27me3), lysine residue K9, or lysine residue K79 at histone H3

Several epigenetic regulators such as EZH2, KDM5A, and KMT2D have been implicated to develop therapeutic resistance in ER-positive tumors. Targeting KMT2D histone methyltransferase leads to decrease in treatment resistance by means of posttranslational modification of ER activation [35]. Similarly, it is indicated that the MYST3 histone acetyltransferase regulates ER alpha activation as a prominent epigenetic regulator [36].

Current studies indicated that ductal carcinoma cells, which have different histologic feature but same somatic mutation, transformed into metaplastic carcinoma cells by means of epigenetic and noncoding gene expression changes [37].

It is important to evaluate gene expression profiling together with epigenetic modifications such as copy-number alteration (CNA), histone modification, DNA methylation, and miRNA expressions for determining tumor phenotype. A more appropriate therapeutic approach can be applied at this point [38–40].

Molecular Portraits of Breast Cancer

PIK3CA, *TP53*, *GATA3*, *CDH1*, *RB1*, *MLL3*, *MAP3K1*, and *CDKN1B* gene mutations are frequently encountered in breast cancer. Although *PIK3CA* mutations are encountered at a rate of 41.3%, *TP53* mutations are encountered at a rate of 16.1%. *TP53* mutations are associated with the high histological grade luminal B breast cancer subtype, whereas *MAP3K1* mutations are related to luminal A breast cancer with a low histological grade. Expression of *Ki-67*, a proliferation marker, in tumor cells may result in resistance against aromatase inhibitors in advanced-stage tumors [41, 42].

Breast cancer is a heterogeneous cancer type in terms of both molecular and clinical characteristics. Even if individuals have similar clinicopathological symptoms, the disease may evolve, resulting in differing outcomes. In this respect, the classification of immunohistochemical staining may have limited utility for evaluation [43].

Investigations using pathological tissues have made it possible to develop immunohistochemical-staining guidelines for classifying tumor cell characteristics. This classification is built upon the basis of ER (estrogen receptor), PR (progesterone receptor), and HER2 (human epidermal growth factor receptor) expression. Positive expression of hormone receptors occurs at a rate of 60–65% in all breast cancer cases, and these cancers can be treated properly with hormone-based therapeutics. In breast cancer, the rate of HER2 positivity is approximately 15–20%; this group is selectively treated with HER2-targeted agents. The remaining 15–20% of breast cancers lack hormone receptors and HER2 and are called triple-negative tumors [44, 45].

Microarray-based assays offer opportunity and resolution in understanding the molecular and genetic features of breast cancer. Perou and colleagues' work suggests five different breast cancer subtypes at the molecular level (molecular portraits) based on gene expression patterns. Among the subtypes they defined were the luminal A and luminal B tumor types, $HER2^+$ tumors, basal-like cancer (triple-negative breast cancer (TNBC)), and normal-like subtypes. The luminal A and luminal B subtypes are typically hormone receptor (ER or PR) positive and have a good prognosis with a 5-year survival rate of 80–85%. Furthermore, hormone receptor-positive breast cancer patients carry *PIK3CA* mutations at a rate of 40%. Despite coexisting *PIK3CA* mutations and the consequent sustained PI3K signaling, these cells show low mTORC1 activity. Basal-like or triple-negative breast cancer subtypes lack ER, PR, and HER2, whereas they express *CK5/6* (cytokeratin 5/6) and *EGFR*. Generally, these subtypes are associated with sustained PI3K activity, *PTEN* deletion, and *BRCA1* and *TP53* mutations, and they ultimately have a poor prognosis. It should be noted that not all basal-like tumors are triple negative and vice versa. $HER2^+$ breast cancer is an aggressive phenotype with a poor prognosis [46, 47].

In one study, Prat and colleagues defined a new molecular-based breast cancer subtype. This subtype is called claudin-low and has low expression of luminal markers such as *ER*, *PR*, *GATA3*, *keratin 18*, and *HER2*; it also shows variable expression of basal markers such as keratins 5, 14, and 17. Claudin-low breast cancer shows little or no expression of epithelial markers such as E-cadherin and claudin 3, making it different from basal-like breast cancer subtypes. The claudin-low tumor cell phenotype can be described as being similar to the mesenchymal phenotype; in other words, it has similar tumor-initiating cell (TIC) characteristics [48, 49].

Microarray-based gene expression profile analyses provide innovative and distinctive data that are beneficial to understand the heterogeneity of the structure of breast cancer. The Oncotype DX 21 gene analysis assay was the first approved assay for clinical use [50]. This assay is useful for determining risk factors of early-stage ER^+, LN^- tumors [51]. The MammaPrint 70 gene analysis assay is another assay for clinical guidance and is useful for determining tumor proliferation and genomic grades of early-stage ER^+ tumors [52, 53]. Furthermore, this assay can be used to ensure prognostic disparity between LN^+ and LN^- tumors. For other assays, such as the 8-gene recurrence score [54], the 14-gene metastatic score, and the 158-gene assay, preclinical studies are ongoing. The 50-gene analysis, which is currently called the PAM50 assay, targets all types of breast cancer and provides the advantage of intrinsic classification as indicated. Although this new assay is not yet applicable to clinics, preclinical studies are ongoing [2, 43, 55, 56].

The implementation of the 21-gene assay data on ER+ breast cancer patients treated with tamoxifen indicates that the 10-year disease-free survival rate is 96.8% for patients with a low recurrence score, 90.9% for patients with a mild recurrence score, and 60.5% for patients with a high recurrence score, with a mean value of 87.8%. Moreover, in ER+ breast cancer patients treated with tamoxifen plus chemotherapy, this assay indicates that the 10-year disease-free survival rate is 95.6% for patients with a low recurrence score, 89.1% for patients with a mild recurrence score, and 88.1% for patients with a high recurrence score; the mean value is 92.2% [57].

In particular, the proliferation rate is a prognostic factor in the luminal subtype of breast cancer. Concordantly, amplifications of *FGD5*, *METTL6*, *DTX3*, *MRPS23, and CKAP2* are well-known prognostic markers. For example, *FGD5* regulates the proangiogenic function of *VEGF* and increases cell proliferation. Among the others, *DTX3* promotes the Notch signaling pathway, *MRPS23* regulates proliferation and oxidative phosphorylation, and *METTL6* is related to drug sensitivity [58, 59]. Ligand-independent pathway activation occurs in ER-positive breast cancer cells with the *ESR1* gene mutation. Furthermore, it is stated that ESR1 chromosomal translocation-bearing breast cancer patients develop resistance to treatment. In that case, targeting the Notch signaling pathway in *ESR* mutant patients is recommended [60–63].

HER2+ breast cancer subtypes are characterized by poor prognosis and different outcomes from systemic chemotherapy. In advanced-stage tumors, PI3K activation is usually deregulated. HER2+ tumors are classified in three different clusters according to ER and LN expression and histological grades performed by 158-gene assay studies. If lymphatic infiltration occurs in early-stage HER2+ tumors, it is associated with a good prognosis. In contrast, low lymphatic infiltration shows that the tumors have a more aggressive phenotype. In addition, studies have indicated that low lymphatic infiltration was associated with increasing PI3K signaling pathway activation and *IGFR1* expression. In HER2+ tumors, *CXCR4*, *PLAU*, *CXCR1*, *TGFBR3*, and *STAT5A* gene expression levels are distinctive markers for evaluating tumor invasion and metastasis [64].

Recently, clarifying mechanisms of acquired resistance to HER2-targeted therapies has been of interest. One of the possible resistance mechanisms is the truncation of the HER2 receptors from the extracellular region; the resultant HER2 becomes a new variant called p95HER2. Thus, the HER2 antibody could not bind and recognize the HER2 receptors. Preclinical studies performed on cell lines show that this mechanism is based on specific HER2-mediated molecules, including *PTEN*, *PI3K*, *mTOR*, *MAPK*, and *VEGF*. However, studies performed on tissue samples show that the *AKT*, *IGFR1*, *p27 Kip1*, and *MUC4* genes are deregulated; in particular, the *PTPN11* gene shows the most alteration after HER2-targeted therapies. In short, resistance mechanisms might also be associated with changes in the expression patterns of these genes [65–67].

Basal-like tumors phenotypically resemble normal basal/myoepithelial breast cells. Basal-like breast cancer cells express basal cytokeratins (5/6, 14, and 17), p-cadherin, and caveolin 1 and have patterns of hormone receptor and HER2 expression similar to those in normal breast cells. Additionally, basal-like breast cancer cells have *TP53* mutations and high expression levels of genes associated with proliferation. Furthermore, studies have reported that the *BRCA1* mutation is associated with this type of cancer [68].

Triple-negative breast cancer lacks hormone receptors and HER2. This type of cancer does not express basal markers and expresses basal prognostic markers less than basal-like tumors. Studies performed with the 14-gene assay show that high levels of aberration occur in the expression levels of *CLIC5*, *MATN1*, and *RPS28* in TNBC cells. Interestingly, high expression of the *IR-7*, *STAT1*, and *IFN* genes is associated with a good prognosis [69]. It is stated that Del-1 mutation in triple-negative breast cancer (TNBC) is associated with poor prognosis [70]. Additionally, molecules such as *KDM4, EVI1*, and *FOXK2,* which play critical role in the metastatic process, are important prognostic markers in TNBC [71–73].

According to 386-gene assay studies, the TNBC subtype can be classified into seven different clusters. These clusters consist of basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal-stem cell-like (MSL), luminal androgen receptor (LAR), and unstable (UNS). BL1 and BL2 clusters are characterized by genes associated with proliferation and the cell cycle. The *AURKA*, *AURKB*, *CENPA*, *CENPF*, *BUB1*, *TTK*, *CCNA2*, *PRC1*, *MYC*, *NRAS*, *PLK1*, and *BIRC5* genes are highly expressed in BL1 and BL2 tumors. Additionally, *EGFR*, *NGF*, *MET*, *Wnt/beta-catenin*, and *IGFR1* gene expression and their associated signaling pathways are activated, particularly in these clusters. The gene ontologies of the IM cluster include the overexpression/activation of TH1/TH2, NK cells, dendritic cells, B and T cell receptor immune cell signaling components, cytokine signaling, IL-12 and IL-17 cytokine signaling components, NF-κB, TNF, and JAK/STAT, which are standard immune response signaling transduction pathways and antigen presentation signal transduction pathways. M and MSL clusters are important in terms of motility and have distinctively aggressive characters compared to the other clusters. Signaling pathways associated with the actin regulatory protein Rho, the extracellular matrix (ECM) receptor interaction, Wnt, anaplastic lymphoma kinase (ALK), and TGF-β are factors that ensure cell motility. These pathways are also characteristic of the EMT marker, stem cell, and

Table 9.1 Molecular subtypes of breast cancer

Intrinsic subtypes by gene expression profiling	ER (IHC)	HER2 (IHC/ISH)	Ki-67 status (IHC)	Key molecular features [48]	Predominant integrated cluster association [43, 44]
Luminal A	ER+	HER2-	Low	*PIK3CA mutations, MAP3K1 mutations, ESR1 high expression, GATA3 mutations, FOXA1 mutations, quiet genomes: gain 1q, 8q, loss of 8p, 16q*	Int cluster 2, Int cluster 3, Int cluster 4, Int cluster 7, Int cluster 8
Luminal B	ER+	HER2±	High	*TP53 mutations, PIK3CA mutations, cyclin D1 amplification, MDM2 amplification, ATM loss, enhanced genomic instability, focal amplifications* (e.g., *8p12, 11q13)*	Int cluster 1, Int cluster 2, Int cluster 6, Int cluster 9
HER2	ER-	HER2+	High	*HER2 amplification, TP53 mutations, PIK3CA mutations, FGFR4 high expression, EGFR high expression, APOBEC mutations, cyclin D1 amplification, high genomic instability*	Int cluster 5
Basal-like	ER-	HER2-	High	*TP53 mutations, RB1 loss, BRCA1 loss, high expression of DNA repair proteins, FOXM1 activation, high genomic instability, focal amplifications* (e.g., *8q24)*	Int cluster 4, Int cluster 10
Claudin-low	ER-	HER2-	Low	*BRCA1 mutations, low expression of luminal markers, variable expression of keratins 5, 14, 17, little or no expression of E-cadherin and claudin 3*	Int cluster 4

+ positive, − negative, *ER* estrogen receptor, *HER2* human epidermal growth factor receptor, *IHC* immunohistochemistry, *ISH* in situ hybridization

claudin-low type of breast cancer. Although the LAR cluster lacks ER, this cluster contains ample hormone regulation pathways for steroid synthesis, porphyrin metabolism, and androgen/estrogen metabolisms. Luminal character markers such as *FOXA1*, *KRT18*, and *XBP1* are the most highly expressed genes in the LAR cluster. The LAR cluster constitutes 11% of TNBC breast cancer [74].

Although TNBC clusters show different clonal expansion patterns, somatic mutations of *TP53*, *PIK3CA*, and *PTEN* are encountered in all these clusters as a common trait [75, 76].

Immunohistochemical staining is essential for the diagnosis of breast cancer. However, because of tumor heterogeneity, immunohistochemical evaluation may not confer all the requirements for the molecular assessment of disease. It is possible to resolve copy-number alterations (CNAs) of breast cancer molecular portraits by array comparative genomic hybridization (aCGH) techniques. By this means, some subtypes can be characterized. Mutated genes can be classified using next-generation sequence technology. The results of genetic analyses in the whole genome provide single-nucleotide polymorphisms (SNPs) and copy-number variants (CNVs) for hereditary profiles and provide single-nucleotide variants (SNVs) and copy-number aberrations (CNAs) for somatic aberration profiles. In recent studies, breast cancer subtypes are classified into ten different integrated clusters by determining the genetic profile of the somatic copy-number alterations. The only common point of ten different integrated clusters is the high expression of mutated *PIK3CA* and *TP53* genes [43].

In the future, further analysis of next-generation sequencing data will improve the understanding of tumor heterogeneity, leading to deciding adequate therapy based on personal genomic breast cancer profiles and providing a more effective prevention of cancer [77].

Mechanisms that are involved in breast cancer susceptibility are presented in Table 9.1.

In a previous study, it was noted that in tumors in which the expression of 97 genes was homogeneous and histological grade 3 was common, the overexpression of *UBEC2C*, *KPNA2*, *TPX2*, *FOXM1*, *STK6*, *CCNA2*, *BIRC5*, and *MYBL2* genes was concluded to be responsible for cell cycle progression and proliferation. However, grade 1 tumors differ from histological grade 3 samples in that they show a stable gene expression profile, whereas grade 2 tumors have heterogeneity in their gene expression profiles [78].

Driver mutations are the main mutations for carcinogenesis, and they provide a clonal selectivity advantage to cancer cells. Then, passenger mutations are triggered and help accelerate carcinogenesis. Previous studies have reported that *AKT1*, *BRCA1*, *CDH1*, *GATA3*, *PIK3CA*, *RB1*, *PTEN*, and *TP53* mutations are driver mutations, and recent studies have added new driver gene mutations to this group, including *AKT2*, *ARID1B*, *CASP8*, *CDKN1B*, *MAP3K1*, *MAP3K13*, *NCOR1*, *SMARCD1*, and *TBX3*. *MAP3K1* and *MAP3K13* gene mutations result in deregulation of the ERK/MAPK signaling pathway. The *AKT* signaling pathway shows a sustained increase in activity in the presence of the *AKT2* mutation. Chromatin regulation is lost by means of *NCOR1*, *SMARCD1*, and *ARID1B* mutations. The *CDKN1B* mutation deregulates the cell cycle and proliferation. In the presence of the *CASP8* mutation, the apoptosis rate decreases. The *TBX3* mutation may affect tissue morphology, but the exact mechanism remains to be clearly defined [79].

Breast cancer is divided into two different classes based on pathological form: invasive ductal carcinoma (IDC) and invasive lobular carcinoma. Analyzing the differences among these classes, the overexpression of *ERBB2*, *JAK2*, *ANKRD32*, and *NRGN* is said to be a prognostic factor for the IDC breast cancer class, and the overexpression of *VWF*, *ELN*, *DPT*, *EMCN*, *FABP4*, *CAV1*, *ADIPOG*, and *ALDH1A1* is reported as a prognostic factor for invasive lobular carcinoma [80].

The interpretation of and the lack of combined clinical and molecular data constitute a serious puzzle for molecular classification in current clustering methods. There is a new clustering method that holds more promise for combining clinical and molecular data. This methodological algorithm presents the results of combining data and is called Molecular Regularized Consensus Patient Stratification (MRCPS). Notably, the *GATA3* gene has been shown to have a major association with other deregulated genes, and mutation of *GATA3* is directly associated with the estrogen receptor [81].

The comparison of genomic profile with proteinase levels plays an important role in the early diagnosis of breast cancer. Proteinases are dictated as important biomarkers for characterization of tumor and response to treatment [82]. Recent studies have pointed out that the combination of genomic and proteomic datasets would be more effective for personalized treatment [83, 84].

In the metastatic mouse model, the integration of genetic, transcriptional epigenetic, and gene expression profiles combined with computational biology tools has been profiled to identify possible genetic genes responsible for metastatic breast cancer development. These gene cluster expressions were compared with the clinical correlations of breast cancer patients. It was aimed to be determining early and late relapse during prognosis of the patient by translating the obtained data [85]. In this context, kinome datasets that assess for protein kinases, metabolomic datasets comparing metabolic deregulations, clusternomic datasets for investigation of heterogeneous gene clusters, and methylomic datasets for recognizing epigenetic regulations were created [86–89]. Bioinformatic tools such as GRAPE and DIRAC are used for this purpose [90, 91].

MicroRNA Signatures in Breast Cancer

MicroRNAs (miRNAs) are small RNAs of approximately 18–25 nucleotides and are endogenously expressed in cells. miRNAs can silence the expression of target genes via repressing translation or leading to the degradation of target mRNAs [92]. Thus, miRNAs modulate various cellular functions including proliferation, apoptosis, and angiogenesis.

Different tissue types have unique expression levels of specific miRNAs. Accordingly, each tumor type appears to have a miRNA signature with aberrant expression [93, 94] (Fig. 9.4).

It is widely accepted that discrepancies among reported miRNA signatures may arise from intrinsic heterogeneity present in breast cancer tumors. Tumor stage, receptor status (HER2, ER, PR), and vascular invasion may contribute to variability [93, 95]. The potential role of miRNAs as a tool for the diagnosis, classification, and treatment of breast cancer is currently under investigation [96–100]. miRNAs may act as oncogenes or tumor suppressors through regulating the mechanisms of proliferation, migration, invasion, metastasis, and apoptosis [101–121]. Table 9.2 presents an illustrative list of regulated miRNAs in breast cancer in association with related pathways.

Long noncoding RNAs (lncRNAs) are gene-specific gene regulators longer than 200 nucleotides [122]. New generation targeted therapies take into account the microRNA and lncRNA expression in breast cancer. The suppression of oncomiRs by therapeutic agents or restoration of tumor suppressor miRNAs by using miRNA mimetics and the use of modified miRNAs are among the miR-mediated therapeutic approaches [123–125]. Targeting lncRNAs such as HOTAIR, SPYR4-IT1, MALAT1, GAS5, and PANDAR, which play considerable role in tumor development, is another current treatment approach [126–128]. On the contrary, low expression of tumor suppressor lncRNAs, such as MEG3 and ANCR, induces metastasis and proliferation in breast cancer [129, 130].

Biology of Breast Cancer

Estrogen Receptors and Breast Cancer

The estrogen receptor (ER) is a steroid hormone receptor that resides in the cytoplasm and participates in cell proliferation, survival, and invasion in ER^+ breast cancer. The binding of estrogen is essential for translocation of the ER from the cytoplasm to the nucleus, where estrogen-bound ER dimerizes and binds to the estrogen response elements of target genes for the activation of gene expression. ER, as a transcription factor, also interacts with coregulatory proteins and other transcription factors. In addition, another form of ER, a membrane-bound or cytoplasmic protein, has nongenomic action. In short, activation of the receptors triggers phosphorylation and the activation of several receptors and signal proteins such as epidermal growth factor receptors, insulin-like growth factor-1R (IGF-1R), Src kinase, Shc adaptor protein, and phosphatidylinositol 3-kinase (PI3K). Thus, there is cross talk among growth factor receptors and estrogen receptor signaling that may contribute to resistance to antiestrogens. ER signaling may be targeted by two methods of inhibition. The common method is binding to the

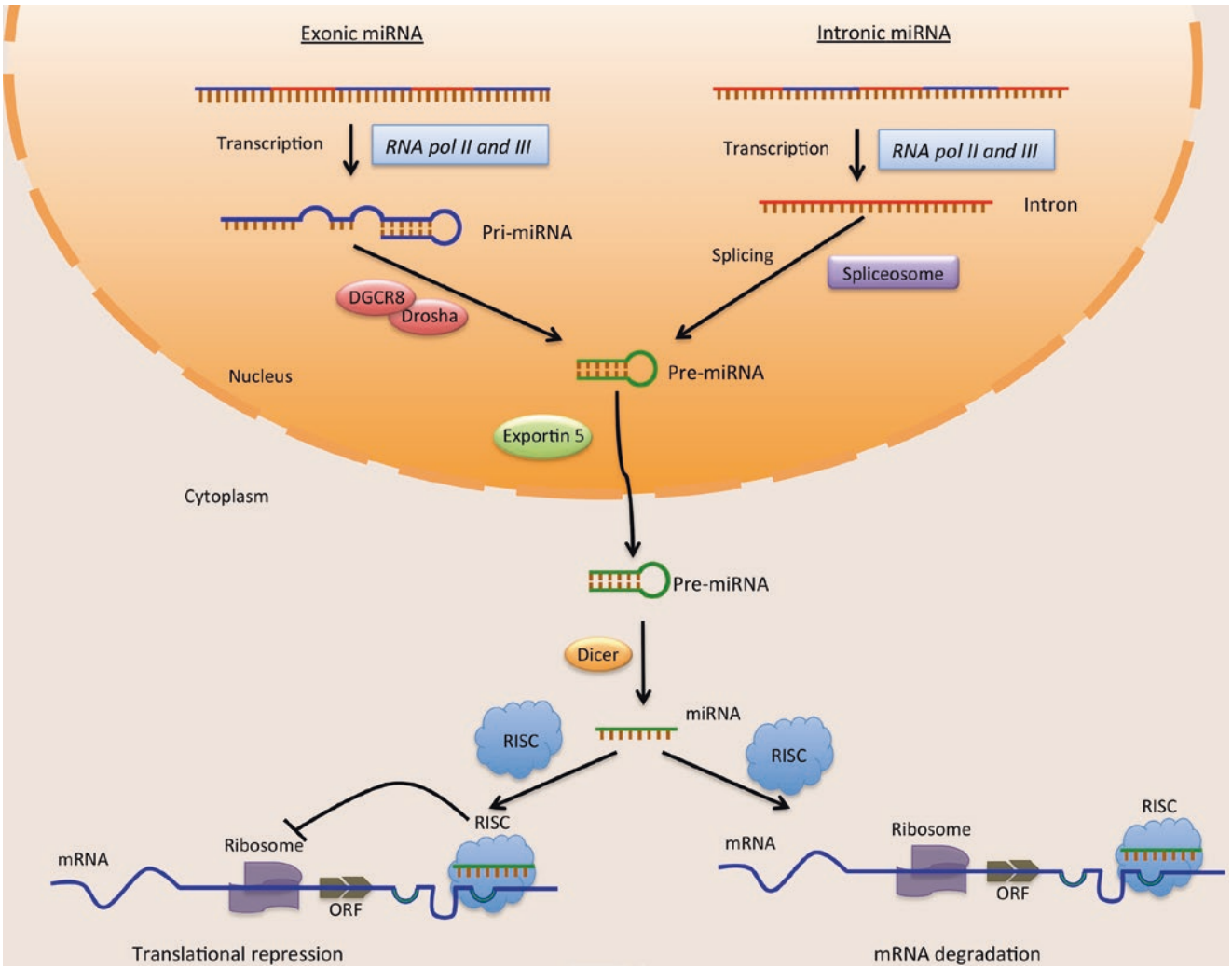

Fig. 9.4 Mechanisms of miRNA expression. MicroRNA (*miRNA*) is transcribed mainly by RNA polymerase (*pol*) II. RNA pol III is also involved in transcription. The pri-miRNA is spliced by Drosha and the DGCR8 enzymatic complex, which leads to pre-miRNA formation. Pre-miRNA is exported into the cytoplasm by the cargo protein exportin-5, and mature miRNA is obtained through cleavage by Dicer. This mature miRNA binds to messenger RNA (*mRNA*), leading to the degradation or blockage of the translation of mRNA. The RNA-induced silencing complex (*RISC*), which is a multi-protein complex, uses miRNA as a template for recognizing complementary mRNA. *ORF* open reading frame

estrogen receptor, e.g., tamoxifen, and the other strategy involves decreasing estrogen production via aromatase inhibitors [131].

The risk of developing resistance to hormone therapy is dramatically increased in patients with high AR and ER level [132, 133]. Current treatment approaches include targeting tumor microenvironment, hormone receptor-related pathways, and proteins in hormone receptor-positive tumors [134–138]. ER alpha-positive tumors can develop resistance to endocrine therapy and chemotherapy where ER beta agonists increase p53 tumor suppressor activity; thus ER beta-positive tumors may be susceptible to therapy [139, 140]. Furthermore, pharmacological activation of ER beta has been shown to suppress metastasis by enhancing the natural immunity [141]. It is stated that, aurora kinase A is positively correlated with HER2 expression and low mean survival in ER-positive cancers [142]. In this case, PI3K inhibition may be a new target for endocrine resistance [143]. RBP2 protein has been shown to develop resistance to tamoxifen by performing several RTK-mediated signaling pathway activations in ER-positive breast cancer tumors [144]. Proteosome inhibitors are novel therapeutic agents that prevent cross talk between ER and HER2 receptors, and consequently continuous activation of intracellular signaling pathways is inhibited [145].

Table 9.2 Some of the regulated miRNAs and their targets in breast cancer

miRNA	Tumor expression level	Validated targets	Pathways
miR-21	Up	*BCL2, TPM1, PDCD4, PTEN, MASPIN, RHOB, MMP3*	Apoptosis, invasion, metastasis
miR-125b	Down	*BAK, HER2, CRAF, MUC1, ERA, RTKN*	Proliferation, apoptosis, migration
miR-155	Up	*FOXO3A, SOCS1, RHOA*	Proliferation, TGF-β signaling
miR-145	Down	*MUC1, ERA, RTKN*	Proliferation, apoptosis, invasion
miR-210	Up	*MNT, RAD52*	Hypoxia
miR-29c	Up	*B7-H3*	Metastasis, invasion
miR-100	Down	*IGF2, β-tubulin*	Proliferation, apoptosis
miR-10b	Down	*TIAM, HOXD10*	Migration, invasion, metastasis
let-7a-2	Down	*RAS, CCR7*	Proliferation, migration, invasion
miR-205	Down	*HER3, VEGFA, EMT*	Proliferation, invasion
miR-125b	Down	*ERBB2, ERBB3, EPOR, ENPEP, CK2-α*	Proliferation, apoptosis
miR-196a	Up	*ANXA1, SPRED1*	Proliferation, apoptosis
miR-497	Down	*BCL2, IGF-1R, cyclin E1, RAF-1*	Proliferation, apoptosis, invasion, metastasis
miR-181	Up	*ATM*	Proliferation, migration, invasion, TGF-β signaling
miR-31	Down/up	*ITGA5, RDX, RHOA*	Metastasis
miR-143	Down	*ERK5, KRAS*	Proliferation, MAPK signaling
miR-191	Up	*BDNF, CDK6, SATB1*	Proliferation, migration
miR-203	Down/up	*SNAI2, BIRC5, LASP1*	Proliferation, invasion, apoptosis, migration
miR-29b	Up	*PI3K, CDC42, PTEN*	Proliferation, migration, invasion, TGF-β signaling
miR-93	Up	*NRF2, LATS2, STAT3*	Proliferation, invasion, metastasis, migration
miR-130b	Down/up	*CCNG2*	Proliferation
miR-455	Down	*CDK14*	Proliferation
miR-24	Up	*HIF1α, FIH1*	Hypoxia, chemotherapy resistance
miR-27a	Up	*SFRP1*	Proliferation, invasion, migration
miR-34a	Up/down	*TWIST1, SLUG, ZEB1/2*	Invasion, migration
miR-137	Up	*BMP7*	Invasion, migration
miR-299-3p	Down	*Oct4*	Apoptosis, invasion
miR-217	Down	*KLF5*	Proliferation, invasion, migration
miR-193a	Down	*WT1*	Proliferation, metastasis

Growth Factor Receptors in Breast Cancer

Growth factors and receptor tyrosine kinases (RTKs) are essential for cell proliferation and survival. The human epidermal growth factor receptor (HER) family consists of four members: EGFR/ErbB1, HER2/ErbB2, HER3/ErbB3, and HER4/ErbB4. In breast cancer, the HER family has been studied extensively. The common structure of HER proteins consists of an extracellular domain for ligand binding, a transmembrane domain, and a cytoplasmic catalytic kinase domain that drives downstream signaling pathways. The main pathways are the PI3K/Akt and Ras/Raf/MEK/MAPK signaling pathways. The growth factor receptor pathways may be constitutively activated as a result of a few aberrations, including the overproduction of ligands, gain-of-function mutations, overexpression and/or gene amplification, and gene rearrangement. Constitutive signal activation leads to oncogenic signaling and resultant uncontrolled proliferation, survival, invasion, and metastasis processes, which are drivers of carcinogenesis. Receptor tyrosine kinases are activated by specific ligands, and activation is followed by homo- or heterodimerization of the receptors [146–149].

The HER2/neu gene is localized on chromosome 17 and encodes a transmembrane tyrosine kinase growth factor receptor that has no ligand-binding domain. HER2/neu gene amplification occurs in 30% of breast cancers. Approximately 100,000 HER2 receptors exist on a normal cell surface, whereas this number reaches 2 million on a breast cancer cell [149, 150]. High levels of aneuploidy, somatic mutations such as the TP53 mutation, FGFR (fibroblast growth factor receptor), EGFR, CDK4 (cyclin-dependent kinase 4), and cyclin D1 amplifications frequently coexist in $HER2^+$ breast cancer [66]. HER2 overexpression ultimately triggers breast and over cancer progression with a poor prognosis. HER2 is

the preferred partner for dimerization with HER1, HER3, and HER4. HER3 lacks a kinase domain. Although ATP binds to the kinase domain of HER3, the phospho-transfer reaction is not driven catalytically. Additionally, HER3 has six phospho-tyrosine residues at the C-terminal that are available for PI3K binding. Thus, HER3 activation by HER2 recruits the PI3K regulatory subunit to the membrane, which initiates signaling [151]. Accordingly, the HER2-HER3 heterodimer is known to be the most potent oncogenic unit in breast cancer [152–154]. As a consequence of mutations in the extracellular domain of HER3, the oncogenic potential of the heterodimer may increase [154]. HER2 overexpression ultimately activates ligand-independent HER2/HER3/PI3K complex formation and kinase activity in tumor cells, whereas trastuzumab, a monoclonal antibody targeting HER2, destabilizes the complex. Additionally, gain-of-function mutations of PI3K and the loss of PTEN may attenuate the effectiveness of the drug. Resistance to trastuzumab can be circumvented through PI3K inhibition [10, 66]. The activation of alternate receptors, such as IGF-1R and c-met, may also lead to resistance to trastuzumab. Targeting HER2 and EGFR simultaneously may have a promise for therapeutic synergy. Tyrosine kinase inhibitors are small-molecule inhibitors that target the cytoplasmic kinase domain of growth factor receptors. Lapatinib, for example, targets HER2 and EGFR simultaneously in breast cancer [155].

Insulin-like growth factors (IGF-1 and IGF-2) and their receptor IGF-1R expression levels have been associated with breast cancer. The upregulation of IGF-1R results in sustained activation of the PI3K/Akt pathway, thereby leading to resistance to HER2-targeted therapy and antiestrogens.

PI3K/Akt/mTOR Signaling Pathway in Breast Cancer

The PI3K/Akt/mTOR pathway is the central signaling mechanism downstream of various RTKs (Fig. 9.5). Activating mutations of *Ras* and *PIK3CA*, the gene encoding the catalytic subunit of PI3K, may lead to aberrant signaling that may be independent of ligands binding to the growth factor receptor [10]. However, the inactivation of the PTEN tumor suppressor gene via mutation and/or deletion is widely detected in various types of tumors, including breast cancer. The loss of PTEN causes phosphatidylinositol-3,4,5-trisphosphate (PIP_3) accumulation, thereby activating the PI3K/Akt pathway [156].

In p110α, the catalytic subunit of PI3K, mutations are observed at a rate of 20–25% in breast cancer. E542K, E545K, and H1057R mutations are common, and PI3K gains sustained oncogenic activity with no need for RTK activation. The *PIK3CA* mutation may be associated with estrogen and progesterone receptor (ER/PR) status, lymph node metastasis, and ErbB2 overexpression in breast cancer with ultimately coexisting PTEN loss [157]. Activating mutations of genes encoding Akt kinases (*Akt1*, *Akt2*, and *Akt3*) are rare in breast cancer. The activation of Akt leads to the inhibition of FOXO transcription factors via the translocation of these factors to the cytosol. The PI3K/Akt pathway activates the downstream mammalian target of rapamycin complex 1 (mTORC1), thereby activating translation and cell growth. Additionally, the Ras/Raf/MEK pathway may be targeted in combinational therapy strategies.

The PI3K/Akt pathway plays crucial roles both in breast carcinogenesis and in targeted therapy. Thus, advances in our knowledge of agents and/or combinations targeting the pathway in the clinic hold promise for the near future [158] (Fig. 9.5).

Hypoxia and Breast Cancer

The dysregulated growth of tumor cells leads to decreased O_2 availability in the tumor mass, which triggers angiogenesis. However, the blood vessels that form in tumoral tissues are abnormal because of distorted vasculature, and breast cancers ultimately contain intratumoral hypoxic regions that result in the activation of the hypoxia-inducible factors HIF-1 and HIF-2 [159, 160]. HIF-1 consists of HIF-1α and HIF-1β subunits. HIF-1α expression and activation is tightly regulated in an oxygen-dependent manner, whereas HIF-1β is constitutively expressed. There are also mechanisms by which HIF-1α is regulated in an oxygen-independent manner. In hypoxic cells, HIF-1α cannot be hydroxylated and degraded, thereby accumulating in the tumor cells. Thus, HIF-1α and HIF-1β heterodimerize and bind to hypoxia response elements located in the target genes. HIF-2 consists of HIF-2α and the HIF-1β heterodimer. HIF-1 and HIF-2 regulate over 1000 target genes encoding major proteins, including vascular endothelial growth factor (VEGF), which is critical for angiogenesis, and glycolytic enzymes, which are involved in the metabolic adaptation of tumor cells to hypoxia. HIF-1α and HIF-2α levels are found to be increased in breast cancer samples and are linked to disease progression and patient outcome [161, 162]. HIF transcriptional activity also contributes to survival, invasion, metastasis, epithelial-mesenchymal transition, stem cell maintenance, and drug resistance in tumor cells.

In hypoxic breast cancer cells, increased HIF-1α transcriptional activity activates the expression of L1 cell adhesion molecule (L1CAM), angiopoietin-like 4 (ANGPTL4), lysyl oxidase (LOX), and LOX-like proteins 2 and 4. L1CAM increases the interaction of tumor cells with endothelial cells (ECs), leading to extravasation and lung metastasis. ANGPTL4 secretion from circulating tumor cells disrupts EC-EC interactions, thereby facilitating the extravasation of

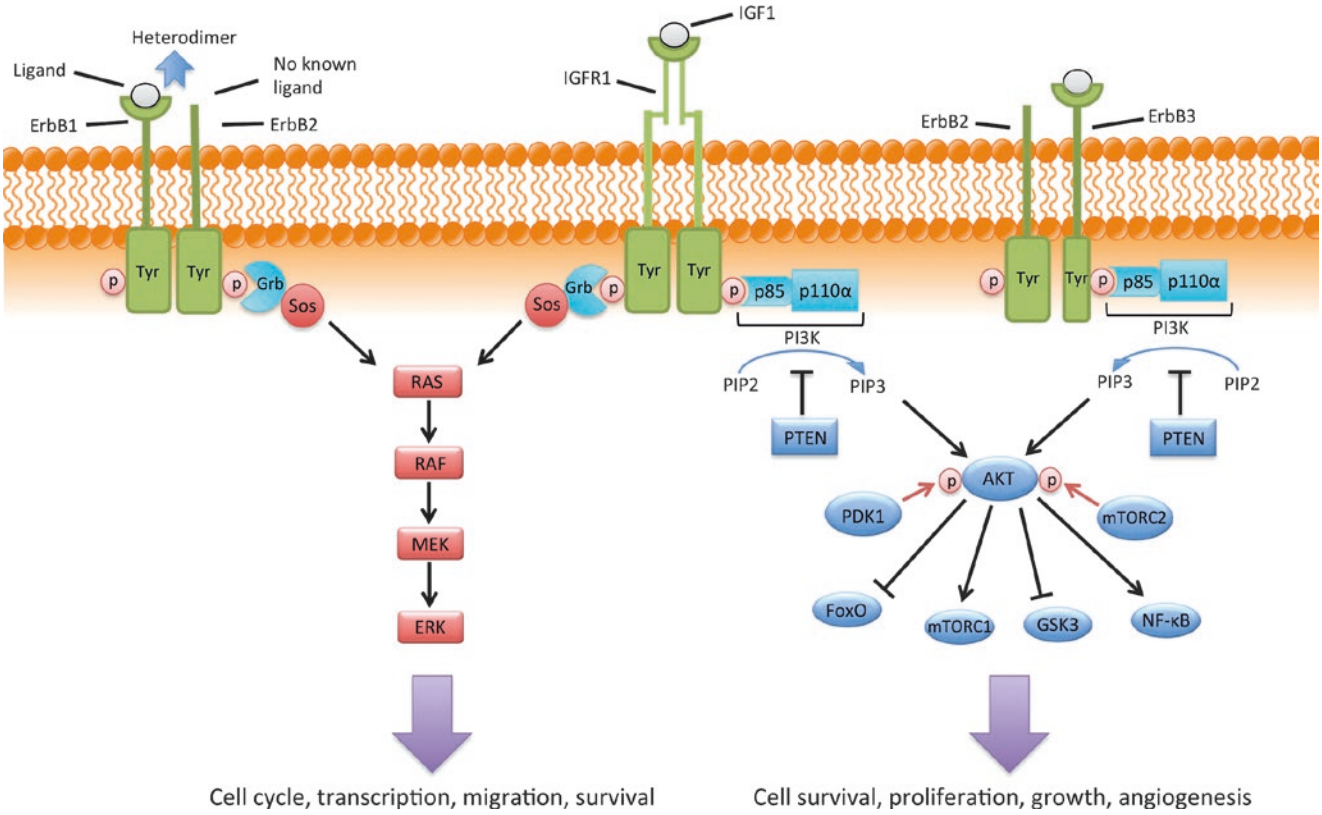

Fig. 9.5 Activation pathways of growth factor receptor tyrosine kinases. *ErbB1* epidermal growth factor receptor 1, *ErbB2* epidermal growth factor receptor 2, *ErbB3* epidermal growth factor receptor 3, *IGFR1* insulin-like growth factor receptor 1, *IGF1* insulin-like growth factor 1, *Tyr* tyrosine, *p* phosphate, *Grb* growth factor receptor-bound protein, *Sos* son of sevenless, *RAS* rat sarcoma, *RAF* rapidly accelerated fibrosarcoma, *MEK* mitogen-activated and extracellular-signal-regulated kinase, *ERK* extracellular-signal-regulated kinase, *PI3K* phosphatidylinositol 3-kinase, *PTEN* phosphatase and tensin homolog, *AKT* protein kinase B, *PDK1* phosphoinositide-dependent kinase 1, *mTORC 1/2* mammalian target of rapamycin complex 1/2, *FOXO* forkhead box protein O, *GSK3* glycogen synthase kinase 3, *NF-κB* nuclear factor kappa B

these cells into lung parenchyma. Breast cancer cells produce LOX and LOX-like proteins, which are involved in extracellular matrix modeling and the formation of premetastatic niches [163–167]. The invasive and proliferative advantage of cancer cell is mediated by HIF1 alpha expression and PHD3 suppression [168, 169]. Furthermore, HIF is induced together with adipocyte increase in the tissue, and as a result prognosis is affected in a worse manner [170].

Angiogenesis

Tumor growth depends on blood vessels and hypoxia acts as an activator of formation of new blood vessels, which is called neoangiogenesis. In breast cancer, angiogenesis is a key process for invasion and metastasis. Angiogenesis is characterized by abnormal vascular architecture with abnormal function and is sustained mainly by proangiogenic factors in the tumor microenvironment, including vascular endothelial growth factor (VEGF). VEGF is involved in the expression of adhesion molecules, the proliferation of endothelial cells, and increased vascular permeability. VEGFRs also act as receptor tyrosine kinases. Tumor cell-endothelial cell interaction is mediated through the signaling unit composed of the VEGF-A-VEGFR2 interaction [171, 172]. Thus, bevacizumab, a humanized monoclonal antibody directed against VEGF-A, has been studied extensively [173].

In breast cancer, VEGF expression has been shown to be correlated with high histological grade, hormone receptor negativity, HER2 overexpression, and lymph node metastasis. Consequently, enhanced angiogenesis and metastasis may be correlated with highly proliferative tumors, such as TN/basal-like breast cancer [174, 175].

Tumor Microenvironment

Cancer cells exhibit increased proliferative activity compared to normal counterparts because of various mutations playing role in carcinogenesis. Additionally, invasiveness of cancer cells toward stroma is accelerated which is in accordance with a modified tumoral microenvironment. Tumor-associated immune cells, cancer-associated fibroblasts, and

endothelial cells provide appropriate environmental conditions for metastasis. Tumor progression occurs rapidly due to the dysregulation of survival and apoptosis pathways in tumor microenvironment [176, 177]. Tumor microenvironment can control cancer cell behavior through cell-stroma interactions. It is a dynamic environment because of cancer cells' interaction with extracellular matrix and soluble factors [178]. The immunotherapeutic agents, which are under investigation, target CTLA-4, PD-L1, and B7-H4 costimulatory molecules in breast cancer microenvironment [179–182]. It is noted that breast cancer patients with high immunogenic expression have less genetic aberration and clonal heterogeneity. This situation confirms the fact that the presence of tumor-infiltrating lymphocytes is an important prognostic marker [183–185].

Clonal Heterogeneity

During the process of carcinogenesis, different clones are formed in tissue and intratumoral heterogeneity evolves. Clonal heterogeneity affects the characteristics of cells in tumor microenvironment and also contributes to treatment response. Briefly, some clones remain in primary tumor tissue, while others can metastasize to distant sites. Advanced new techniques can detect circulating free DNA fragments and circulating tumor cells. Different tumor clones can be identified by this way and may shed light onto more effective treatment strategies [186, 187]. These techniques are currently being used for determination of molecular and phenotypic tumor clones of breast cancer [188–190]. The study performed in circulating tumor cells obtained from metastatic breast cancer patients indicated that the presence of ADAM17 and KRT19 gene expression is critical in determining cancer progression and treatment response [191].

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Clinical Aspects of Estrogen and Progesterone Receptors and ERBB2 Testing

10

Ebru Cilbir and Suayib Yalcin

Introduction

Breast cancer comprises a heterogeneous group of tumors with a wide spectrum of morphologically and molecularly different subtypes, resulting in different biological behaviors, presentation, and prognosis. The major issue in making treatment decisions is to identify the subgroup of patients who will particularly benefit from a given treatment; this concept is now being developed as precision medicine. Aside from the stage of the disease, the evidence of distant metastasis, the organ of distant metastasis, and the age, performance status, and menopausal status of the patient, the histology, grade, and molecular pattern of the tumor are also important in deciding which treatment modality is the best for an individual patient. Among the molecular alterations associated with breast cancer, estrogen receptors (ERs), progesterone receptors (PRs), and v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (*ERBB2*) or neuroblastoma−/glioblastoma-derived oncogene homolog which is also called human epidermal growth factor receptor 2 (HER2) status are the most important molecular markers and are firmly established in the standard care of all primary and recurrent/metastatic breast cancer patients.

Here, we will review the clinical utility of hormonal receptor (HR) and ERBB2/HER2 testing in breast cancer, how this information is translated to treatment decision-making, the valid assays for these markers, and the guidelines for testing these important biological markers.

E. Cilbir
Division of Medical Oncology, Diskapi Yildirim Beyazit Treatment and Research Hospital, Ankara, Turkey

S. Yalcin (✉)
Department of Medical Oncology, Hacettepe University Cancer Institute, Ankara, Turkey
e-mail: syalcin@hacettepe.edu.tr

Hormonal Receptors

Historical Information

Breast cancer is a hormone-dependent malignancy. It arises from breast tissue that is normally responsive to endogenous hormones, and its course can often be influenced by the administration or removal of these hormones [1]. In 1896, Beatson et al. first reported dramatic regression of breast cancer after bilateral oophorectomy in a premenopausal woman with advanced disease [2]. Unfortunately, oophorectomy is not effective in all patients. In 1900, Stanley Boyd [3] reported that only one-third of patients responded to ovarian ablation, and those responses lasted an average of 1–2 years. Although these data were initially disappointing, endocrine therapy became a standard of care in the treatment of breast cancer. However, because only one-third of patients responded, the question was which patients would respond to endocrine therapy and whether a marker could be used to predict this response. Identifying this marker would allow the avoidance of ineffective ablative surgery in some patients [4].

Estrogenic hormones produced in the ovary were discovered by Allen and Doisy [5]. Then, in the early 1960s, radiolabeled estrogens were first observed to be preferentially concentrated in estrogen target organs. These observations gave rise to the concept of an "estrogen receptor", opening the door to molecular targeting in the treatment and prevention of breast cancer [4].

The finding that estrogen target tissues contained ERs and that nontarget tissues did not led to the question of whether these concepts could translate to the clinic to predict the endocrine responsiveness of breast cancer. Thus, if ERs are necessary for estrogen-stimulated growth, evaluating the presence or absence of ERs in a tumor specimen may be informative [4].

Jensen et al. reported in 1971 that the measurement of ER levels could predict the response to hormone therapy [6]. In general, these data were consistent with all of the clinical correlations presented at a 1974 workshop in Bethesda, Maryland, sponsored by the Breast Cancer Task Force [7]. Of the patients evaluated, only 8% of ER-negative (ER-) tumors responded to additive or ablative therapy, whereas 60% of patients who

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were ER positive (ER+) had an objective response to endocrine therapy. The results of the 1974 workshop established the ER assay as a valuable predictive test for the endocrine treatment of advanced breast cancer [4].

The identification of the ER has not only proved to be a successful therapeutic target for the treatment and prevention of breast cancer but has also proved to be a selective molecular model for all subsequent efforts to design targeted therapeutics in cancer [4].

Biology of ERs and PRs

Human breast cancers depend on estrogen and/or progesterone for growth, and this effect is mediated through ERs and PRs. ERs are ligand-regulated receptors that belong to the steroid nuclear receptor family. They function as transcription factors and transduce hormonal signals into a large variety of physiological responses in various organs [8]. The two structurally related ERs, ERα and ERβ, are the products of two separate genes that are differentially expressed in tissues. ERα is responsible for estrogen-induced mitogenic signaling in epithelial cells in breast, uterine, and ovarian tissues [9]. In the normal mammary gland, estradiol binds to both ERα and ERβ to control cell proliferation and differentiation [10]. The two ER isoforms are expressed at similarly low levels in the normal breast, whereas breast cancer cells express more ERα than ERβ [11].

The key components of ERs are the DNA-binding domain, which binds with high affinity and specificity to DNA sequences (estrogen response elements (EREs)) to regulate the transcription rates of target genes, and the ligand-binding domain, which binds estrogens [12]. The two ERs and the PR also form complexes with a number of coregulatory proteins that coordinately act to influence the transcription of estrogen-responsive genes. Mechanisms regulating ERα and ERβ function can occur at three levels: differential translation of exons, splicing of their messenger RNA (mRNA), and posttranslational modifications.

The effects of estrogen are mediated not only through nuclear ERs but also through cytoplasmic/membrane ERs and G protein-coupled ERs [11]. The ER proteins are generally believed to shuttle between the cytoplasm and the nucleus, and in vitro experiments have demonstrated that ligand-free ERα is maintained in a non-DNA-binding form in a multichaperone complex organized around Hsp90 (heat shock protein 90) [13]. ER-mediated transcription is a highly complex process involving multiple coregulatory factors and cross talk between different signaling pathways [11].

Genomic ER-Mediated Transcription Mechanisms

ERs and PRs function as transcription factors in the nucleus (Fig. 10.1). In the absence of hormones, histone deacetylase (HDAC) and the receptor corepressors NCoR and SMRT are

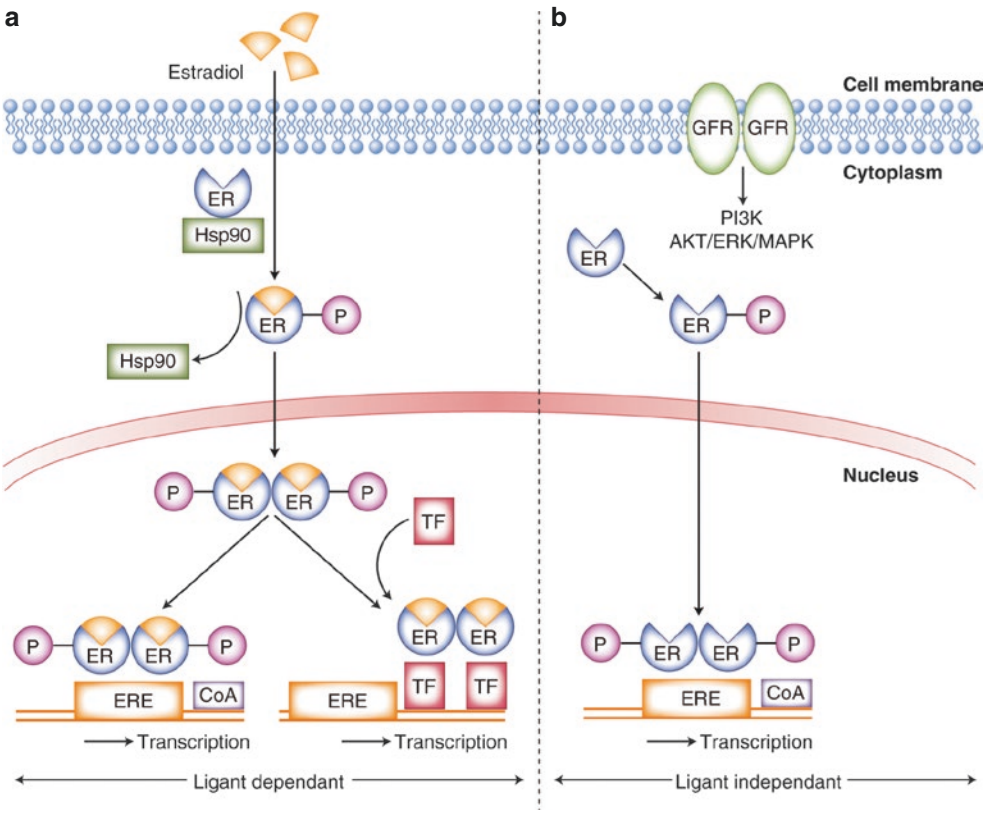

Fig. 10.1 Genomic ER-mediated transcription mechanisms. (**a**) Classical genomic ligand-dependent mechanisms; (**b**) ligand-independent genomic mechanisms. Abbreviations: *ER* estrogen receptor, *GFR* growth factor receptor, Hsp90 heat shock protein 90, *TF* transcription factor, *ERE* estrogen response element, *CoA* coactivator

bound to the receptor, forming a multichaperone complex. DNA is wrapped tightly. Estradiol binding to ERα activates the receptor through phosphorylation and dissociates proteins such as HSP90 [14]; during this process, the receptor undergoes conformational changes. Several kinases in the growth factor signaling networks can also activate the ER and its coregulatory proteins, and this process is termed ligand-independent activation [15]. The ER then binds to the 13-base-pair ERE sequence within the promoter. ER dimers dynamically and sequentially recruit various regulatory protein complexes that contribute to chromatin remodeling, thereby strongly enhancing transcriptional activity [16]. The nuclear receptor coactivators that are associated with ERs include the general transcription factor P300/CBP. P300/CBP is ubiquitously expressed and serves as a coadaptor between nuclear receptors and DNA. P300/CBP plays a critical role in cell cycle regulation, cell differentiation, and apoptosis, and it exhibits histone acetyltransferase (HAT) activity [17, 18]. Importantly, HATs are required for the full activation of ER-mediated transcription. Methyltransferases, including CARM1 and PRMT1, are also ERα-associated coactivators. Members of the p160 protein family, namely, steroid receptor coactivator 1 (SRC1), SRC2, and SRC3, also play various roles [19]. After ligand activation, protein-protein interactions with other transcription factors can also result in gene regulation by indirect binding to DNA (outside EREs) [20].

Estrogen-regulated promoters are affected by dynamic and complex processes and are involved in chromatin remodeling. Consequently, these events contribute to the hormonal regulation of gene expression. Phosphorylation of coactivators can affect ER-dependent transcription, even in the absence of ligands (ligand-independent ER-mediated transcription) or in the presence of antiestrogens, by increasing their subcellular nuclear localization, their interaction with the ER, and their ability to recruit transcriptional coregulators such as the CBP/P300 coactivator to the receptor-promoter complex [11].

Estradiol-ERα complexes affect the transcription of genes that are involved in proliferation, differentiation, survival, stimulation of invasion, metastasis, and angiogenesis. Some of these genes, like those involved in cell cycle progression (such as c-myc, cyclins D, A, and E), are activated [21]. Consequently, the growth of ERα-expressing (ER+) cells from breast tumors is estradiol dependent, and the removal of estradiol leads to regression. The presence of ERβ inhibits both ERα-mediated transcription and estradiol-induced proliferation in various types of cancer cells [22–24]. Therefore, ERβ in breast cancer lesions may be associated with more benign tumors. ERα and ERβ differentially regulate both the proliferation and apoptosis of normal mammary epithelial cells [25]. The ERα/ERβ ratio is currently hypothesized to be a key element in the regulation of estradiol activity in breast cancer cells [26].

Posttranslational Modifications

Posttranslational modifications of ERs and PRs include phosphorylation, ubiquitylation, acetylation, and methylation. Among the multiple kinases that can phosphorylate ERα are p38 mitogen-activated protein kinase (MAPK), cyclin A-CDK2, CDK7, c-Src, and pp90rsk1 [27–31]. ERα can also be phosphorylated by signaling molecules such as Akt (also known as protein kinase B), extracellular regulated kinase (Erk) 1/2, MAPK, protein kinase A (PKA), and p21-activated kinase 1 (PAK-1), thereby producing various responses to ligands [32–35]. Phosphorylation of the ER results in a variety of activities in different tumors. These effects involve receptor turnover, cellular localization, and transcriptional activity and are complex and interdependent.

The PR can be phosphorylated, with distinct phosphorylation sites coordinately regulated by ligands or kinases [36]. Phosphorylation of the PR, similar to ERα, can regulate ligand hypersensitivity [37].

Mutations in the ER Gene

ERα is encoded by ESR1 gene. A few mutations have been reported in the ERα gene, including a somatic mutation that causes a single amino acid change in the ERα hinge domain (lysine 303 to arginine, called K303R ERα). The receptor resulting from this mutation is hypersensitive to the growth-promoting effects of estrogen. Tumors with this mutation are associated with older age, larger tumor size, more lymph node-positive disease, and poor outcomes. Its incidence differs among studies [38–40]. Somatic base-pair missense mutations in ESR1 may confer hormone independence. Large-scale studies like The Cancer Genome Atlas (TCGA) project shows ESR1 mutations in only 0.5% of the primary breast cancer samples [41, 42]. With the more prevalent use of next-generation sequencing (NGS) and liquid biopsies, ESR1 mutations are much more prevalent in metastatic ER+ breast cancer patients with prior aromatase inhibitor therapy [43–45]. These mutations are most commonly missense mutations in codons 537 and 538 of the ligand-binding domain. They cause ligand-independent constitutive activation of ER. The most prevalent ones are Y537S and D538G [45, 46].

Nongenomic Pathways

In addition to classical ER genomic activity, which alters gene expression in the nucleus, estrogens have a more rapid class of effects (Fig. 10.2), for which plasma membrane estrogen-binding sites have been described [47]. Many studies have shown that, in response to their ligands, ERs and

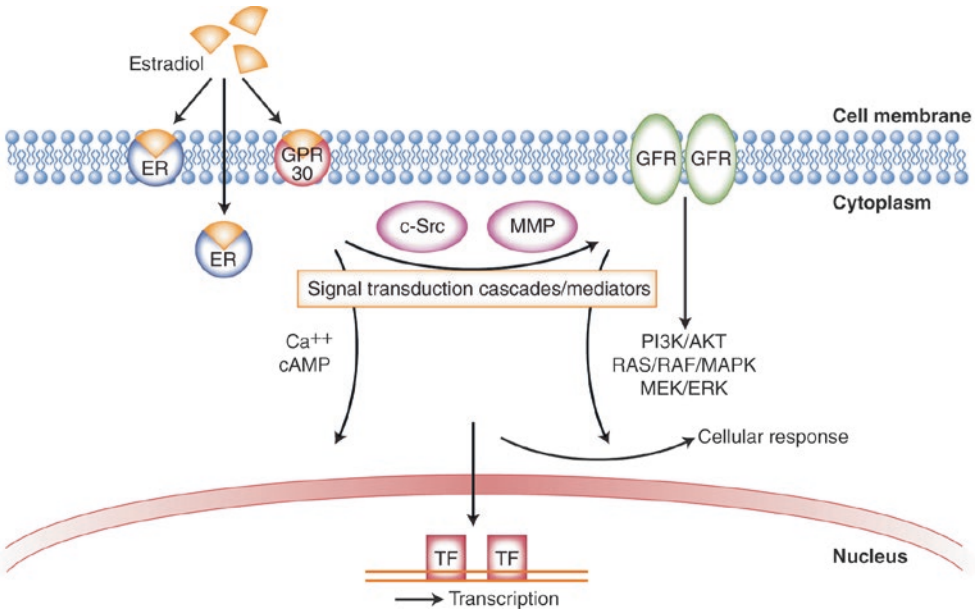

Fig. 10.2 Nongenomic ER-mediated transcription mechanisms. Abbreviations: *ER* estrogen receptor, *GPR30* G protein-coupled receptor 30, *GFR* growth factor receptor, *MMP* matrix metalloproteinase, *cAMP* cyclic adenosine monophosphate, *MAPK* mitogen-activated protein kinase, *Erk* extracellular regulated kinase, *TF* transcription factor

PRs can mediate signaling cascades originating from the membrane or the cytoplasm through direct interaction with signal-transduction mediators [48]. This nongenomic ER action (also called rapid, nonnuclear, or nonclassical) occurs within seconds to a few minutes and is independent of gene transcription [49].

A small subpopulation of the classic ERα and β subtypes that are located outside the nucleus or closely related nonclassical short forms of the ER transduce rapid estrogen signaling [50–52]. Membrane receptors distinct from the classic ER, especially G protein-coupled receptor 30 (GPR30), may also contribute to the rapid effects of estrogen [53, 54]. Membrane and cytoplasmic ERs transmit their signals through kinase cascades. These cascades include growth factor receptors and cellular tyrosine kinases as well as their downstream pathways. Membrane and cytoplasmic ERs also use calcium, cyclic adenosine monophosphate (cAMP), and other second messengers for signal transduction. This signaling leads to rapid cellular responses without gene transcription, but they also regulate nuclear transcription [48, 52, 55–57]. In addition, ERs have a role in the mitochondria, where they mediate cell survival signaling [52, 58, 59].

For progesterone-induced rapid effects, distinct nonclassical and classical receptor forms are present in the membrane and the cytoplasm [60].

ER Cross Talk with Other Signal-Transduction Pathways

In breast tumors, estrogens promote the activity of growth factor signaling pathway components, including ligands (e.g., transforming growth factor (TGF) α, insulin-like growth factor (IGF)-II), and receptors (EGF and IGF-I receptors) and key signal-transduction molecules (e.g., insulin receptor substrate-1) while also reducing the expression of growth-inhibitory factors (e.g., TGFβ) and inhibiting expression of tyrosine phosphatases. Altogether, these estrogens lead to a net increase in growth factor mitogenic activity. These observations imply a positive feedback loop that augments essential signaling pathways of both the estrogen/ER and growth factors/receptor systems [55, 61, 62].

In vitro studies have shown that membrane ERs can activate various growth factor receptors, including EGFR, HER2, and IGF-IR [52, 55, 63]. This pathway involves sequential activation of G proteins, the cellular tyrosine kinase c-Src, and matrix metalloproteinases (MMPs), followed by the release of heparin-binding EGF-like growth factor (HB-EGF). HB-EGF then binds to and activates adjacent EGFR and its downstream kinase cascades (e.g., Ras/Mek/MAPK and PI3K/Akt) [55]. These cascades then activate transcription [62]. Thus, genomic and nongenomic downstream pathways of ERs potentiate each other, resulting in proliferation and cell survival.

The Clinical Importance of ERs and PRs in Breast Cancer

Human breast cancers are dependent upon estrogen and/or progesterone for growth, and this effect is mediated through ERs and PRs. The symbol ER that we use as a predictive marker for endocrine therapy generally refers to ERα. We will use only ER in place of ERα in the rest of this chapter.

Up to two-thirds of invasive breast cancers of women younger than 50 years of age are ER or PR positive, and

approximately 80% of tumors in women older than 50 years of age are ER positive [64]. Measurement of these receptors has become a routine part of the evaluation of breast cancers.

The responsiveness of a tumor to hormone therapy is an important parameter in breast cancer management. However, not all patients with breast cancer benefit from hormone therapy. Tumor expression of ERs and/or PRs can best identify those women who are most likely to benefit from hormone therapy. Tumors that are negative for ERs and PRs are unlikely to respond to hormone therapy and respond better to cytotoxic chemotherapy. ERs and PRs can be utilized as both predictive and prognostic factors. A prognostic factor is any parameter available at the time of diagnosis that correlates with disease-free or overall survival. Thus, it indicates inherent biological aggressiveness of the tumor and correlates with natural history of the disease. A predictive factor indicates the likelihood of a response to a given therapy (here, ER and PR/hormone therapy).

We also know that adjuvant hormone therapy can halve the recurrence rate of patients with ER+ breast cancer [65]. Hormone therapy is relatively nontoxic, and responses can last for many years in some patients with metastatic disease. Thus, hormone therapies offer many significant advantages to particular subsets of breast cancer patients. The measurement of ER and PR levels in patients can identify those tumors that are most likely to benefit from hormonal agents.

ER status is strongly influenced by tumor grade and histology [64]. In one previous study, grade 1 tumors having histologies of pure tubular, colloid, and classic lobular carcinoma were ER positive [66]. Patients with ER+/PR+ tumors have a better prognosis than patients with ER+/PR- tumors, who in turn have a better prognosis than patients with ER-/PR- tumors [67]. ER expression is associated with most known prognostic factors but not with nodal metastases [68]. Thus, ER status is an important marker of growth rate rather than metastatic potential.

ER and PR Expression as Predictive Factors for Hormone Therapy

Efficacy of ER and PR Expression in Predicting the Benefit of Hormone Therapy in the Advanced Disease Setting

Approximately 30–40% of patients with ER+ metastatic disease will respond to first-line hormone therapies, and another 20% will experience disease stabilization [69–72]. The small proportion of patients who respond to hormone therapy with ER- disease may be mostly due to false-negative receptor assay results. All metastatic patients who receive hormone therapy show progression, and they often respond to a second line of hormone therapy [73]. Responses to subsequent lines of hormone therapy decline gradually but remain in the range of 20–30%, and this response is also dependent on ER positivity. Hormone therapy provides good palliation, better quality of life, and improved survival [74, 75]. Beyond simply designating a result as "positive," the expression level of the ER in the tumor is also very important in endocrine responsiveness. Response rates to hormone therapies are directly correlated to the level of ER expression [76, 77]. As with ERs, increasing PR levels are also associated with better response, longer time to treatment failure, and longer survival [78, 79]. Although the ER and PR expression levels are correlated, PR positivity is correlated with higher response rates independent of ER. ER+/PR+ tumors have higher response rates than ER+/PR- tumors [78]. As a result, PR status provides important information about the responsiveness to hormone therapies.

Discordance between the hormone receptor status of the relapse/metastases and the primary tumor is an important issue. A conversion rate of 20–30% has been reported from ER+ to ER- status, with less frequent conversion reported for ER- to ER+ status [80–84]. A significantly shorter median survival was noted with the loss of the ER in the metastasis in one of these studies [82]. Because metastases are the targets of therapy, the hormone receptor status of the metastases should be more predictive than that of the primary tumor. A previous study showed that 74% of patients with concordant ER+ results between primary tumor and metastases responded to hormone therapy, whereas only 12% of patients with ER+ primary tumors and ER- metastases responded [81]. A separate study that investigated biopsies from patients who developed resistance to tamoxifen reported changes in hormone receptor status and HER2 expression [85]. PR expression in metastatic lesions can also show discordance from the primary tumor. As with ERs, most of these conversions are of PR+ tumors losing PR expression [82, 83]. Sequential biopsies from patients given subsequent lines of hormone therapy have shown that loss of PR expression is associated with poor survival [86]. The metastatic tumor's hormone receptor status is a better predictor of survival than the primary tumor's hormone receptor status. Beyond the probable technical causes of false-negative or false-positive results, several explanations have been suggested for this discordance. Possible explanations include intratumoral heterogeneity, which can lead to clonal selection of different clones with distinct hormone receptor properties that can change over time, changes within single cells themselves as an adaptive mechanism to prior treatments and selection of more resistant clones, and tumor dedifferentiation with the development of metastasis [84, 87, 88]. Therefore, before making treatment decisions, the molecular markers of breast cancer should be retested in the metastatic lesions when possible.

Efficacy of ER and PR Expression in Predicting the Benefit of Adjuvant Hormonal Treatment

The Oxford meta-analysis reviewed data from 48,000 women from randomized clinical trials with 15–20 years of follow-up. This meta-analysis showed that the benefit of 5 years of adjuvant tamoxifen treatment is dependent on the ER and PR status of the tumor. ER status was a significant predictive factor for endocrine therapy [65]. More recent large prospective trials confirmed that tamoxifen reduces the risk of distant relapse, death, and contralateral breast cancer in ER+ breast cancer [89, 90].

More recently, aromatase inhibitors have been studied in adjuvant treatment of postmenopausal patients either before, immediately after, or long after tamoxifen. For example, a TransATAC trial retrospectively collected the samples of monotherapy arms of an ATAC trial and then tested ER expression in a central laboratory. This study showed a marginally significant relationship between ER level and time to recurrence. This effect was significant in anastrozole-treated patients ($p = 0.0009$) but less significant in tamoxifen-treated patients ($p = 0.078$). There was no significant interaction between ER level and benefit of anastrozole over tamoxifen treatments [91]. ER expression of tumors in patients from the BIG1–98 trial monotherapy arms (letrozole versus tamoxifen) was also analyzed centrally. Disease-free survival (DFS) varied based on ER expression levels [92].

However, meta-analyses of early breast cancer adjuvant tamoxifen therapy have not shown any benefit of PR expression among ER+ patients. An exception was a small subgroup of patients with ER-/PR+ tumors, who also showed benefits from hormone therapy. However, ER-/PR- patients have not shown any benefit [65, 93]. Several studies have suggested that tumors in this small subset are biologically different from ER+/PR+ tumors and represent a group of tumors with worse clinicopathologic features and clinical outcome. However, whether this group truly exists or whether it represents tumors that are actually ER+, with technical challenges producing this misclassification, remains unknown [94–97]. The discrepancies in these results may exist because relatively few studies included measurements of PR in the meta-analysis, and there may be PR measurement errors. A large retrospective study of patients receiving adjuvant tamoxifen showed that patients who had ER+/PR+ tumors experienced a 15–30% lower risk of recurrence and death than patients who had ER+/PR- tumors [67]. Other large studies of adjuvant tamoxifen have also shown that both ER and PR expressions are predictors of the benefits of hormone therapy [95, 98–99]. Recently, the MA.17 trial, which randomized postmenopausal women after 5 years of tamoxifen to the aromatase inhibitor letrozole versus placebo, also found that the benefit of letrozole over placebo was confined to ER+/PR+ tumors and was not seen in the ER+/PR- tumors [100].

Other than ERs, many cellular signaling networks contribute to endocrine responsiveness. These molecular pathways can modulate the ER pathway or provide alternative mitogenic and survival stimuli for the cells. Thus, resistance to hormone therapy may develop in ER+ tumors. Therefore, ER positivity alone is not sufficient for response to endocrine therapies. Some multigene predictive scores have been developed to predict hormonal responsiveness. One example is the Oncotype DX 21 gene assay, which includes several downstream ER-regulated genes and several proliferation genes in addition to ER mRNA [101].

Efficacy of ER and PR Expression in Predicting the Benefit of Adjuvant Chemotherapy

Luminal A (ER/PR+, HER2-)-type tumors receive less benefit from adjuvant chemotherapy, even if they have high risk of recurrence, because of lymph node positivity. The studies showing the benefit of adding taxanes to adjuvant treatment of node-positive breast cancer showed benefits irrespective of hormonal receptor status, but they did not take into account the endocrine effects of chemotherapy-induced amenorrhea in premenopausal patients [102–104]. Hormone receptor-positive breast cancer patients benefit less from adjuvant chemotherapy than hormone receptor-negative patients [105]. A study comparing adjuvant DAC (docetaxel-Adriamycin-cyclophosphamide) with FAC (fluorouracil-Adriamycin-cyclophosphamide) showed a benefit of adding taxanes regardless of ER status [103]. Premenopausal patients constituted more than half of the patients in this study, and TAC was associated with a greater incidence of chemotherapy-induced amenorrhea than was FAC. Thus, the advantage of adding a taxane may have been due to its endocrine effects by inducing amenorrhea.

The meta-analysis of adjuvant chemotherapy trials supports that ER- tumors derive more benefit from chemotherapy than ER+ tumors. The ovarian ablative effects of chemotherapy are not observed in postmenopausal patients. In postmenopausal patients, the benefits of chemotherapy in reducing recurrence and mortality of ER- patients are more prominent. However, the meta-analysis still showed a significant benefit of adjuvant chemotherapy in ER+ patients, but the ER level and the PR and HER2 status were not included in the meta-analysis [65].

More recent trials (Cancer and Leukemia Group B (CALGB) and Breast Cancer Intergroup trials) also evaluated the effects of ER status on patient outcome. They compared the effect of adding taxanes, dose-dense regimens, or increasing doses of doxorubicin with standard AC (Adriamycin-cyclophosphamide). Only ER- patients showed a benefit from increasing the dose of doxorubicin, adding taxanes, or using dose-dense adjuvant chemotherapy in terms of relative risk of recurrence and mortality. These aggressive adjuvant chemotherapy regimens tended to bene-

fit ER+ patients, but the effect was not significant [106]. When both ER and HER2 status are considered, patients with HER2-, ER+ breast cancer had no benefit from the addition of paclitaxel to adjuvant AC chemotherapy. However, addition of paclitaxel was beneficial in HER2+ patients, regardless of ER status [107].

Neoadjuvant chemotherapy trials have also shown the effect of ER status in pathologic complete response (pCR) rates. pCR rates in the ER- groups are significantly higher than in ER+ patients [108].

Multigene profiles, such as the Oncotype DX 21 gene profile, provide additional information for highly endocrine-sensitive tumors and identify those patients who will not benefit from chemotherapy. Patients with ER+ tumors who also have a low-risk 21-gene recurrence score receive no benefit from CMF (cyclophosphamide-methotrexate-fluorouracil) chemotherapy [109–110]. In another study, postmenopausal patients with ER+ tumors and a low-risk score with the 21-gene assay but having node positivity showed no benefit from adding chemotherapy (FAC-cyclophosphamide-doxorubicin-fluorouracil) to tamoxifen [111].

Targeting Other Pathways in Hormone Receptor-Positive Breast Cancer

Studies on resistance to hormonal therapies and ER biology show the role of signaling pathway cross talk. Adaptive upregulation of growth factor signaling, for example, PI3K/AKT/mTOR pathway, may confer resistance to selective ER modulators and ER degraders [112, 113]. The mammalian target of rapamycin (mTOR) pathway has some role in hormone-resistant ER+ disease. mTOR has downstream position at PI3K/AKT and Ras/Raf/Mek/Erk signaling pathways [114]. An mTOR inhibitor everolimus has demonstrated activity when combined with a steroidal aromatase inhibitor exemestane as a hormonal therapy [115].

Activation of the CDK4/CDK6/E2F axis, which has a central role in cell cycle progression, is a common feature of ER+ breast cancer. Preclinical studies show that ER+ cell lines are most sensitive to CDK 4/6 inhibition, and they show synergy when combined with hormonal therapy [116–118]. CDK4/6 inhibitors, palbociclib, ribociclib, and abemaciclib, show activity in combination with an aromatase inhibitor or selective ER downregulator, in patients with advanced HR+/HER2- breast cancer, both in the second- and first-line settings [118–122].

ER and PR Expression Levels as Prognostic Factors

Multiple studies show a relation between the hormonal status of the tumor and patient outcome. Patients with stage I ER+ breast cancer who receive no systemic therapy after surgery have a 5–10% lower likelihood of recurrence at 5 years in comparison with ER- patients [123–124]. Studies with longer follow-up show that as more time passes, the difference in the rate of relapse and death significantly diminishes and eventually disappears [125–127]. ER expression is associated with a number of other well-established prognostic indicators but not with nodal metastases [68], and its prognostic significance reduces over time. ER status does not predict the metastatic potential of the tumor. However, ER expression predicts an indolent and slowly growing tumor with longer time to disease recurrence. ER+ tumors are more frequently found in older patients [64, 128], and they are well differentiated histologically [129]. They have a lower fraction of dividing cells [130], are more often diploid, and are less likely to exhibit a mutation, loss, high expression, or amplification of breast cancer-related genes such as TP53 [131], HER2 [97], or EGFR [97]. ER status is also prognostic for the site of metastasis. ER+ tumors more frequently metastasize to the bone, soft tissue, or the reproductive and genital tracts, whereas ER- tumors metastasize more often to visceral organs or the brain [132, 133].

Methods for Measuring ER and PR Status

Assessment of ER and PR status is an essential factor in the evaluation of every newly diagnosed breast cancer. Various assay methods have been used to measure ER values in breast cancer specimens. These tests should be accurate and reliable because the results of such testing help to direct therapy. Thus, women who might benefit from endocrine therapy are provided with treatment, and those who are unlikely to derive benefit do not receive these treatments and are not exposed to unnecessary side effects [134].

The dextran-coated charcoal/ligand-binding assay (DCC/LBA) was the first method that became the standard for ER detection and measurement. Other assays, such as enzyme immunoassay and enzyme-linked immunosorbent assay (ELISA), then became available. By the late 1980s and early 1990s, immunohistochemistry (IHC) of formalin-fixed paraffin-embedded (FFPE) specimens began to replace the DCC assay because IHC has distinct advantages over DCC. These advantages include the need for smaller amounts of tissue, the ability to conduct testing on FFPE tissue (obviating the need for fresh/frozen tissue), the ability to correlate staining with histology, and the storage and retrieval of archived slides for later testing [134]. However, much data underlying the knowledge that the relationship between the response to hormone therapy and the amount of ER expression in the tumor tissue are based on studies using LBAs, and the cutoff values of positive and negative ER status were

developed with reference to LBA results. IHC methods have been subject to retrospective comparisons with established methods such as LBA, and published reports have also indicated that IHC may be more predictive than LBAs in identifying patients who will derive benefit from hormone therapy [77, 135–138].

IHC is performed by first treating thin sections of tissue using a variety of antigen retrieval methods. Next, the tissue is incubated with a primary antibody directed against the ER or PR. Then, secondary detection systems (secondary antibodies) that are conjugated to an enzyme (e.g., horseradish peroxidase) can be used to amplify the chromogenic signal. The sections are counterstained and viewed microscopically.

ER and PR levels can also be determined by the evaluation of messenger RNA either by individual assay (Northern blot analysis or reverse transcription polymerase chain reaction (RT-PCR)) or as part of multigene expression assays such as Oncotype DX and MammaPrint [109, 139]. However, due to the scarcity of data directly correlating these results with clinical outcomes, it is too early to recommend their routine use.

Optimizing IHC

Optimization includes evaluating the effects of preanalytic variables (i.e., variables of testing, involving the collection, fixation, and storage of samples), analytic variables (i.e., variables associated with the method of testing itself), thresholds to define results, and postanalytic variables (i.e., variables associated with handling of results, such as reporting) [134].

The American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) reported the latest guidelines for ER and PR testing in 2010 to provide clarification about testing parameters and establish mandatory proficiency testing and inspection criteria to improve the accuracy of these tests [140].

Regarding IHC assays for ERs and PRs, no gold-standard assay is available. A relevant standard would be any assay whose specific preanalytic and analytic components conformed exactly to assays whose results had been validated against clinical benefit from hormone therapy. ER and PR status should be determined in all newly diagnosed invasive breast cancers and recurrent and metastatic lesions, if appropriate. Newly diagnosed cases of ductal carcinoma in situ (DCIS) are also commonly tested for ER and PR expression because a retrospective subset analysis of the National Surgical Adjuvant Breast and Bowel Project B-24 clinical trial showed a significant reduction in subsequent breast cancer diagnosis restricted to patients with ER-positive DCIS [140].

Because the results may vary substantially between laboratories because of differences in specimen handling, tissue fixation, antigen retrieval, and antibody type, standardization of these variables is necessary.

Standardization of the Assays

A sample is accepted as positive for ER or PR expression if $\geq$1% of tumor cell nuclei are immunoreactive. Both the average intensity (weak, moderate, strong) and extent of staining (as a percentage) are reported. A sample is accepted as negative for ER or PR expression if <1% of tumor cell nuclei are immunoreactive in the presence of evidence that the sample expresses ER or PR in intrinsic controls. A sample is uninterpretable for ER or PR expression if there are no immunoreactive tumor nuclei and the internal epithelial elements (internal control) lack any nuclear staining [140].

Optimal Preanalytic Standardization

Tissue Handling

Issues related to testing variation begin as soon as the breast tissue is removed from the patient. Both the warm and cold ischemic times are important variables in the analysis of labile macromolecules such as proteins, RNA, and DNA from clinical tissue samples. The time from the interruption of the blood supply to the tumor by the surgeon to the excision of the tissue specimen is defined as warm ischemia, and the time from excision of the tissue to the initiation of fixation is defined as cold ischemia. Studies have documented the progressive loss of these labile molecules after the surgical interruption of blood flow, through tissue ischemia, acidosis, and enzymatic degradation [141, 142]. Breast resection specimens should be fixed as quickly as possible in an adequate volume of fixative (optimally tenfold greater than the volume of the specimen). The time of tissue collection (defined as the time when the tissue is handed from the surgical field) and the time the tissue is placed in fixative should be recorded. The time from tumor removal to fixation should be kept to $\leq$1 h [140].

After being received in the pathology laboratory, specimens should be oriented and carefully inked for surgical margin assessment, sectioned at 5 mm intervals, and placed in 10% neutral (phosphate) buffered formalin (NBF). If the excised specimen was obtained remotely from the grossing laboratory, the sample should be bisected through the tumor and promptly placed in NBF prior to transport. Although less optimal than immediate gross examination of the fresh sample by the pathologist, this process is preferable to storage of the sample in the refrigerator unfixed or in fixative without sectioning [140].

Type of Fixative

Only 10% NBF should be used as the fixative for breast tissue specimens. Higher or lower concentrations of NBF are not acceptable [140].

Duration of Tissue Fixation

Breast tissue specimens must be fixed in 10% NBF for no less than 6 h and for not more than 72 h before processing [143, 144]. Formalin penetrates tissue at a rate of approximately 1 mm/h, which is why breast excision samples must be incised in a timely fashion to initiate formalin fixation throughout the tissue. Fixation does not begin until formaldehyde has penetrated into the tissue. However, permeation of tissue by formalin is not the same as the chemical reaction of fixation, which involves protein cross-linking by formaldehyde. Underfixation of breast tissue may lead to false-negative ER results. Overfixation is likely to be less problematic than underfixation, but it can also potentially lead to false-negative results due to excessive protein cross-linking by formaldehyde [140, 143, 145].

Analytic Standardization

Antibody Selection for ER and PR Testing

Testing for ER and PR expression by IHC must be performed using antibodies that have been clinically validated and show good correlation with the outcomes of patients receiving endocrine treatment. An ASCO/CAP panel provided a list of antibodies with acceptable clinical validation. Antibodies sold for research use only or investigational use only or developed by the testing facility may not be used in ER and PR testing [140]. A laboratory performing ER testing should initially validate its proposed or existing assay against one of the clinically validated assays and demonstrate acceptable concordance. To be considered acceptable, the results of the assay must be initially 90% concordant with those of the clinically validated assay for the ER+ and PR+ category and 95% concordant for the ER- or PR- category [140].

Control Samples for IHC Assays

External and internal controls can be used to ensure that the IHC test has been performed properly. Positive and negative external controls should be included in every batch of assays. Acceptable external controls are cell lines previously defined as highly positive, intermediate, and negative for the analyzed receptor to ensure that assay is working properly. Internal control defines the staining of normal epithelial elements of the analyzed tissue to ensure that handling procedures are performed properly. If an external or internal control does not produce the expected reaction, the result of patient testing must not be reported, and retesting is warranted [140].

Postanalytic Standardization

The percentage of cells with nuclear staining is reported by either estimation or quantitation. Quantitation may be performed either manually or by image analysis. The entire slide should be reviewed to assess the tumor-containing areas. Cytology samples with limited tumor cells and little tumor staining must have at least 100 cells counted. If cytoplasmic staining occurs, the assay should be repeated or performed on another sample. The sample should be rejected if there are obscuring artifacts such as decalcification of the sample or staining only of necrotic debris. If the test result is negative but that particular histologic type of breast cancer is unlikely to be ER- (tubular, mucinous, or lobular morphology or Nottingham score of 1), the tumor should also be subjected to confirmatory testing, such as sending the same specimen to a reference laboratory for retesting or by repeating the assay on another block or on a separate breast cancer specimen [140]. A comprehensive quality control program for ER/PR IHC analyses should be established. This analysis should include the analysis of whether positive results in a given period could reflect the ER+ breast cancers in the patient population served by the laboratory [140].

HER2 Testing

HER2 encodes a member of the epidermal growth factor receptor family of receptor tyrosine kinases, and its amplification with resultant overexpression plays a major role in sustaining multiple pathways in cancer growth. These roles include self-sufficiency in growth signals, sustained angiogenesis, increased cell division, and enhanced invasion [146, 147].

The HER2 gene encodes a 185 kDa protein that has tyrosine kinase activity. HER2 is a membrane protein that is expressed at low levels in all epithelial cells in normal fetal and adult tissues [148]. The HER2 gene is amplified in a variable percentage of human breast [149, 150], ovarian [150], bladder, endometrial [151], salivary gland [152], and gastric [153] cancers. HER2 gene amplification has been associated with increased levels of expression of HER2 mRNA and protein product [150].

HER2 Signal-Transduction Pathways

The HER family consists of four structurally related members, the epidermal growth factor receptors (EGFR) HER1, HER2, HER3, and HER4, which are transmembrane receptor tyrosine kinases that regulate cell growth, survival, differentiation, migration, and other cellular responses. HER family receptors are activated by ligand-induced dimerization or receptor pairing [154]. They are able to homodimerize or heterodimerize with other HER family members, producing multiple receptor combinations. Dimerization leads to activation of the intrinsic tyrosine kinase domain of the receptor. Phosphorylation of tyrosine residues leads to the activation of downstream

signaling pathways such as the RAS/RAF/MEK/ERK pathway and the PI3K/Akt/mTOR pathway [155]. HER2 and HER3 are unique in that HER2 has no known ligand that stimulates its activity, and HER3 has very little enzymatic kinase activity and mainly functions as a ligand-activated dimer partner for the other family members [156]. HER heterodimers are more potent in signal transduction than are homodimers. Heterodimerization follows a strict hierarchical principle, with HER2 representing the preferred dimerization partner for all other HER receptors [157]. HER2-containing heterodimers display increased potency due to the relatively slow rate of ligand dissociation and slow rate of receptor internalization. Thus, signaling by HER2-containing heterodimers is prolonged and results in enhanced activation of signaling pathways. The most potent heterodimer is HER2/HER3 [158].

In addition to their function as receptors on the cell surface, HER family proteins are present in the nucleus to act as kinases and transcriptional regulators. EGFR can be transported to the nucleus and act as a tyrosine kinase. HER2 can be transported to the nucleus via endocytic vesicles. The intracellular domain of HER4 is cleaved and transported to the nucleus [159–161].

Clinical Significance of HER2 Amplification/ Overexpression

HER2 is amplified in approximately 15–20% of breast cancers [162]. HER2 overexpression is associated with important clinical outcomes in breast cancer patients. HER2 overexpression is a poor prognostic factor in the absence of adjuvant treatment independent of tumor size, grade, and hormone receptor status. It has been associated with shorter DFS and shorter overall survival (OS) in primary invasive node-negative breast cancer patients treated with surgery alone [163]. HER2 is also an important predictive marker for certain treatments.

Response to HER2-Targeted Therapies

Much clinical evidence has shown that only breast cancers with HER2 amplification or overexpression respond to HER2-directed therapies such as monoclonal antibodies directed against HER2 [164, 165]. Agents that target HER2 are remarkably effective in both metastatic and adjuvant settings. Trastuzumab, a humanized monoclonal antibody, improves response rates, time to progression, and survival when used alone or added to chemotherapy in metastatic breast cancer [166]. Further, prospective randomized trials have shown that adjuvant trastuzumab reduces the risk of recurrence and mortality in patients with early-stage breast cancer [167–171]. Other HER2-targeted drugs, including the tyrosine kinase inhibitor lapatinib, the antibody pertuzumab, and the antibody drug conjugate ado-trastuzumab emtansine (T-DM1), improve outcomes in HER2-positive metastatic breast cancer [172–174].

Response to Hormone Therapies

HER2 positivity may be associated with resistance to hormone therapies. Although this association remains controversial, some data suggest that this effect may be specific to selective estrogen modulator (SERM) therapy such as tamoxifen and perhaps not to estrogen depletion therapies such as aromatase inhibitors [92].

Preclinical studies suggest physiological cross talk between the HER2 and ER signal-transduction pathways. HER2 expression in human breast cancer cells is downregulated by estrogens [175]. Conversely, transfection and overexpression of HER2 in vitro promote estrogen-independent growth and tamoxifen resistance in ER+ human breast cancer cells [176, 177]. Amplification and/or overexpression of HER2 may be associated with primary resistance to hormone therapy. The hypothesized mechanisms underlying the hormone independence of HER2-expressing cells include phosphorylation of the ER, ligand-independent ER activation, and regulation of hormone receptor expression [177, 178].

Some retrospective and nonrandomized clinical studies showed lower response rates of hormone therapy in HER2-overexpressing tumors. However, randomized trials in either adjuvant or metastatic settings failed to provide supporting evidence. Although some trials have concluded that patients with HER2-overexpressing tumors are relatively resistant to adjuvant hormone therapy [179–182], a similar number could not demonstrate such a relationship [183–186].

The adverse influence of HER2 expression in response to hormone therapies is hypothesized to be limited to therapies based on a ligand-binding agent such as SERMs. Further, the response to ligand-depleting therapies such as ovarian ablation or aromatase inhibitors is not affected by HER2 overexpression. In a study of neoadjuvant endocrine therapy analyzing 250 postmenopausal women receiving either letrozole or tamoxifen for 4 months, women in the tamoxifen group with HER2+ tumors showed significantly lower response rates than those with HER2- tumors (17% versus 40%, respectively). However, patients receiving letrozole showed no difference in response rates between HER2+ and HER2- groups (69% versus 53%, respectively) [187]. Multiple studies have assessed the response rates to hormone therapy among women with HER2+ versus HER2- tumors. Although the results are mixed, most show an adverse influence of HER2 overexpression in response to treatment with tamoxifen [188–192]. However, these data may not be sufficient for using HER2 status as a factor for hormonal treatment selection (aromatase inhibitors versus tamoxifen) [193, 194].

Response to Chemotherapy

HER2 may be associated with either sensitivity or resistance to some chemotherapeutic agents. HER2 positivity is associated with better outcomes in response to adjuvant anthracycline-containing regimens in most studies [195–197]. A meta-analysis of eight trials comparing anthracycline- versus non-anthracycline-based chemotherapy concluded that the benefits of adjuvant anthracycline use were confined to women with HER2+ tumors [198]. This effect may be secondary to coamplification of HER2 with topoisomerase II (topo2), which is the direct target of anthracyclines [199]. In a study involving patients with HER2+ metastatic breast cancer who were treated with chemotherapy with or without trastuzumab, topo2α coamplification was associated with significantly improved survival when only anthracycline-containing chemotherapy was used for treatment, compared with outcomes in HER2+ cancers lacking topo2α coamplification [200]. Another meta-analysis of five randomized trials comparing adjuvant anthracycline-containing treatment with CMF (cyclophosphamide-methotrexate-fluorouracil) assessed whether HER2 expression or topo2 expression could predict response to anthracyclines [201]. Trastuzumab was not administered to patients in any of the trials included in this analysis. There was a significant improvement in both event-free survival and OS in patients with HER2 overexpression, and similar results were observed for patients with topo2 alterations. In patients without HER2 overexpression and in patients with normal topo2 levels, only improvement in event-free survival was observed, with no improvement in OS. Now, because adjuvant trastuzumab is routinely used in HER2-overexpressing breast cancer patients and because both agents are cardiotoxic, the results are more conflicting. Although individual studies of an anthracycline-based regimen plus trastuzumab versus a non-anthracycline-containing regimen plus trastuzumab have shown equivalent survival outcomes for HER2+ patients, these data are controversial [167, 200]. Many clinicians still believe that anthracyclines provide additional benefits in combination with trastuzumab for patients with HER2+ breast cancer [202]. Cardiac toxicity is a serious concern in patients with early-stage disease who are treated with both trastuzumab- and anthracycline-containing chemotherapy regimens. For these reasons, accurate determination of HER2 alterations in breast carcinomas is crucially important.

HER2 amplification or overexpression has been correlated with responsiveness to paclitaxel-containing chemotherapy [107], but the data are inconsistent and contradictory. In a study of metastatic breast cancer patients who received either paclitaxel or cyclophosphamide plus epirubicin, women whose tumors were HER2+ had a significantly longer progression-free survival and OS with a taxane-containing regimen, while there were no differences between the two regimens in those whose tumors were HER2 negative [203]. In the adjuvant setting, the interaction between HER2 status and benefit from taxanes was evaluated in a subset of 1322 women in the Cancer and Leukemia Group B (CALGB) 9344 study. This study showed that four cycles of adjuvant paclitaxel after AC significantly improved DFS and OS compared with four cycles of AC alone in women with node-positive breast cancer. There was a significant benefit from the addition of paclitaxel after AC in women with HER2+ tumors regardless of ER status and in ER- tumors regardless of HER2 status. However, there was no evidence of benefit in the subgroup of patients with ER+ and HER2- tumors [102]. Other trials conducted in the adjuvant setting have failed to note an association between HER2 overexpression and benefit from taxanes [204, 205]. Reports from neoadjuvant taxane therapy are conflicting. One suggests a worse response rate [206], and two others report no association between HER2 gene amplification or overexpression and response [207, 208].

Methods for Measuring HER2

Because HER2 gene amplification is directly correlated with HER2 expression levels at the mRNA and protein levels, HER2 status could potentially be evaluated at any of these levels and should correspond to the HER2 status determined using any of the other measures.

Several assays have been used for HER2 determination in tissue. The studies that led to the approval of the humanized mouse 4D5 monoclonal antibody trastuzumab in HER2+ metastatic breast cancer selected the patients with an IHC assay method, known as the clinical trial assay (CTA) [166, 209]. This assay used two different antibodies, 4D5 (the mouse monoclonal antibody used to produce humanized trastuzumab) and CB11 (a mouse monoclonal antibody). However, these so-called home-brew assays were not validated and are not considered appropriate for commercialization. Since the development of the CTA, a number of commercially available testing kits have received approval from FDA for the assessment of patients for whom trastuzumab may be a suitable treatment [210].

The available assays are as follows:

- Overexpression of the HER2 protein product: Western blotting, ELISA, or IHC.
- Overexpression of HER2 RNA: Northern blotting or reverse transcription polymerase chain reaction (RT-PCR).
- HER2 gene amplification by in situ hybridization (ISH): fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), silver-enhanced in situ hybridization (SISH), or differential PCR.

Because there is insufficient evidence to support the use of mRNA and DNA microarray assays to determine HER2 status in unselected patients, these techniques will not be discussed here.

Recommendations for tissue handling as well as preanalytic, analytic, and postanalytic factors in ER/PR testing are also suitable for HER2 testing. Therefore, these points will not be discussed again.

Immunohistochemistry Tests

Grading of IHC assays is based on a 0, 1+, 2+, and 3+ scoring system. Tumor specimens that demonstrate complete, intense circumferential membrane staining in >10% of tumor cells are classified as 3+ on IHC and constitute a positive result. The definition of 2+ (IHC equivocal) is revised in 2018 as weak to moderate complete membrane staining observed in >10% of tumor cells [211] and requires confirmation of HER2 status by an alternative method, usually ISH, or may order a new test on a new specimen if available, using IHC or ISH. If there is faint or barely perceptible and incomplete membrane staining in >10% of tumor cells, the sample is classified as IHC 1+, and this finding constitutes a negative HER2 result. If there is either no staining at all or there is faint or barely perceptible incomplete membrane staining in ≤10% of tumor cells, the score is IHC 0 and is reported as HER2 negative [162, 211].

The HercepTest, an IHC test that was approved by FDA at the same time as trastuzumab, uses a rabbit polyclonal antibody against HER2. Two other FDA-approved IHC systems are the Pathway anti-HER2/neu test (Ventana Medical Systems Inc., Tucson, AZ), which utilizes a rabbit monoclonal antibody (4B5), and the Bond Oracle HER2 IHC System (Leica Biosystems, Newcastle upon Tyne, UK), which uses the mouse CB11 clone [212].

In Situ Hybridization

HER2 FISH assays were initially approved by the FDA for the identification of women with node-negative disease who were at high risk of recurrence or disease-related death, as well as for selection for doxorubicin chemotherapy [213]. Later, the selection criteria were expanded to cover the assessment of patients for whom trastuzumab treatment was being considered.

When measured against an external "gold-standard" molecular characterization of HER2 status, FISH is more accurate, reproducible, and robust than IHC [213]. However, for practical and historical reasons, IHC has been more widely used as the primary test for HER2 status. IHC is comparatively quick and is viewed using a conventional bright-field microscope, and stained tissues do not degrade over time [214]. IHC testing also permits parallel viewing of tumor morphological features. In contrast, FISH is technically more demanding and requires the use of fluorescence microscopy [214].

Emerging technologies for HER2 testing include CISH and SISH. CISH uses a peroxidase enzyme-labeled probe for chromogenic detection by diaminobenzidine. SISH uses the same system with a silver-based detection system. Because these processes do not use fluorescent dye, a standard bright-field microscope can be used [214]. Automated ISH techniques may enable more rapid testing. CISH can be stored because the signal is stable [214]. An additional emerging technology is dual-color, dual-hapten, bright-field in situ hybridization (DDISH), which has all the advantages of SISH. Further, SISH requires two separate slides to detect both HER2 and CEP17, whereas DDISH uses double-stranded probes labeled with two haptens to detect both markers on a single slide [215].

When interpreting ISH results, both the average HER2 copy number signals per cell and ratio between HER2 and the chromosome 17 enumeration probe (CEP17) should be considered. When HER2 testing is performed by a validated single-probe ISH assay, HER2 is positive if the average HER2 copy number is ≥6.0 signals/cell; HER2 is equivocal if the average HER2 copy number is ≥4.0 and <6.0 signals/cell (ISH equivocal); and HER2 expression is negative if the average HER2 copy number is <4.0 signals/cell. It is recommended that concomitant IHC review should become part of the interpretation of single-probe ISH result. If not already assessed by the institution or laboratory performing the ISH test, IHC testing for HER2 should be performed using sections from the same tissue sample used for ISH and the slides from both ISH and IHC should be reviewed together to guide the selection of areas to score by ISH. The use of dual-probe ISH assays is also recommended to use preferentially. The update on recommendations for HER2 testing with ISH method cancelled an equivocal result. Instead, pathologists are forced to make a judgement as positive or negative using combination of repeated IHC and dual-probe ISH method. If a single-probe ISH assay is equivocal, perform IHC and/or dual-probe ISH. If IHC is 3+, the result may be reported as positive. If concurrent IHC is 2+, perform dual-probe ISH for final results. If concurrent IHC is 0 or 1+, consider the final result as negative [211].

When HER2 testing is performed by a validated dual-probe ISH assay, the results are grouped as Groups 1–5. A positive ISH result is defined as an average HER2 copy number of ≥4.0 signals/cell and a HER2/CEP17 ratio ≥2.0. This is regarded as Group 1. If HER2/CEP17 ratio is ≥2.0 and average HER2 copy number is <4 signals/cell, this is regarded as Group 2. If HER2/CEP17 ratio is <2 and average HER2 copy number is ≥6.0 signals/cell, this is regarded as Group 3. If HER2/CEP17 ratio is <2 and average HER2 copy number is ≥4.0 and <6.0 signals/cell, this is regarded as Group 4. If HER2/CEP17 ratio is <2 and average HER2 copy number is <4.0 signals/cell, this is regarded as Group 5, and the final ISH result is negative. Regarding Groups 2, 3, and 4, IHC

testing for HER2 should be performed using sections from the same tissue sample used for ISH, and the slides from both ISH and IHC must be reviewed together to guide the selection of areas to score by ISH. If IHC is 3+, the final result is HER2 positive. If IHC is 0 or 1+, Her2 is regarded as negative with comments. If IHC 2+, ISH is recounted by having an additional observer, blinded to previous ISH results, count at least 20 cells that include the area of invasive cancer with IHC 2+ staining. If reviewing the count by the additional observer changes the result into another ISH category, the result should be adjudicated per internal procedures to define the final category. If the count remains same as the initial observer, for Group 2 patients, diagnosis is HER2 negative; for Group 3 patients, diagnosis is HER2 positive; for Group 4 patients, diagnosis is HER2 negative with a comment [211].

If available, the test should be performed on a core biopsy specimen from the newly diagnosed patient. If the test result is clearly positive or clearly negative, no retesting is needed. If the test is negative and there is apparent histopathologic discordance or if specimen handling has not been consistent with guideline recommendations, a section of the tumor from the excisional specimen should be retested. If the result is positive, no further testing is needed. However, if the test is negative and there remains significant clinical concern about the result after consultation between the pathologist and the medical oncologist, it may be appropriate to repeat the test in a different block from the patient's tumor [162].

The HER2 test result must be reported as indeterminate if technical issues prevent the test from being reported as positive, negative, or equivocal. These conditions include inadequate specimen handling, artifacts (crush or edge artifacts) that make interpretation difficult, or analytical testing failure. Under these circumstances, another specimen should be requested [162].

Laboratories performing these tests should follow all accreditation requirements, one of which is the initial testing validation. This requirement conforms to the 2010 ASCO/CAP recommendations for ER/PR testing [162].

Laboratories are responsible for ensuring the reliability and accuracy of their testing results by complying with accreditation and proficiency testing requirements for HER2 testing results. They should review and document external and internal controls with each test and each batch of tests [162].

Histopathologic Features Suggestive of Possible HER2 Test Discordance

- A new HER2 test should be ordered if the initial HER2 test was positive in any of the following:
  1. Histologic grade 1 carcinoma of infiltrating ductal or lobular carcinoma, ER+ and PR+.
  2. Tubular/mucinous/cribriform (at least 90% pure), adenoid cystic carcinoma (90% pure), often triple negative [146].
- A new HER2 test may be ordered on the excision specimen if the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative in a tumor exhibiting any of the following:
  3. Grade 3 tumor.
  4. Small amount of invasive tumor in the core biopsy.
  5. The carcinoma upon definitive resection contains a high-grade carcinoma that is morphologically distinct from that in the core biopsy.
  6. The core biopsy result is equivocal for HER2 after testing by both ISH and IHC.
  7. There is doubt about the specimen handling of the core biopsy [162].

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Prognostic and Predictive Factors

11

Sitki Tuzlali and Ekrem Yavuz

Prognostic and Predictive Factors

A variety of pathological parameters are used to assess prognosis and predict the therapeutic response of breast cancer patients. These parameters include tumor size, axillary lymph node status, histological features (especially histological grade and lymphovascular invasion), hormone receptor status, HER2 status, and the proliferative capacity of the tumor. Considering these factors in combination is of greater clinical value than viewing each in isolation, and the combined approach forms the basis of a number of schemas used to group patients into various risk categories, such as the St Gallen criteria, the NIH consensus criteria, the Nottingham Prognostic Index, and Adjuvant! Online (www.adjuvantonline.com) [1].

Tumor size and axillary lymph node status are the components of the TNM tumor staging system published by the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) [2, 3].

Tumor Size

Pathological measurement of tumor size is considered the gold standard and ideally should be performed before fixation and checked with microscopic size. For maximum correlation with prognosis, tumor size should only be assessed on pathological specimens since clinical evaluation is inaccurate. It has been shown in many studies that patients with smaller tumors have better long-term survival than those with larger tumors [4–7]. Tumor size is based on the maximum size of the invasive component of the tumor [8, 9]. Maximum invasive tumor size (T) is a reasonable estimate of tumor volume [3].

In cases with an accompanying in situ component, the in situ area that is outside the invasive tumor is not included in the tumor size "T." However, if the in situ component is intermingled with the invasive areas, T will include these in situ areas. If there are multiple areas of invasion, the size of the largest invasive carcinoma is used in the T staging. Occasionally multiple invasive foci occur in close proximity to each other, creating difficulty in determining the invasive tumor size. Correlation of radiologic and gross findings with the microscopic appearance may be necessary. Sometimes, the choice of T staging may also depend on the pathologist's own judgment. Small, microscopic satellite foci around the primary tumor do not appreciably alter tumor volume and are not added to the maximum tumor size.

In cases in which the tumor is transected in a previous biopsy, the sizes of the tumors in the separate specimens should not be added, and an estimation with the help of imaging studies should be performed [9].

Lymph Node Status

The status of the axillary lymph nodes is the most important single prognostic parameter in breast carcinomas. Lymph node staging should be based on histological evaluation of the excised lymph nodes since clinical evaluation is not sufficient for accurate staging. Numerous studies have shown that patients with histologically confirmed axillary lymph node involvement have a significantly poorer prognosis than those without nodal involvement. The extent of axillary invasion by level also has strong prognostic significance, with involvement of higher levels of the axilla having a worse prognosis [10]. Surgical removal of positive nodes does not appear to have a major role in survival but is required for accurate staging and local control [11].

S. Tuzlali (✉)
Tuzlali Private Pathology Laboratory, Istanbul, Turkey

Department of Pathology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

E. Yavuz
Department of Pathology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey
e-mail: yavuze@istanbul.edu.tr

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Sentinel lymph node biopsy and the importance of low-volume metastases are mentioned in detail in Chap. 15.

Although basal-like carcinomas belong to the poor prognostic group, they are the least likely to exhibit extensive nodal involvement. For these patients, other prognostic markers are more important than nodal staging [8].

Pathology reports should include the following [9]:

- The total number of sentinel lymph nodes, if a SLNB procedure is performed
- The total number of lymph nodes identified
- The number of metastatic lymph nodes (sentinel or non-sentinel)
- The size of the largest metastatic deposit
- Extranodal extension

Grading

The Nottingham (Elston-Ellis) modification of the Scarff-Bloom-Richardson grading system, also known as the Nottingham grading system (NGS) [12], is the grading system recommended by various professional organizations, such as the World Health Organization (WHO) [8], American Joint Committee on Cancer [AJCC], the Royal College of Pathologists (UK RCPath) [13], and College of American Pathologists (CAP) [9]. NGS provides a simple, inexpensive, and routinely applicable overview of the intrinsic biological characteristics and clinical behavior of tumors [12]. In NGS, the subjectivity of previous grading systems is minimalized by strict definitions of the evaluation criteria.

Multiple independent studies have shown that NGS has a prognostic value equivalent to that of LN status and greater than that of tumor size [14–17].

NGS refers to the semiquantitative evaluation of some morphological characteristics on an adequately prepared hematoxylin-eosin-stained tumor tissue section. This assessment should be performed by an appropriately trained pathologist using a standard protocol. NGS when adequately carried out, provides a simple, inexpensive and validated method for assessing patient prognosis especially in parts of the world where alternative molecular tests are not available [15].

NGS is based on the evaluation of three morphological features [8, 12, 13]:

(a) Degree of tubule or gland formation
(b) Nuclear pleomorphism
(c) Mitotic count (found in ten consecutive high-power fields (HPFs) in the most mitotically active part of the tumor)

Feature	Score
Tubule/acinar/gland formation	
>75% of the tumor	1
10–75% of the tumor	2
<10% of the tumor	3
Nuclear pleomorphism	
Small, regular uniform cells	1
Moderate increase in size and variability	2
Marked variation	3
Mitotic counts	
Dependent on microscope field area	1–3

Final Grading

Add the scores for tubule formation, nuclear pleomorphism, and mitotic count

Grade 1 – well differentiated	3–5 points
Grade 2 – moderately differentiated	6–7 points
Grade 3 – poorly differentiated	8–9 points

Histological Type

The favorable prognosis of certain histological types of invasive carcinoma of the breast is well established. Tubular carcinoma, mucinous carcinoma, and invasive cribriform carcinoma have all been reported to have a favorable prognosis [18]. Other special types of breast cancer carrying an unfavorable prognosis are metaplastic carcinomas and invasive micropapillary carcinomas.

Lymphovascular Invasion

Lymphovascular invasion (LVI) is the finding of carcinoma in the small vessels outside the main tumor mass. LVI is strongly associated with lymph node status and is also an independent prognostic indicator of both local and distant recurrences and survival [19, 20]. The presence of both LVI and nodal metastases confers a worse prognosis than either alone [8]. The presence of LVI after neoadjuvant chemotherapy is also found to be strongly associated with a poor prognosis [21].

Tumor emboli are usually identified within thin-walled vascular channels. It is not possible to determine whether these spaces are lymphatics, capillaries, or venules, and the broad term “lymphovascular invasion” is used.

Vascular invasion should only be assessed in the breast tissue surrounding the tumor and not within it. The most common area to find LVI is within 0.1 cm of the edge of the carcinoma.

Suboptimal fixation is the major reason for misinterpretation of both ductal carcinoma in situ and shrinkage artifacts as LVI. With optimal fixation, processing and sectioning, LVI can be reliably identified in hematoxylin and eosin sections. Therefore, immunohistochemistry is not necessary.

Hormone Receptors

The estrogen receptor (ER) is a nuclear transcription factor that is a regulator of cellular growth, proliferation, and differentiation in the breast epithelium. In addition to its prognostic value, ER is the most important biological marker of clinical response to hormonal therapies, such as tamoxifen [22]. The progesterone receptor (PR) is an estrogen-regulated gene, and its expression therefore indicates a functioning ER pathway. The best response is seen in patients whose tumors express both ER and PR [23].

Immunohistochemical determination of these receptors is the standard tool in current pathology-oncology practice. By immunohistochemistry (IHC) nuclear expression of the ER protein is detected in approximately 80% of breast cancer (Fig. 11.1). Approximately 40% of ER-positive tumors are PR-negative. The lack of PR expression in ER-positive tumors may be a surrogate marker of aberrant growth factor signaling that could contribute to tamoxifen resistance [24].

A cutoff of 1% of tumor cells is recommended for a specimen to be considered as positive for ER/PR because clinical data have indicated that these patients can respond to hormonal treatment [25].

ASCO/CAP guidelines recommend the use of only 10% neutral-buffered formalin as the fixative for breast cancer specimens. The fixation time should not be less than 6 h and not more than 72 h before processing [25, 26].

All tumor-containing areas on a given slide should be evaluated, and the percentage of tumor cells staining positively should be recorded and reported. Only nuclear staining is considered as positive. The intensity of staining is also recorded as weak, moderate, or strong; this measurement represents an estimate of the average staining intensity of the positively stained tumor cells in comparison with the positive control section [25].

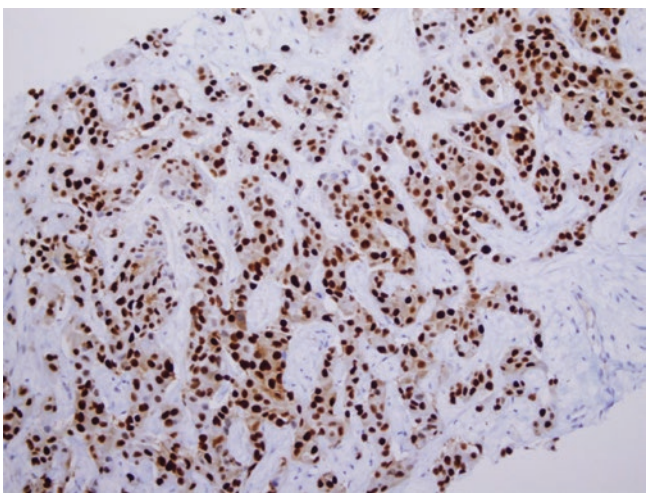

Fig. 11.1 Immunohistochemical determination of estrogen receptor in breast cancer. Brown-stained nuclei are positive for estrogen receptor. Few nuclei with bluish staining lack estrogen receptor

Validated antibodies demonstrating good correlation with patient outcomes in published reports should be chosen for accurate results. The ASCO/CAP panel recommends [25] clones 1D5, 6F11, SP1, and 1D5 + ER.2.123 (cocktail) for ER and clones 1294, 312 and 1A6 for PR.

Receptor assessment should be performed in recurrent disease [13] because a meta-analysis of 47 studies revealed discordance rates of 14% (range 0–67%) for ER and 21% (range 0–62%) for PR between primary and metastatic tumors [27]. Loss of receptor expression is more common (9.17%) than gain (4.51%), and the discordance rates for ER are highest in bone metastases [27].

HER2

The HER2 (ErbB2) gene is located on chromosome 17 and encodes the protein p185, which is a growth factor receptor on the surface of normal breast epithelium. Studies have revealed that this gene is amplified in approximately 15–20% of breast cancers, with resultant elevation of protein expression. Overexpression of HER2 is associated with aggressive histological features and poor prognosis [28].

More important is the use of the HER2/neu oncoprotein as a target for therapy. Trastuzumab (Herceptin) is a humanized monoclonal antibody that targets the extracellular domain of the HER2 receptor. Several randomized clinical trials have demonstrated substantial survival benefits in patients with HER2-positive breast cancer treated with anti-HER2-targeted therapies, such as trastuzumab [28].

The most commonly used methods to evaluate HER2/neu in breast cancer are IHC and in situ hybridization (ISH). ISH determines the number of HER2 copies using a DNA probe coupled to a fluorescent (FISH), chromogenic (CISH), or silver (SISH) detection system.

In clinical practice, accurate assessment of HER2 is essential for selecting patients that are candidates for anti-HER2 treatment. Relatively low and unacceptable concordance rates between local and central laboratories in determining the presence of HER2 protein necessitated refinement of test performance parameters [29]. There is difficulty in interpreting equivocal immunohistochemistry and borderline FISH cases even in highly experienced and validated laboratories [30], which is also one of the major reasons for the need for quality-control procedures. Many trials have also revealed that there is significant variation in HER2 testing, resulting in considerable false-negative and false-positive rates [31]. To overcome these difficulties, ASCO and CAP collaborated to develop HER2 testing guidelines to standardize preanalytical and analytical procedures and quality assurance measures. The adoption of

the ASCO/CAP guidelines in 2007 resulted in the following improvements [32]:

Concordance with FISH has improved; the number of FISH-inconclusive cases decreased from 10.8% to 3.4% (a 64% reduction) [33], resulting in a lower incidence of false-positive IHC results [34].

In 2013, an update of the ASCO/CAP guidelines was published [35], and in May 2018, the same expert panel published another update with the title "HER2 Testing in Breast Cancer – 2018 Focused Update" [36].

According to these guidelines, four categories exist for reporting the results of IHC testing of HER2:

1. Negative
2. Positive
3. Equivocal
4. Indeterminate

These categories are briefly described below [35]:

Negative

Score 0: No staining observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the invasive tumor cells.

Score 1+: Incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumor cells.

Equivocal

Score 2+: Weak to moderate complete membrane staining in >10% of invasive tumor cells.

Cases with circumferential membrane IHC staining that is intense but in ≤10% of tumor cells (heterogeneous but limited in extent) can be considered 2+ equivocal, but additional samples may reveal different percentages of HER2-positive staining [37].

Positive

Score 3+: Circumferential membrane staining that is complete and intense in >10% of invasive tumor cells (Fig. 11.2).

Samples scoring 3+ are regarded as unequivocally positive, and those scoring 0/1+ are regarded as negative. Equivocal scores (2+) mandate further assessment using ISH.

Indeterminate

This category was added in the 2013 update [35]. The test should be reported as indeterminate if technical issues prevent one or both tests (IHC and ISH) from being reported as positive, negative, or equivocal. Examples include inadequate specimen handling, artifacts (e.g., crushing or marked edge artifacts) that make interpretation difficult, analytical testing failure, or if controls are not as expected. The test should be repeated if possible.

ISH Reporting

Recent updated recommendations for various possibilities of average HER2 and chromosome 17 (centromeric probe) signal ratios (HER2/CEP) are summarized below (36). The categorization of ISH groups listed below is recommended when using a double-probe assay:

Group 1: Ratio ≥ 2.0; HER2 ≥ 4.0 signals/cell (Fig. 11.3)
Group 2: Ratio ≥ 2.0; HER2 < 4.0 signals/cell
Group 3: Ratio < 2.0; HER2 ≥ 6.0 signals/cell
Group 4: Ratio < 2.0; HER2 ≥ 4.0 and < 6.0 signals/cell
Group 5: Ratio < 2.0; HER2 < 4.0 signals/cell

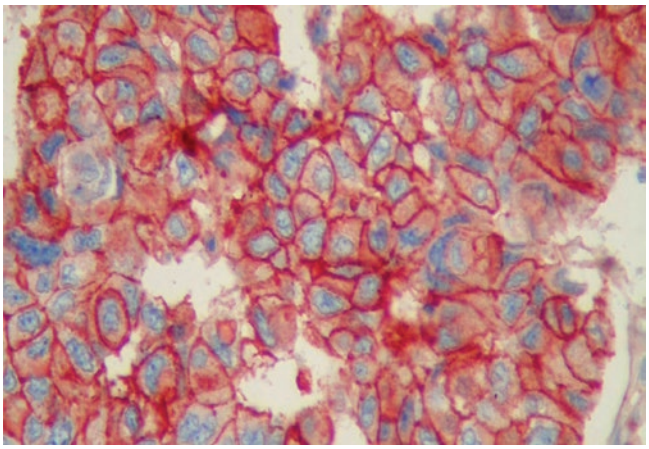

Fig. 11.2 Immunohistochemical score 3+ staining for c-erbB2. Strong, complete membranous staining with the chicken-wire appearance

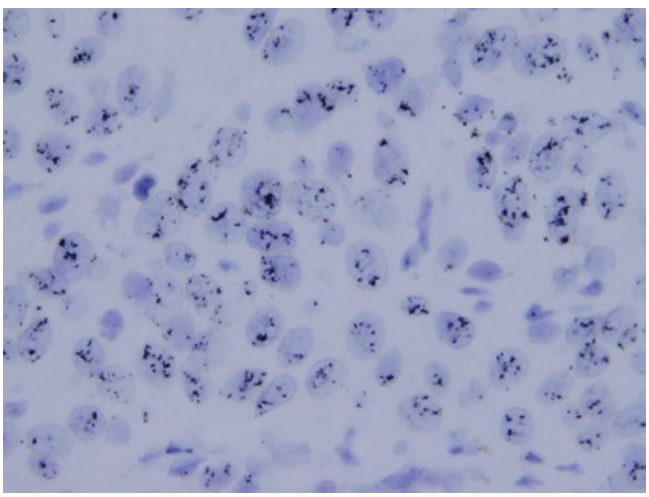

Fig. 11.3 Gene amplification breast carcinoma shown in silver in situ hybridization (SISH). Numerous signals per nuclei forming many clusters

Positive

Group 1
Group 2 AND concurrent IHC 3+
Group 3 AND concurrent IHC 2+ or 3+
Group 4 AND concurrent IHC 3+

Negative

Group 2 AND concurrent IHC 0–1+ or 2+
Group 3 AND concurrent IHC 0–1+
Group 4 AND concurrent IHC 0–1+ or 2+
Group 5

Regarding preanalytical and analytical measures, these guidelines recommend that the cold ischemic time be as short as possible, i.e., less than 1 h. Only formalin-fixed, paraffin-embedded tumor tissue samples are considered appropriate for assay. Surgical specimens should be incised as soon as possible through the tumor to allow the penetration of fixative. The specimens are fixed in 10% neutral-buffered formalin for 6 to 72 h, and routine processing and staining or probing are performed according to standardized analytically validated protocols. Cytological samples prepared from cytological fine needle aspirates of metastatic lesions should also be fixed in 10% neutral-buffered formalin [35].

Measures of Proliferation

There are several methods for evaluating tumor proliferation: the mitotic count, thymidine labeling index (TLI), S-phase fraction by flow cytometry, and IHC using antibodies against specific cell cycle antigens, such as Ki-67 and proliferating cell nuclear antigen (PCNA).

A meta-analysis revealed that evaluations of PCNA, TLI, Ki-67, and the S-phase fraction were all related to worse survival outcomes in early breast cancer.

Ki67

Ki67 antigen is the most commonly used immunohistochemical marker of cell proliferation. Ki67 antigen is expressed by proliferating cells in the late G1, S, and G2/M phases of the cell cycle. Several studies have shown that its expression correlates with other well-known markers of proliferation, such as mitotic index, S-phase fraction, tyrosine kinase, and bromodeoxyuridine incorporation.

Clinical utility of Ki67 immunostaining has been reported in both the adjuvant setting as a prognostic and predictive marker and as an endpoint for neoadjuvant systemic therapy studies [37]. However, its routine clinical use is controversial due to problems with both preanalytical parameters and methodological differences in scoring.

The St Gallen Breast Cancer Consensus Panel endorses Ki67 for differentiating Luminal A from Luminal B tumors. Acknowledging that the cut point between Ki67 "high" versus "low" tumors varies between laboratories, the Panel accepted a level of <14%, which has the best correlation with gene expression on the basis of the results of a single reference laboratory [38, 39].

The International Ki67 in Breast Cancer Working Group is cautious in recommending the routine use of Ki67 [40]. Because of the lack of standardization of evaluation methods Ki67, IHC is not recommended by CAP or ASCO [9].

Like other biomarkers, many variables, e.g., the length of fixation, antigen retrieval method, and choice of antibody clone, affect the results of Ki67 scoring. Among several antibodies against Ki67, only the mouse monoclonal antibody MIB1 has been widely adopted for approximately two decades, but a recent rabbit monoclonal antibody, SP6, has similar performance to MIB1 for visual analysis and improved performance for image analysis [41].

Substantial variability in Ki67 scoring is observed among some of the world's most experienced laboratories, with moderate concordance at best [42], due to differences in scoring, such as tumor region selection, counting method (hot spot versus average), and subjective assessment of staining positivity.

Despite these difficulties, Ki67 still provides useful information in pathology reports. When very low (a few percent), Ki67 can corroborate a Luminal A phenotype in the context of high ER and PR contents; a very high Ki67 index can corroborate a Luminal B phenotype regardless of the percentage of the ER/PR content; and in high-grade triple-negative tumors, a Ki67 index of >50% is almost universal [37, 38]. The recent St Gallen Panel, despite indicating caution regarding the reproducibility of IHC for Ki67 and its use to make clinical decisions due to the variability of this assay, agreed that either grading or Ki67 could be used to distinguish between Luminal A- and B-like tumors [43]. The same Panel [43] also agreed that, when available, gene expression signatures were preferable to standard pathology **when adequate reproducibility is not granted**.

Gene Expression Tests

Several gene expression profiling assays have been developed in an attempt to predict the survival and response of patients to breast cancer therapies. These assays are based on the identification of prognostic gene signatures by microarray.

Perou [44] and his colleagues were the first to distinguish four molecular classes of breast cancer with their "intrinsic" classification:

Luminal cancers: almost all ER positive, express cytokeratin 8 and 18 typical for the breast glands, and are divided into:

Luminal A, which are mostly histologically low grade and express the highest levels of ER and ER-related genes and lowest levels of proliferation-related genes.

Luminal B, which tend to be of higher grade with a worse prognosis, low expression of hormone receptor genes, and high expression of proliferation cluster genes (*MKI67*) and cell cycle-associated genes (*CCNB1* and *MYBL2*) [45].

HER2**-enriched cancers** show amplification and overexpression of the ErbB2 gene, do not express hormone receptors, and have a poor prognosis.

A substantial proportion of breast cancers are HER2 positive but also express ER. These breast cancers are classified as "Luminal B" cancers.

Basal-like breast cancers overlay markedly with ER-, PgR-, and HER2-negative (triple-negative) tumors with a poor prognosis and expression of cytokeratins of the basal layer (e.g., CK 5/6). These cancers are characterized by the expression of genes usually found in the basal/myoepithelial layer of the normal breast, with high levels of proliferation-related genes.

Tumors that were initially classified as "normal breast-like" are now accepted as an artifactual group arising from intermixing of the normal breast epithelium within the tumor.

More recently, additional subtypes have also been described [46]:

The molecular apocrine subtype features activation of androgen receptor signaling.

The interferon subtype is characterized by the high expression of interferon-regulated genes, including STAT1.

The claudin-low subtype comprises tumors that have transcriptomic features suggestive of a "cancer stem cell-like" phenotype with high epithelial-mesenchymal transition (EMT) markers.

Studies have revealed that the most stable separation is between basal-like tumors and tumors classified as of another intrinsic subtype. Approximately 70–75% of cancers classified as basal-like by microarrays are triple negative by IHC, and only 70–75% of cases that are triple negative by IHC are basal-like by microarrays [47]. Furthermore, there is substantial discrepancy in HER2 status by IHC/FISH and microarray results [48].

Many groups have attempted to develop genomic tests based on genomic profiling with the expectation of better predicting clinical outcomes compared to standard pathological and clinical markers. The most common tests are listed below:

MammaPrint

This assay, which was developed by The Netherlands Cancer Institute in 2002, was the first prognostic signature to be described. Gene expression microarray analysis of breast cancer specimens from 78 node-negative patients less than 55 years of age was used to develop the 70-gene prognostic signature [49, 50]. By comparing the expression profiles of tumors from patients who developed distant metastasis within 5 years and those who did not, the researchers identified a prognostic signature. This signature was found to be a predictive parameter of outcome and also predictive of chemotherapy response in patients with poor prognosis. This signature has been validated in several independent cohort studies and shown to add prognostic information beyond standard clinicopathological factors in both node-negative and node-positive patients [51–54].

The commercially available MammaPrint categorizes patient into two groups: (a) low risk and (b) and high risk for breast cancer distant relapse within 10 years of the initial diagnosis. MammaPrint was developed originally for fresh frozen tissue but now has FDA clearance for formalin-fixed paraffin-embedded (FFPE) tissues.

The international, prospective, phase III trial "microarray in node-negative and 1–3 positive lymph node disease may avoid chemotherapy" (MINDACT, NCT00433589) is designed to address whether chemotherapy can be safely avoided in patients who are predicted to be at low risk by the MammaPrint test but at high risk by the clinical assessment Adjuvant! Online [55]. MINDACT showed that approximately 46% of patients who were at high clinical risk for recurrence defined using Adjuvant! might not require chemotherapy. These women had a low genomic risk for recurrence according to MammaPrint [55].

Oncotype DX Test (Genomic Health, Redwood, CA, USA)

Oncotype DX is a quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay that measures gene expression in FFPE samples. Oncotype DX measures a panel of 21 genes, including 16 cancer-related (prognostic) genes plus 5 reference genes, and generates a recurrence score (RS) that classifies patients as of low (RS <18), intermediate (RS 18–30), or high (RS ≥31) risk of recurrence [56]. The 10-year distant recurrence rates of each category are 6.8%, 14.3%, and 30.5%, respectively.

The test was originally designed to predict distant recurrence in 10 years in hormonal receptor-positive and node-negative breast cancers, and its role in lymph node-positive patients remains controversial [57].

Oncotype DX is included in the St Gallen, American Society of Clinical Oncology, and National Comprehensive Cancer Network (NCCN) guidelines as a decision tool enabling the identification of patients who are most likely to benefit from adjuvant chemotherapy and is indicated for women with node-negative, ER-positive breast cancer to determine prognosis in patients recommended to receive at least a 5-year course of endocrine therapy.

The Trial Assigning IndividuaLized Options for Treatment (Rx) (TAILORx) study demonstrated that a group of TAILORx trial participants with low 21-gene recurrence score (Oncotype DX® Recurrence Score®) results of 10 or less who received hormonal therapy alone without chemotherapy had a less than 1% chance of distant recurrence at 5 years [57].

PAM50 (Prosigna)

The PAM50 ROR (NanoString Technologies, Seattle, WA, USA) score is based on a 50-gene test that was developed to identify intrinsic breast cancer subtypes. The ROR is derived from the expression profile of the 50 genes and includes information on tumor size as well. The ROR score has been validated in women with node-negative or node-positive disease and has been shown to classify women into low- or high-risk groups and add prognostic information beyond that of clinical or IHC4 factors [58–60].

In the transATAC trial, the PAM50 ROR score provided more prognostic information than RS, with fewer patients being categorized as intermediate risk and more as high risk. The PAM50 ROR score also provided at least as much information as IHC4 and may provide more information in the node-negative/ HER2-negative group [61]. The ROR score was also evaluated in the ABCSG-8 (Austrian Breast and Colorectal Cancer Study Group 8) trial, in which postmenopausal women with early-stage breast cancer were randomly assigned to receive tamoxifen or anastrozole for 5 years. In this large study, the ROR score was found to add significant prognostic information beyond that of clinical parameters for distant recurrence in the overall population and all subgroups. Better discrimination between the low- and high-risk groups was also confirmed in all subgroups [62].

The Genomic Grade Index (GGI) (MapQuant Dx) (Ipsogen, Marseille, France) is a 97-gene microarray signature that assigns a molecular grade.

The Breast Cancer Index (BCI) (BioTheranostics, San Diego, CA, USA) is a centrally performed qRT-PCR-based assay for use on FFPE tumor blocks.

The EndoPredict test (Sividon Diagnostics GmbH, Koln, Germany) is also a qRT-PCR-based multigene assay that measures the expression of eight cancer genes and three housekeeping control genes (plus one gene to measure the presence of contaminating genomic DNA), which are then combined with the classic prognostic factors of tumor size and node status (EPclin score) to stratify patients with ER-positive Her2-negative cancer into a low or high risk of recurrence if treated with adjuvant endocrine therapy alone.

A trial comparing multiparameter tests (MammaPrint, Oncotype DX, Prosigna, IHC4, and IHC4-AQUA) [63] concluded that according to existing evidence, the different tests provide broadly equivalent risk information for the population of women with ER-positive breast cancers. However, for individual patients, the tests may provide differing risk categorization and subtype information. There is marked disagreement across all tests. Indeed, for all tests, the level of agreement was "moderate."

Major Disadvantages of These Tests

- They are informative only in hormone-receptor-positive, lymph node-negative cases.
- The long-term recurrence risk cannot be predicted except as shown in a study for Prosigna [63].
- The cost-effectiveness of these tests is another concern. These tests are performed in central laboratories, except Prosigna, which can be performed in appropriate local laboratories [60].

In the recent version of the American Joint Commission of Cancer (AJCC) guidelines for breast cancer, prognostic gene signatures are integrated into the staging scheme as prognostic staging [3]. According to this prognostic staging, patients with hormone receptor-positive, Her-2-negative, and lymph node-negative tumors and prognostic gene signatures (Oncotype DX, MammaPrint, EndoPredict, BCI) with a low risk score places the tumor into the same prognostic category as T1a-T1b N0M0 regardless of T size, and the tumor is staged using the AJCC prognostic stage group table as stage I [3].

Potential Markers

Several pathological variables (e.g., EGFR and p53) have been studied to evaluate their importance as prognostic or predictive markers in breast cancer but have not gained any

clinical utility. The most promising examples are tumor-infiltrating lymphocytes (TILs) and programmed cell death ligand (PD-L1) and anti-PD-L1.

Tumor-Infiltrating Lymphocytes (TILs)

Studies have revealed a strong linear relationship between an increase in tumor-infiltrating lymphocytes (TILs) and improved recurrence-free survival for triple-negative and HER2-positive early-stage breast cancers [64].

Higher levels of TILs are also associated with increased rates of pathologic complete response to neoadjuvant therapy in all molecular subtypes [64, 65]. Increased TILs were found to be an adverse prognostic factor for survival in Luminal-HER2-negative breast cancer [65].

An International TILs Working Group established the criteria for the pathological evaluation of TILs to standardize the assessment [66].

PDL-1

Blocking the immune checkpoint receptor "programmed cell death-1" (PD-1) and its ligands, "PD-L1 and PD-L2", is a promising strategy for cancer immunotherapy. The expression of PD-L1 and PD-1 in early breast cancer is associated with higher TIL scores as well as a pathologic complete response. The expression of these proteins correlates with the tumor grade and subtype and is highest in triple-negative breast carcinomas [67].

Due to the promising results for the PD-1 inhibitor pembrolizumab and PD-L1 inhibitor atezolizumab in early-phase studies, many phase III clinical trials are testing their benefit in metastatic TNBCs with or without chemotherapy [67].

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Gene Arrays, Prognosis, and Therapeutic Interventions

12

Cagatay Arslan, Zeki G. Surmeli, and Y. Yavuz Ozisik

Introduction

Breast cancer accounts for one-third of cancer cases among women and is the second most frequent cause of death [1]. It consists of heterogeneous subtypes that differ in clinical presentation and disease course. Improvements in treatment agents and screening procedures have increased the diagnosis of early breast cancer and survival rates. Chemotherapy, endocrine treatment (ET), and trastuzumab comprise the main armamentarium for the adjuvant treatment of breast cancer. Adjuvant chemotherapy and ET decrease the mortality of early breast cancer by approximately 50% [2]. However, early breast cancer patients do not benefit equally from adjuvant ET and/or chemotherapy. The recurrence risk of disease for hormone receptor-positive early breast cancer with tamoxifen after surgery is 15% in 10 years, and the survival benefit of adjuvant chemotherapy in the same group of patients is 3–10% [3, 4]. Patients at high risk are classically identified based on clinicopathological factors, such as age, tumor size, histopathological grade, nodal status, hormone and HER2 receptor expression, and menopausal status. However, using these standard clinicopathological factors might not thoroughly show the individual risk of disease recurrence and the benefit from adjuvant systemic chemotherapy for early breast cancer patients. Many early breast cancer patients do not benefit from adjuvant systemic chemotherapy [5]. Quality-of-life issues, acute and long-term side effects of systemic chemotherapy, and cost of the unnecessary treatments are the main concerns for this group of patients. During the past two decades, the level of knowledge regarding the molecular pathways and underlying genetic changes in breast cancer has improved. However, treatment decisions still often rely on classical histopathological and immunohistochemical techniques. Numerous biomarkers have been studied to define the residual risk of recurrence, but none of them have been recommended for routine use [6–11].

Quantitative approaches for defining prognosis and individualization of treatments are thus required. In recent years, molecular signatures of gene expression have been correlated with the risk of breast cancer recurrence [12–15]. Questions of reproducibility and the need for fresh or fresh-frozen tissue have limited their clinical application. A number of genomic expression tests were developed regarding this unmet medical need. Several genomic expression tests have been developed and validated on specimens of previous phase III studies to improve the prognostication of early breast cancer patients and/or the prediction of the utility of adjuvant systemic treatment (Table 12.1) [16]. In this chapter, using satisfactory data from the literature, we review the most commonly used genomic expression-based tests for predicting the prognosis of and chemotherapy benefit for early breast cancer.

Gene Array Tests

Oncotype DX

The Oncotype DX (Genomic Health, Inc., Redwood City, CA) uses real-time reverse-transcriptase chain reaction (RT-PCR) to quantify the expression levels of 21 genes in formalin-fixed, paraffin-embedded (FFPE) breast cancer tissue samples [17]. The test, which is regulated by Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP), is performed in a central laboratory in the USA. This assay uses a calculation model to generate a risk score ranging between 0 and 100 based on 5 reference and 16 cancer-related genes. The reference genes, *GAPDH*, *ACTB* (*β-actin*), *RPLPO*, *GUS*, and

C. Arslan (✉)
Department of Internal Medicine and Medical Oncology, Bahcesehir University Faculty of Medicine, Istanbul, Turkey

Z. G. Surmeli
Department of Medical Oncology, Ankara Medical Park Hospital, Ankara, Turkey

Y. Y. Ozisik
Department of Medical Oncology, Hacettepe University Cancer Institute, Ankara, Turkey
e-mail: yozisik@hacettepe.edu.tr

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Table 12.1 Summary of selected approved genomic tests for breast cancer [16]

	Oncotype DX™	MammaPrint™	Prosigna™
Generic name	21-gene signature	70-gene signature	PAM50
Company	Genomic Health	Agendia	NanoString
Method	qRT-PCR	Microarray	qRT-PCR nCounter™ (for Prosigna)
Target genes	16 genes 5 controls	70 genes	50 genes 5 controls
Specimen	FFPE	Fresh/frozen FFPE (2011)	FFPE
Analyses	Central Lab.	Central Lab.	Localized
Prognostic index	Risk score (RS)	MammaPrint index	Risk of recurrence (ROR)
Indication	Prognostic Predictive	Prognostic Predictive	Prognostic Intrinsic subtype classifier
Population studied	N0-1, ER positive	N0-1, <61 years old	N0-1, ER positive, postmenopausal
Evidence	NSABP B14 NSABP B20 ECOG 2197 TransATAC NSABP B28 SWOG 8814 JBCRG Northern California	TRANSBIG	NCIC MA.12 NCIC MA.5 ABCSG-8 TransATAC

TFRC, are used for normalization. The cancer-related genes used in the assay include genes related to proliferation (*Ki-67*, *Survivin*, *MYBL2*, *CCNB1* [cyclin B1], and *STK15*), invasion (*CTSL2* [cathepsin L2] and *MMP-11* [stromolysin 3]), and hormone receptors (*ER*, *PR*, *BCL2*, and *SCUBE2*) as well as HER2 (*GRB7* and *HER2*), *GSTM1*, *BAG1*, and *CD68*.

A multistep approach was adopted for developing the assay for expression levels of tumor-related genes by the Genomic Health researchers. Routinely used tumor blocks were used for this purpose and to validate the assay. High-throughput RT-PCR was used to quantify gene expression levels in FFPE tumor tissue sections [18]. In the second phase, they chose 250 candidate genes from the published literature, genomic databases, and experiments based on DNA arrays performed on fresh-frozen tissue [12–14, 19]. In the third phase, data from 447 breast cancer patients from 3 different studies, including patients from the tamoxifen-only arm of the NSABP B-20 trial, were used to test the correlation of 250 candidate genes with the recurrence of breast cancer [20–22]. In the fourth phase, a panel of 16 cancer-related genes and 5 reference genes were selected from the results of three studies based on the strength of their performance in the previous studies and the consistency of the primer and probe performance in the assay. An algorithm was designed based on the expression of these 21 genes for computing a recurrence score (RS) for each tumor sample [17]. The possible RSs ranged between 0 and 100, where a higher recurrence score indicated a higher likelihood of recurrence. The RS was derived from the reference-normalized expression measurements of the 16 cancer-related genes. Reproducibility within and between blocks was also assessed. Based on their RS, patients were divided into three risk categories, including low (<18), intermediate (18–30), and high (≥31). The RS was prognostic for estrogen receptor (ER)-positive early breast cancer patients with positive (1–3 lymph nodes involved) and negative lymph node involvement who were treated with tamoxifen. A low RS predicted no likelihood of recurrence in 10 years and little benefit from chemotherapy. Adjuvant chemotherapy showed benefits in high-RS patients but not in patients with a low RS.

Oncotype DX was tested in a community-based population from Northern California [23]. The 21-gene assay was prognostic in ER-positive patients with and without tamoxifen treatment ($p = 0.003$ and $p = 0.03$, respectively). There were 220 patients and 570 controls in this study. Archived tumor tissues were tested. Nearly 50% of the patients were in the low-risk group. The risk for death from breast cancer within 10 years in tamoxifen-treated patients was 2.8 (95% confidence interval (CI) 1.7–3.9), 10.7 (95% CI 6.3–14.9), and 15.5% (95% CI 7.6–22.8) in the low-, intermediate-, and high-risk patients, respectively. The risk of death within 10 years in patients with no tamoxifen treatment was 6.2 (95% CI 4.5–7.9), 17.8 (95% CI 11.8–23.3), and 19.9 (95% CI 14.2–25.2) in low-, intermediate-, and high-risk patients, respectively.

The Oncotype DX assay was further tested in hormone receptor-positive early breast cancer patients from Japan [24]. All patients were treated with tamoxifen. Among the total patients, 280 patients had tumor tissues that were adequate for the assay. Of these patients, 48% were in the low-risk group, 20% were in the intermediate-risk group, and 33% were in the high-risk group. Distant recurrence risks in 10 years were 3.3 (95% CI 1.1–10), 0, and 24.8% (95% CI 15.7–37.8) in low-, intermediate-, and high-risk lymph node-negative patients, respectively. Differences between the low-risk and high-risk groups for distant recurrence were significant (log rank $p < 0.001$). There was also a significant difference in overall survival (OS) between the low-risk and high-risk groups ($p = 0.008$).

Pivotal Trials of Oncotype DX

NSABP B14 Trial

The data obtained from the large, multicenter NSABP B14 trial and the FFPE tumor tissues were used to validate this 21-gene RT-PCR assay for RS detection in early-stage, node-

negative, ER-positive breast cancer patients, who had been treated with tamoxifen [17]. The RS was calculated as low, intermediate, or high for each patient, as previously defined. The data from the prospective NSABP B14 trial were used for validation. Cutoff points were determined based on the results of the NSABP B14 study. According to the study results, the rates of recurrence in 10 years were 6.8 (95% CI 4–9.6), 14.3 (95% CI 8.3–20.3), and 30.5% (95% CI 23.6–38.4) for the low-, intermediate-, and high-risk groups, respectively. The risk of recurrence in the high-risk group was similar to that for the lymph node-positive patients [25]. In this study, 51% of the patients were in the low-risk group, and 27% of the patients were in the high-risk group. Age and tumor size are standard factors used for predicting recurrence. However, when RS was added to the multivariate Cox model, recurrence could no longer be predicted based on age or tumor size. Moreover, all patients with tumors smaller than 1 cm ($N = 109$) were not in the low-risk group. Forty-four of these patients were in the intermediate- or high-risk groups, which means a risk of 15–20% for recurrence in 10 years. A subgroup of the patients with low-grade tumors also showed high RS and high rates of recurrence in this study. Additionally, concordance of pathologists was moderate for poorly differentiated tumors and low for the well- and intermediate-differentiated tumors. HER2 amplification was detected in 55 of 668 patients (8%). The 10-year recurrence was 75% (95% CI 63.2–86.9) in HER2-amplified and 86% (95% CI 83.1–88.9) in HER2-nonamplified breast tumors ($p = 0.08$). In the Cox model including RS and classical factors (estrogen, progesterone receptors, HER2 DNA amplification), any RS was a significant predictor of distant recurrence. The RS provided significant predictive power independent of age or tumor size ($p < 0.001$). The RS was also prognostic ($p < 0.001$) and could be used as a continuous function to predict the recurrence in each patient [17]. It is important to note that all patients were treated with tamoxifen; thus, outcomes must be evaluated considering the effects of both tamoxifen and the natural disease course.

TransATAC Trial

ATAC is a phase III trial that included 9366 postmenopausal early breast cancer patients, including both ER-positive and ER-negative patients. This study compared 5 years of adjuvant treatment with tamoxifen alone, anastrozole alone, and tamoxifen in combination with anastrozole [26]. In the translational arm of the ATAC study (TransATAC), the risk of recurrence was evaluated using the Oncotype DX assay in axillary node-negative or node-positive, hormone receptor-positive postmenopausal breast cancer patients who were treated with tamoxifen and anastrozole. RNA was extracted from 1372 tumor blocks from patients in the monotherapy arms of this study. Available scores were obtained from 1231 patients (node positive, $N = 306$; node negative, $N = 872$; node status unknown, $N = 52$). Multivariate analysis showed that RS was significantly associated with time to disease recurrence ($p < 0.001$ and $p = 0.002$ for node-negative and node-positive patients, respectively). There was a poor correlation between RS and "Adjuvant! Online" for estimating prognosis ($p < 0.001$). In node-negative patients, the disease recurrence rate within 9 years was 4, 12, and 25% for the low-, intermediate-, and high-risk groups, respectively. In node-positive patients, the 9-year disease recurrence rates were 17, 28, and 49% for the low-, intermediate-, and high-risk groups, respectively. The hazard ratio (HR) for disease recurrence was 2.7 for high-RS and 1.8 for low-RS lymph node-positive patients. Similar results were obtained for OS. For any RS, the risk of distant recurrence was higher in node-positive than node-negative patients and in patients with 4 or more positive nodes than in patients with 1–3 positive nodes. Prognostic value was similar in the tamoxifen and anastrozole groups. In the original study, anastrozole showed a 16% risk reduction for distant recurrence compared with tamoxifen [27]. However, in this study, HRs for distant recurrence was similar in the tamoxifen and anastrozole treatment arms, and RS did not interact with any treatment arm. Relative risk reductions for anastrozole compared with tamoxifen were similar in all RS groups. A higher risk reduction with anastrozole in patients with high RS might be expected. However, the number of cases was too small to allow for such an analysis.

Studies of Prediction for Chemotherapy

NSABP B20 Trial

In the original NSABP B20 trial, there were 2363 ER-positive, axillary lymph node-negative early breast cancer patients [28]. The aim was to examine the benefit of CMF (cyclophosphamide, methotrexate, 5-fluorouracil) or MF (methotrexate, 5-fluorouracil) chemotherapy followed by 5 years of tamoxifen treatment. The Oncotype DX assay was studied in these tumor blocks, and prospective clinical outcomes were investigated in that study group to examine whether the Oncotype DX assay could predict the benefits of chemotherapy. Tumor blocks with sufficient tumor tissue for the RS assay were obtained in 670 patients, and adequate samples were obtained from 651 tumor blocks. Of the total patients, 227 (29.5%) were treated with tamoxifen, and 424 (27.7%) were treated with chemotherapy plus tamoxifen. Among the 651 assessable patients, the proportions of patients without distant recurrence in 10 years were 92.2% in the chemotherapy plus tamoxifen group and 87.8% in the tamoxifen group. Disease recurrence (locoregional or distant) was observed in 90.1% of the chemotherapy plus tamoxifen patients and in 83.5% of the tamoxifen patients. The 10-year survival estimate was 89.5% in patients treated with chemotherapy plus tamoxifen and 86.4% in patients

treated with only tamoxifen. There were 353 (54.2%) patients with low RS, 134 (20.6%) with intermediate RS, and 164 (25.2%) with high RS.

This study showed that the benefit of chemotherapy was not equivalent across all ER-positive and axillary lymph node-negative early breast cancer patients in the NSABP B20 study. The Oncotype DX assay was shown to predict the chemotherapy (CMF or MF) benefit in this group of patients. The magnitude of the benefit of chemotherapy for distant recurrence was greater for the high-RS group than for the intermediate- and low-RS groups. A Kaplan-Meier estimate indicated that 10 years of freedom from the disease recurrence improved from 60% to 88% in patients in the high-RS group. There was no demonstrable risk reduction with chemotherapy regarding the 10-year disease recurrence rates in the low-RS group (relative risk, 1.31; CI, 0.46–3.78). A significant risk reduction (27.6% reduction in absolute risk) was shown with chemotherapy in the high-RS group (relative risk, 0.26; CI, 0.13–0.53). The benefit of chemotherapy was not clear in the intermediate-RS group (relative risk, 0.61; CI, 0.24–1.59). In a multivariate analysis of Cox models containing chemotherapy treatment and RS, the interaction between chemotherapy treatment and RS was significant ($p = 0.038$).

NSABP B28 Trial

The current standard adjuvant treatment of ER-positive, axillary lymph node-positive breast cancer in pre- or postmenopausal patients is ET plus chemotherapy (ET + CT) [2]. Nevertheless, exploratory analyses show that breast cancer patients with high levels of ER positivity and a lack of HER2 overexpression may not derive substantial benefit, even if those patients show positive axillary nodes [29, 30].

The original phase III NSABP B28 trial included 3060 pre- or postmenopausal ER-positive ($N = 2019$) and ER-negative/borderline ($N = 1041$) axillary lymph node-positive, early breast cancer patients [31].

In this trial, 2687 patients received concurrent ET. Patients received tamoxifen, and patients were randomized into AC (doxorubicin plus cyclophosphamide) and AC plus paclitaxel chemotherapy groups. RS analysis was performed in 1065 tumor blocks of ER-positive patients treated with endocrine therapy [32]. Of the 1065 patients, 386 (36%) had low RS (<18), 364 (34%) intermediate RS (18–30), and 315 (30%) high RS (≥31). RS was a significant predictor of locoregional recurrence risk (LRR), DFS event, and mortality in univariate analyses both in AC and AC plus paclitaxel-treated patients. Median follow-up time was 11.2 years. Ten-year cumulative incidence rates of events for DFS, distant recurrence, and OS were different between low-, intermediate-, and high-RS groups. Adding paclitaxel to AC derived no benefit regarding outcomes in 10 years in low-RS group of patients. Disease recurrence rate was similar in AC and AC plus paclitaxel treated patients in low-RS group for adjuvant therapy (19.2 (95% CI 14–25.8) and 19.1 (95% CI 14.1–25.5), respectively) (HR 0.95; 0.62–1.45). Death rate was also similar in AC and AC plus paclitaxel-treated patients in low-RS group (8.5 (95% CI 5.2–13.7) and 11.5 (95% CI 7.7–16.9), respectively) (HR 1.28; 0.72–2.27). However paclitaxel benefit was evident in intermediate- and high-RS group of patients. In patients with low RS, adding paclitaxel to anthracycline-based adjuvant treatment did not provide any additional benefit to the final outcome. Based on these results, aggressive chemotherapy may not be warranted for subgroup of patients with low RSs [32].

A subgroup of ER-positive breast cancer patients shows a low risk of disease recurrence even with positive axillary nodes. These patients are unlikely to benefit from adjuvant chemotherapy. Oncotype DX RS was also shown to be prognostic in axillary lymph node-positive, concordant with axillary lymph node-negative tamoxifen-treated breast cancer patients. Oncotype DX RS may also predict patients who will not benefit from the addition of chemotherapy (i.e., patients with low RS).

SWOG 8814 Trial

Recent data from the SWOG 8814 trial showed Oncotype DX to be prognostic and predictive in ER-positive, axillary lymph node-positive breast cancer patients [33]. The SWOG 8814 study, a parent trial, was a phase III trial and showed that adjuvant CAF (cyclophosphamide, doxorubicin, 5-fluorouracil) chemotherapy plus tamoxifen was superior to tamoxifen alone for DFS and OS in postmenopausal, estrogen and/or progesterone receptor (ER/PR)-positive, axillary lymph node-positive breast cancer patients. The Oncotype DX assay was applied to specimens from a tumor bank for RS analysis. Genomic Health, Inc., performed that study, and the investigators were blinded to patients' clinical data and outcome. Clinical data and outcome were combined with the Oncotype DX RS results after all assays were completed. Tumor samples were available for 664 of 1477 patients (45%). The parent trial included three arms: tamoxifen only, concomitant CAF and tamoxifen, and sequential CAF and tamoxifen. In this translational study, the concomitant CAF and tamoxifen arm was omitted due to inferior efficacy. Sufficient RNA was obtained from 149 patients in the tamoxifen arm and 219 patients in the CAF plus tamoxifen arm (367 total patients) for RT-PCR analysis. The 21-gene RS was prognostic in tamoxifen-treated, ER-positive, axillary lymph node-positive patients and predictive for adjuvant chemotherapy with CAF regimen in patients with high RS [33]. The RS was prognostic for DFS in tamoxifen-treated patients ($p = 0.006$). There was a significant benefit of chemotherapy in the high-RS group (log rank $p = 0.03$; HR, 0.59; 95% CI, 0.35–1.01), but no benefit was detected in patients with low RS (log rank $p = 0.97$; HR, 1.02; 95% CI,

0.54–1.93) regarding DFS. A low RS identifies patients who may not benefit from adjuvant chemotherapy despite positive nodes. The benefit of adjuvant chemotherapy in the high-RS group was independent from the number of positive nodes. The benefit of chemotherapy on DFS was significant for the first 5 years of follow-up. However, there was no additional prediction of chemotherapy benefit for DFS beyond 5 years, despite the continued presence of the cumulative benefit after 10 years. Similar results were obtained for the prognostic value of the 21-gene RS. Ten-year OS estimates were 77, 68, and 51% for patients with high, intermediate, and low RS, respectively. Breast cancer-specific survival (BCSS) and OS were significantly better in patients with high RS but not low and intermediate RS when treated with CAF and tamoxifen compared to tamoxifen only. In the exploratory analysis after adjustment for classical risk factors, including age, histopathological grade, race, tumor size, PR status, and HER2 status, both treatment and RS remained significant. The limited number of samples obtained from patients included in the original study was an important caveat of this study. The probability of a chemotherapy effect in low-RS patients cannot be ruled out completely due to the small sample size and broad CI ranges in this study. This study challenged the standard adjuvant chemotherapy treatment of patients with axillary lymph node-positive, ER-positive breast cancer.

ECOG 2197 Trial

In the original ECOG 2197 trial, there were 2185 pre- or postmenopausal ER-positive and lymph node-negative and node-positive (1–3 nodes) breast cancer patients treated with doxorubicin-containing chemotherapy or docetaxel plus ET [34]. The Oncotype DX assay was tested in the doxorubicin-containing chemotherapy arm ($N = 465$) and was a significant prognostic marker of disease recurrence in this study group. There was a significant correlation between RS and 5-year disease recurrence rates in both lymph node-positive and node-negative patient groups ($p < 0.001$). Of the lymph node-positive patients, 46% were in the low-RS group. Patients with zero or one positive node had a 5-year disease recurrence rate under 3%, and patients with two or three positive nodes had a 5-year disease recurrence rate of 8%. However, good outcomes may be attributed to low RS, chemotherapy, or both. Ten-year follow-up analysis also showed that RS was still a prognostic marker for disease recurrence for lymph node-positive and lymph node-negative breast cancer patients [35].

Based on all of these studies, Oncotype DX has shown a high level of utility for estimating the risk of distant recurrence in 10 years and the benefit of chemotherapy in ER-positive, HER2-negative early breast cancer patients with up to three metastatic lymph nodes. The Oncotype DX assay has been adopted by the international guidelines of ESMO, ASCO, NCCN, and St. Gallen [36–39].

Impact on Treatment Decision

The Oncotype DX assay changed treatment decisions made on classical clinicopathological factors in several studies. Its impact on decision-making seems to increase with the results of the prospective trials. In a survey study in the USA, oncologists were asked about the treatment recommendation for their most recent ER-positive, lymph node-positive breast cancer patient after getting the Oncotype DX assay result [40]. The vast majority of the patients had one to three positive lymph nodes (92.5%), and most (96.5%) had a tumor size smaller than 5 cm. Of the 160 physicians who responded (16% of the original sample), 86% made decisions before obtaining the RS. However, 51% of those oncologists changed their decision after receiving the RS result. Treatment decisions were changed to ET alone from ET + CT in one-third of those cases. Chemotherapy was eliminated in 49% of the intermediate-RS group and in 21% of the low-RS group of patients. Additional chemotherapy decisions were made in 9% of the cases.

In a study from Israel, 951 patients with one to three positive nodes received ET with or without chemotherapy [41]. Treatment decisions for 282 patients were made according to Oncotype DX, and there were 669 controls. In the Oncotype DX group, chemotherapy was given to all patients with high RS (100%), 37% of patients with intermediate RS, and 7% of patients with low RS. Chemotherapy was given to 24.5% of all Oncotype DX patients and 70.4% of controls ($p < 0.001$). However, the patients' clinicopathological features were not balanced between the groups, and patients in the control group had bigger tumor sizes, higher grade, and more positive lymph nodes. After adjustment according to these factors, Oncotype DX was associated with a 65% decrease in chemotherapy usage.

A study of 50 lymph node-positive breast cancer patients from Australia showed a 26% change in the treatment decision with Oncotype DX, and the majority of the treatment decisions were to omit chemotherapy [42]. A study of 42 axillary lymph node-positive ER-positive breast cancer patients (22 with macrometastasis) from Spain showed that 73% of the patients had low RS [43], and the recommendation of chemotherapy decreased from 55% to 17% with the use of Oncotype DX ($p = 0.021$).

In a recent meta-analysis of eight studies (1437 patients) on the impact of Oncotype DX on treatment decisions, the adjuvant therapy recommendation changed in 33.4% of patients due to Oncotype DX RS versus the decision recommended based on clinicopathological factors [44]. After Oncotype DX, the adjuvant chemotherapy recommendation was 83.4% in patients with high RS,

37.4% with intermediate RS, and 5.8% with low RS. The overall chemotherapy recommendation was 28.2% after using the Oncotype DX assay.

Prospective Clinical Trials

The phase III SWOG 1007 (RxPONDER, NCT01272037) trial is designed to determine the effect of chemotherapy with adjuvant endocrine therapy in patients with ER-positive, 1–3 axillary lymph node-positive breast cancer and low or intermediate RS (less than or equal to 25) [45]. This study will provide important information about the patients in whom chemotherapy can be omitted in the low- and intermediate-RS groups. It will also address issues of quality of life and long-term side effects such as premature menopause and weight gain.

The prospective TAILORx trial was designed mainly to investigate the benefits of chemotherapy in the intermediate-RS group (scores of 11–25) of ER-positive, axillary lymph node-negative early breast cancer patients [46]. The Oncotype DX assay was applied prior to treatment, and patients were divided into three RS groups: low (RS <11), intermediate (RS 11–25), and high (RS >25). Patients in the low-RS group were treated with adjuvant ET, and those in the high-RS group were treated with ET + CT. Enrollment was completed in 2010, and 10.253 patients were recruited in the study. Early results of low-RS (<11) subset of patients have been reported [47]. Overall, 1626 (15.9%) patients had a low (<11) RS. At 5 years, invasive disease-free survival rate was 93.8%, and recurrence-free survival rate was 98.7%. These results support that low-RS scores may identify low-risk patients with ER-positive, node-negative breast cancer, who have very good prognosis and may not benefit from adjuvant chemotherapy. Overall survival results of 6711 (69%) intermediate-risk (11–25) patients from TAILORx trial treated with endocrine compared to chemoendocrine treatments were published in June 2018 [48]. Endocrine treatment was not inferior compared to chemoendocrine treatment in intermediate-risk patients. However, with a recurrence score of 16–25, a subgroup analysis showed survival benefit of chemotherapy in 50 years of age or younger. WSG PLAN B is a phase III trial run by the West German Study Group. In this study, two different chemotherapy regimens were compared in 3198 patients with node-positive or high-risk node-negative HER2-negative early breast cancer [49, 50]. Oncotype DX was performed in hormone receptor-positive tumors, and chemotherapy was omitted if the RS was low (≤11). Of the 2568 hormone receptor-positive tumors, 18.1%, 60.4%, and 21.6% were classified as low (≤11), intermediate (12–25), and high RS(>25), respectively. In patients with low RS, who had pN0 to pN1 breast cancer and were treated with ET alone, 3-year DFS was 98.4%. Three-year DFS was 97.5% and 94.9% in intermediate- and high-risk patients treated with chemotherapy.

WSG ADAPT is another phase II/III trial in pre- and postmenopausal early breast cancer patients [49]. It aims to individualize the adjuvant treatment decision in early breast cancer patients and is using the Oncotype DX assay with conventional prognostic factors (nodal status). Dynamic changes in proliferation rates and apoptosis were checked after a short course of treatment. The aim was to establish early predictive surrogate molecular markers for outcome by assessing the response to a repeated biopsy after 3 weeks of induction treatment.

MammaPrint

MammaPrint is based on DNA microarray technology. Using gene expression profiling, the Netherlands Cancer Institute™ and its spin-off company Agendia™ developed a 70-gene prognostic signature called MammaPrint™ for axillary lymph node-negative early breast cancer patients. These 70 genes are involved in the cell cycle, invasion, proliferation, angiogenesis, metastasis, and signal transduction. Oncotype DX assesses none of these genes. The 70-gene signature was developed for a dichotomous risk classification as low or high risk of disease recurrence in 5 years in a cohort of node-negative breast cancer patients, who were not treated systemically [14]. This gene signature defined the low- and high-risk groups for 5-year disease recurrence [14]. The low-risk group was identified as having a 13% risk of distant metastasis in 10 years, and the high-risk group was identified as having a 56% risk of distant metastasis in 10 years without adjuvant treatment. The first validation study was performed with the samples from the tumor bank of the Netherlands Cancer Institute. A total of 295 tumor samples were obtained from breast cancer patients (ER negative and ER positive, lymph node negative and node positive, untreated, treated with chemotherapy and/or endocrine therapy) [15]. Metastasis-free survival and OS were higher in the low-risk group than in the high-risk group in lymph node-negative and node-positive patients, and all patients were evaluated using the MammaPrint assay. Of the patients with low-risk profiles, 85% were disease recurrence-free, and 50.6% of the patients with high-risk profiles were disease recurrence-free in 10 years. In the multivariate analysis, independent prognostic markers for disease recurrence were high-risk profile with MammaPrint™, tumor size, and absence of chemotherapy. This 70-gene expression profile was developed on microarrays containing 25,000 60-mer oligonucleotides that are not designed for routine clinical practice. This 70-gene prognosis profile, which was translated to a customized microarray (MammaPrint™), contains a reduced set of 1900 probes suitable for high-throughput processing [51]. It allows the use of less RNA and a short processing time of 5 days. To validate its prognostic value, the RNA of 162

patients from two previous studies was used for hybridization with this custom array. Custom microarray results were compared with the original analysis, and they showed an extremely high correlation of prognosis prediction ($p < 0.0001$). This first study showed that this small, custom microarray could be a reliable diagnostic tool for predicting the outcome of disease in breast cancer patients. MammaPrint™ was intended for use in women under 61 years of age with stage I and II, ER-positive or ER-negative early breast cancer. The US FDA approved it in 2007 for determining the risk of distant recurrence at 5 and 10 years but not for predicting the benefit of chemotherapy. Based on the evidence supporting the prognostic value of MammaPrint™, it has been incorporated into the ESMO and St. Gallen guidelines as a prognostic tool [37, 38]. The test was initially developed and validated for fresh or fresh-frozen tumor tissue [15, 52]. A recent study of 211 matched samples showed 91.5% consistency between using FFPE and using fresh or frozen specimens [53].

Another MammaPrint™ validation study was performed by TRANSBIG researchers in 307 patients with early, axillary lymph node-negative breast cancer, with a median follow-up of 13.6 years, in 5 different European countries [54]. MammaPrint™ results were compared with other clinicopathological risk classification systems. Patients were defined as low risk if their 5-year probability of distant metastasis-free survival was above 90%. The clinicopathological low-risk group was defined as a 10-year OS probability greater than 88% (for ER-positive patients) or 92% (for ER-negative patients). MammaPrint was identified as an independent prognostic marker for distant metastasis-free survival and OS. In the univariate analysis of the 70-gene signature in high- versus low-risk patients, the HR for time to distant metastasis was 2.32 (95% CI, 1.35–4; $p = 0.002$), HR for OS was 2.79 (95% CI, 1.6–4.87; $p < 0.001$), and the HR for DFS was 1.56 (95% CI, 1.04–2.16; $p = 0.032$). MammaPrint was a more powerful prognostic tool for distant metastasis-free survival and OS in comparison with clinicopathological factors defined by the Nottingham Prognostic Index, St. Gallen Criteria, or Adjuvant! Online. When adjusted for the clinical risk groups, the 70-gene risk score results showed HRs of 2.13 (95% CI 1.19–3.82), 2.63 (95% CI 1.45–4.79), and 1.36 (95 CI 0.91–2.03) for time to distant metastasis, OS, and DFS, respectively.

Data for Patients with Positive Lymph Nodes

The 70-gene assay result was also prognostic in lymph node-positive breast cancer patients and predicted the outcome better than the other classical clinicopathological factors. A validation study was conducted with 241 patients from two European institutes, with one to three positive lymph nodes and operable T1–T3 early breast cancer. Adjuvant treatment decisions were made according to national guidelines from the two different countries (the Netherlands and Italy). MammaPrint™ predicted that patients in the low-risk group would have 91 and 96% distant metastasis-free survival and BCSS, respectively, in 10 years [55]. For the poor prognostic group, the 10-year distant metastasis-free survival and BCSS were 76 and 76%. The 70-gene risk score was superior to other clinicopathological factors for predicting BCSS. In the multivariate analysis, the 70-gene risk score HR was 7.19 (95% CI 1.8–28.43; $p = 0.005$) for BCSS.

The MammaPrint™ assay was studied using frozen tumors from 173 N2 (with 4–9 axillary lymph node metastatic) breast cancer patients from the Netherlands and Italy [56]. Seventy patients (40%) were classified as genomic high risk, and 103 (60%) were classified as genomic low risk. Patients in the genomic high-risk group were more often grade 3 (60%), hormone receptor negative and HER2 positive (25%). The 5-year OS was 97 and 76% in the low- and high-risk groups, respectively ($p < 0.01$). Distant metastasis-free survival in 5 years was 87% for low-risk patients and 63% for high-risk patients ($p < 0.01$). In the luminal A subgroup, the 70-gene assay result was the only independent risk factor for distant metastasis and breast cancer-specific death in breast cancer patients with 4–9 positive lymph nodes. MammaPrint can be integrated into the selection of treatment strategy in this group of patients.

Data on Adjuvant Treatment Decisions

The performance of the 70-gene signature was assessed in a prospective observational community-based study that included 427 early breast cancer (cT1-3N0M0) patients [57]. Adjuvant systemic treatment decisions were given considering the 70-gene signature, the Dutch CBO 2004 guidelines, and the preferences of the physicians and patients. The median follow-up duration was 62 months. In the 70-gene signature group, 15% (33/219) of low-risk patients and 81% (169/208) of high-risk patients received adjuvant chemotherapy. The 5-year probability of disease recurrence-free survival according to the 70-gene score was 97.0% for low-risk and 91.7% for high-risk patients. The 5-year probabilities of disease recurrence-free survival for the Adjuvant! Online low- and high-risk groups were 96.7 and 93.4%, respectively. There were 124 patients in the 70-gene signature low-risk and Adjuvant! Online high-risk groups. Of these patients, 94 (76%) did not receive adjuvant chemotherapy, and the 5-year disease recurrence-free interval was 93.4%. The adjuvant chemotherapy decision would decrease by 32% (94/295) if the 70-gene signature had been used in the Adjuvant! Online high-risk group of patients. The prognostic value of the 70-gene signature was again shown for the 5-year probability of disease recurrence-free survival, showing that in the low-risk 70-gene signature group, omission of chemotherapy did not compromise outcome.

The 70-gene assay was studied for the predictive value of chemotherapy in 541 patients whose tumor samples were obtained from 7 previously reported studies conducted between 1984 and 2006 with known adjuvant treatment data [58]. There were 315 patients in the ET group and 226 patients in the ET + CT group. According to the 70-gene assay, 252 (47%) patients were in the low-risk group, and 289 (53%) patients were in the high-risk group. BCSS at 5 years was 97% in patients treated with ET and 99% in patients treated with ET + CT in the low-risk group (HR, 0.58; 95% CI, 0.07–4.98, $p = 0.20$). In the high-risk group, BCSS was 81% in patients treated with ET and 94% in patients treated with ET + CT (HR, 0.21; 95% CI 0.07–0.59, $p < 0.01$). Distant disease-free survival in the low-risk group was 93% in patients treated with ET and 93% in patients treated with ET + CT (HR, 0.26; 95% CI, 0.03–2.02, $p = 0.2$). In the high-risk group, distant disease-free survival was 76% and 88% in the patients treated with ET and ET + CT, respectively (HR, 0.35; 95% CI, 0.17–0.71, $p < 0.01$). There was a significant benefit of adding CT to ET for the group identified as high risk according to the 70-gene signature (Fig. 12.1). This benefit was not significant in patients with low risk according to the 70-gene signature. High-risk ER-positive breast cancer patients may be treated with more intensive treatment (i.e., chemotherapy) strategies in addition to adjuvant endocrine therapies.

MammaPrint can affect the adjuvant chemotherapy recommendation. Using the MammaPrint™ assay may decrease the variability of adjuvant treatment decisions. A cohort of 194 patients from 4 different countries in Europe was used to measure the impact of MammaPrint™ on adjuvant treatment decisions [59]. Patients' clinicopathological data were sent to different multidisciplinary teams with and without the MammaPrint™ assay result, and adjuvant treatment decisions were provided for each patient. The Dutch, Belgian, Italian, and Spanish teams changed treatment decisions in ER-positive and HER2-negative patients in 37, 24, 28, and 35% of cases, respectively. MammaPrint™ increased the interinstitutional agreement in treatment advice (i.e., whether to utilize chemotherapy) from 51% to 75%.

Prospective Trials

The MINDACT (Microarray In Node-negative and 1–3 node-positive Disease may Avoid ChemoTherapy) trial was designed to investigate the clinical utility of MammaPrint™ (70-gene profile) with clinicopathological criteria for the selection of ER-positive early breast cancer patients with one to three positive nodes for adjuvant chemotherapy. The clini-

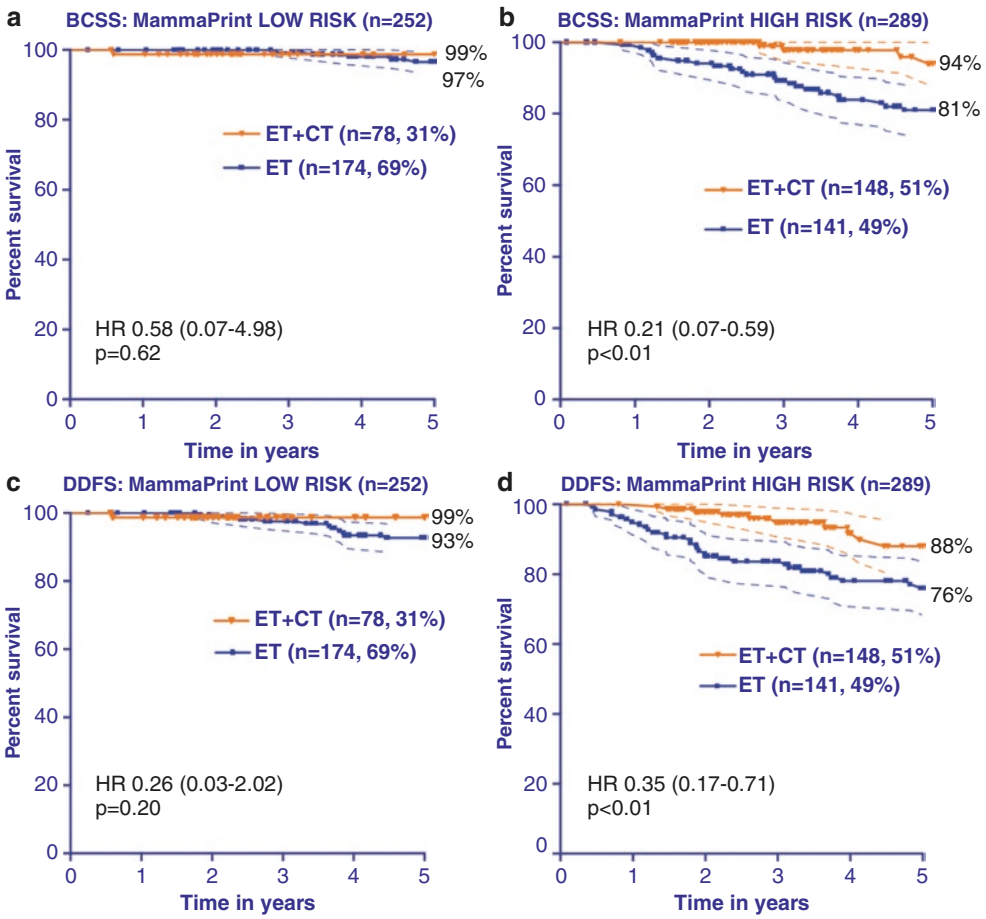

Fig. 12.1 Panel (**a**, **b**), 5-year breast cancer-specific survival by treatment within the 70-gene signature groups (70-gene low risk on the *left*, high risk on the *right*). Panel (**c**, **d**), 5-year distant disease-free survival by treatment within the 70-gene signature groups (70-gene low risk on the *left*, high risk on the *right*). Abbreviations: *BCSS* breast cancer-specific survival, *DDFS* distant disease-free survival, *n* number, *ET* endocrine therapy, *ET + CT* endocrine + chemotherapy, *HR* univariate hazard ratio. (Reproduced from Reference [58] with kind permission of Springer Science + Business Media)

copathological risk definition was made using a modified version of Adjuvant! Online. MammaPrint™ also defined patients as low or high risk. The MINDACT trial hypothesizes that using the MammaPrint™ assay will outperform the clinicopathological classification through the better classification of patients as high or low risk and by reducing chemotherapy usage by 10–20% without impairing the outcome. Patients who were defined as low risk by both Adjuvant! Online and MammaPrint™ were given ET, and adjuvant ET and CT was given to patients characterized as high risk by both Adjuvant! Online and MammaPrint™. Patients were randomized to the ET or chemotherapy arms if the risk groups differed between Adjuvant! Online and MammaPrint™. The study was designed to address whether a non-anthracycline-containing regimen (docetaxel plus capecitabine) may be used instead of an anthracycline-containing regimen. There were two arms for ET: letrozole only and tamoxifen followed by letrozole.

The pilot phase of the study included 800 patients [60]. Of those patients, 386 (48%) were in the low-risk group based on both Adjuvant! Online and MammaPrint, and 198 (24.8%) patients were in the high-risk group based on both Adjuvant! Online and MammaPrint™. There were 216 (27%) discordant patients, of whom 75 (9.4%) were in the low-risk group from Adjuvant! Online and the high-risk group from MammaPrint™ and 141 were in the high-risk group from Adjuvant! Online and the low-risk group from MammaPrint™. There was an 8.25% difference (95% CI 4.7–11.8%; $p < 0.001$) between the high-risk groups defined by Adjuvant! Online (42%) and MammaPrint™ (34%). There was a high consistency among the treatments in both groups (>92%).

MINDACT trial included 6693 patients [61]. Clinical and genomic risks were concordant in about two-thirds of the patients; 41% of the patients had low, and 27% of the patients had high clinical and genomic risks. High clinical and low genomic risk was detected in 23.2%, and low clinical and high genomic risk was detected in 8.8% of the patients. Among patients with high clinical risk, 46% had low genomic risk, and this group was the primary focus of the study. Patients with high clinical and low genomic risks who did not receive chemotherapy had a 5-year distant metastasis-free survival (dMFS) rate of 94.7% (95% CI 92.5–96.2) and OS of 97.3% (95% CI 95.6–98.4). Of note, half of the patients in this group had at least one positive axillary lymph node. The lower boundary of 5-year survival without distant metastasis was higher than the prespecified noninferiority margin of 92%; therefore, the study met its primary endpoint by demonstrating the noninferiority of omitting chemotherapy in patients with high clinical and low genomic risks. Accordingly, among patients with high clinical risk features, Mammaprint™ can identify those with good prognosis who may not benefit from chemotherapy. dMFS and OS rates were 1.9 and 1.5 percentage points higher in patients at high clinical and low genomic risk, respectively, who underwent chemotherapy, but the study was underpowered to assess statistical significance of these differences. On the other hand, in patients at low clinical and high genomic risk, use of chemotherapy did not result in any significant differences in 5-year outcomes; therefore, MammaPrint™ may not be useful in guiding treatment in low clinical risk patients.

Prosigna

The NanoString Prosigna assay uses the expression of 50 target genes and eight constitutively expressed normalization genes. The test can be performed using qRT-PCR, but Prosigna™ relies on the NanoString nCounter Analysis System, which delivers direct, multiplexed measurement of gene expression. The assay is highly sensitive and precise. It uses 250 ng of RNA from FFPE tumor tissue. Assay controls are included along with test samples, and the process meets the predefined quality criteria. The PAM50 test was generated as a second-generation multigene expression assay and was developed to define the intrinsic breast cancer subtypes as luminal A/B, HER2-enriched, and basal-like from FFPE tissue [62]. PAM50 is more effective than classical immunohistochemical methods and clinicopathological factors for subtyping breast cancer. It uses a set of 50 genes and 5 control genes for analysis. In addition to classifying the tumor's intrinsic subtype, PAM50 gives a numeric score for the patient's distant recurrence probability by calculating the molecular subtype correlations, a subset of proliferation genes and pathological tumor size. The PAM50 test was adopted for performance by nCounter Analysis in a local molecular pathology lab (Prosigna™ Breast Cancer Gene Signature Assay, NanoString Technologies, Seattle). Training is required for the operators to demonstrate proficiency in studying this locally used test. The embedded software automatically applies all of the quality thresholds to the data. A clinically validated algorithm is used for expressing the risk of recurrence and the intrinsic subtype, both of which are prognostic indicators of the risk of disease recurrence for breast cancer. This test works with either frozen or FFPE samples and uses multiplexed gene-specific fluorescently labeled probe pairs to measure gene expression.

PAM50 reflects the underlying biology associated with the ER and HER2 pathways, and it also includes proliferation genes and markers of basal phenotype. Luminal A and B breast cancers are the most frequent subtypes. Luminal A tumors are characterized by lower expression levels of proliferation genes and ERBB2 and by lower recurrence rates compared with luminal B tumors, and these characteristics can be shown by the PAM50 risk of recurrence score.

RNA is extracted from tumor tissue, and the samples are hybridized without reverse transcription or amplification for both capture and reporter probes for the measured genes and assay controls. After hybridization, the target-probe complexes are processed on the nCounter Analysis System. A minimum threshold of expression for normalization genes must be met by the test sample data to ensure that the assay signal is high enough to produce precise results.

The test was prognostic in untreated (no systemic treatment) or tamoxifen-treated early breast cancer patients [62, 63]. It was validated in 786 stage I and II ER- and/or PR-positive postmenopausal patients (in this validation study, only 40 of 789 patients were premenopausal) and provided accurate information for subtyping and prognosis by risk of recurrence [63]. This risk of recurrence score gave the estimated 10-year recurrence probability in postmenopausal early breast cancer patients with ET. Despite clinical ER positivity, ten of the cases were assigned to nonluminal subtypes by PAM50 in this study. PAM50 expression for intrinsic subtyping provided more prognostic information than standard clinical factors and IHC.

PAM50 was validated on over 2400 patients from two large retrospective studies [64, 65]. Using the mRNA of 1017 ER-positive early breast cancer patients from the ATAC trial, the PAM50 risk of recurrence score was studied and compared with Oncotype DX and IHC4 results [64]. IHC4 is a distant recurrence index derived from immunohistochemical analysis of estrogen and progesterone receptors, HER2, and Ki67. Additional prognostic information beyond that in the clinical treatment score, which integrates the prognostic information from nodal status, tumor size, histopathological grade, age, and treatment, was greater in the PAM50 risk of recurrence than the Oncotype DX RS for the overall population and for each subgroup (lymph node-negative and node-positive and HER2-negative/lymph node-negative subgroups). In the node-negative/HER2-negative subgroup, prognostic information obtained using the PAM50 risk of recurrence score was more accurate than the Oncotype DX RS. The correlation between risk of recurrence and clinical treatment score was similar to what was previously found in the TransATAC study between Oncotype DX RS and Adjuvant! Online. The risk of recurrence includes more clinicopathological information than the other factors. The PAM50 risk of recurrence score can be applied in the context of clinicopathological factors involved in the clinical treatment score.

One of the other large validation studies was on the patients from the ABCSG-8 trial. PAM50 was evaluated for obtaining the risk of recurrence and defining subtypes from the FFPE tumor tissues of 1478 patients from the ABCSG-8 trial [65]. The original study was a phase III prospective design of 3901 patients to test tamoxifen versus tamoxifen followed by anastrozole in the adjuvant treatment of ER-positive early breast cancer patients. Both risk of recurrence and intrinsic subtypes (luminal A/B, basal-like, and HER2-enriched) were defined using PAM50. There were 1004 (67.9%) patients in the luminal A, 418 (28.3%) patients in the luminal B, 48 (3.3%) patients in the HER2-enriched, and 8 (0.5%) patients in the basal-like subgroups according to the PAM50 test. The aim was to test whether the risk of recurrence score adds prognostic value in predicting disease recurrence beyond standard clinical factors. For 10-year disease recurrence risk, lower than 10% was defined as low risk, and higher than 20% was defined as high risk (Fig. 12.2). The risk of recurrence score added prognostic information to the clinical predictors in all subgroups ($p < 0.0001$). The luminal A subgroup had a lower risk of recurrence at 10 years compared with the luminal B subgroup ($p < 0.0001$). Ten-year distant recurrence-free survival rates were higher in the luminal A subgroup than in the luminal B (HR, 2.85; 95% CI, 2.04–4; $p < 0.0001$) (Fig. 12.3). Low-risk and high-risk groups were discriminated using the risk of recurrence score in all subgroups of patients. As a result, the PAM50 test was validated in this study for predicting disease recurrence in ER-positive postmenopausal early breast cancer patients. A 10-year disease recurrence under 3.5% in the risk of recurrence low category makes it unlikely that additional chemotherapy would improve the outcome.

The combined analysis of the TransATAC and ABCSG-8 trials with 2137 patients showed that the risk of recurrence adds significant prognostic information for late recurrences (>5 years) in women with hormone receptor-positive early-stage breast cancer [66]. Median follow-up time was 10 years for that analysis. Predefined risk stratification

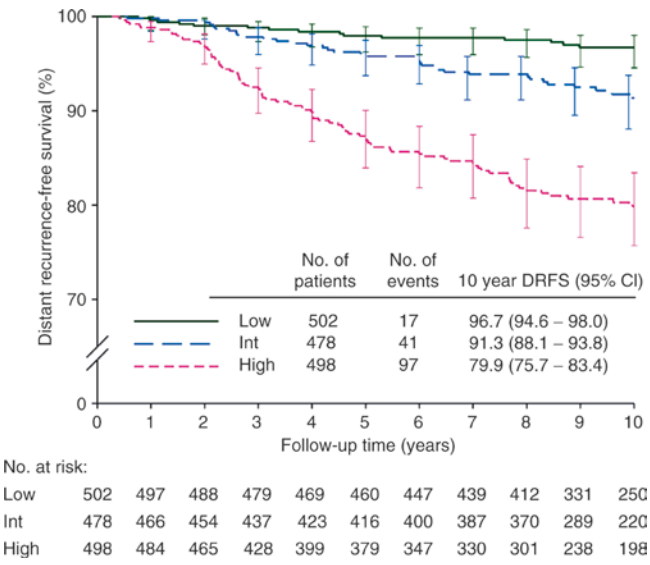

Fig. 12.2 Prognosis based on PAM50 classifier for DRFS. (Reproduced from Reference [65] with permission of Oxford University Press on behalf of the European Society for Medical Oncology. © The author (Michael Gnant) 2013)

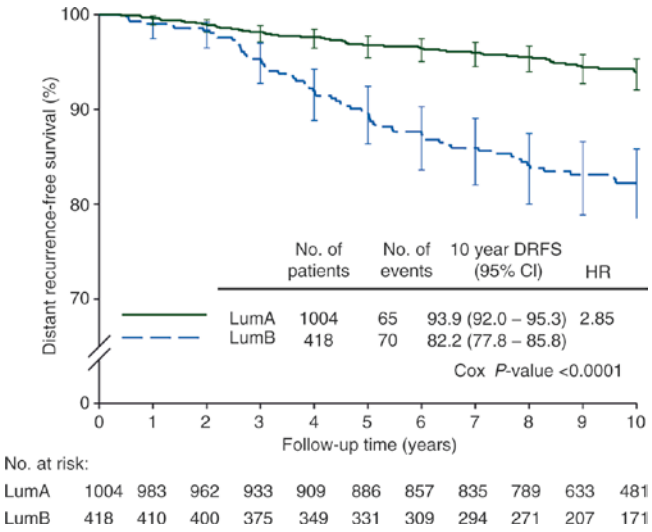

Fig. 12.3 Distant recurrence-free survival: Kaplan–Meier plot of luminal (*A*, *B*) subtypes with 95% CI. (Reproduced from Reference [65] with permission of Oxford University Press on behalf of the European Society for Medical Oncology. © The author (Michael Gnant) 2013)

showed significant differences between risk groups based on the risk of recurrence for 10-year distant recurrence rates. Patients with recurrence in the first 5 years were excluded from this analysis. The risk of recurrence score for the patients with recurrence (53.7 ± 20.4) in the first 5 years was higher than the patients with no recurrence (41.89 ± 19.5) ($p < 0.001$). There were more late recurrences and more patients with poor differentiation, larger tumor size, and >3 positive lymph nodes in the TransATAC study than the ABCSG-8 trial. Of the 2137 patients analyzed, 1530 (73.8%) women had luminal A, and 542 (26.2%) women had luminal B breast cancer. Patients in the luminal B subgroup had a 2.9-fold higher risk of distant recurrence (HR, 2.9; 95% CI, 2.07–4.02; $p = 0.001$). Patients were divided according to the 10-year recurrence probability as having a low, intermediate, and high risk of recurrence (lower than 10, 10–20%, and higher than 20%, respectively). The risk of distant recurrence in 5–10 years was 2.4% (95% CI 1.6–3.5), 8.3% (95% CI 6.1–11.2), and 16.6% (95% CI 13.1–20.9) in patients in the low, intermediate, and high risk of recurrence groups, respectively. The risk of late recurrence was 6.9 (HR, 6.9; 95% CI, 4.54–10.47) times higher in the high-risk group than in the intermediate-risk group and 3.3 (HR, 3.26; 95% CI, 2.07–5.13) times higher in the intermediate-risk group than the low-risk group. Based on the risk of recurrence score, patients in the high-risk group may be separated for extended therapy.

Tissue samples from the MA.12 NCIC CTG (National Cancer Institute of Canada Clinical Trials Group) prospective trial, which compared tamoxifen and placebo in early premenopausal breast cancer patients, were used for PAM50 risk of recurrence evaluation. The aim was to evaluate intrinsic breast cancer subtypes and prognosis with PAM50 and IHC [67], and 395 patients were evaluated. PAM50 gave significant prognostic information for both DFS and OS, but IHC analysis could not. The 5-year DFS and OS rates were higher in the luminal A group and lower in the HER2-enriched group according to PAM50 ($p = 0.0003$). Classification of the patients into intrinsic subtypes using PAM50 was also more effective than using IHC. This study also concluded that PAM50 was predictive for adjuvant tamoxifen treatment in node-negative and node-positive premenopausal breast cancer patients. There was significant interaction with 10-year DFS probability and the risk of recurrence score in lymph node-negative and node-positive early breast cancer patients treated with tamoxifen.

The phase III GEICAM/9906 study population ($N = 814$) was evaluated for PAM50 breast cancer subtyping and clinical standard markers [68]. The standard IHC panel for breast cancer (ER, PR, and HER2) could not adequately define the PAM50 expression subtypes in this study. There was high agreement between biomarker scoring by protein IHC and gene expression, but gene expression determinants for ESR1 and ERBB2 status were more prognostic.

In a population-based study, 1319 women with breast cancer from the LACE and Pathways cohort were tested for intrinsic subtyping [69]. According to PAM50 subtyping, 53.1% of the patients were luminal A, 20.5% were luminal B, 13% were HER2-enriched, 9.8% were basal-like, and 3.6% were normal-like. Among low-risk hormone receptor-positive patients with classical clinicopathological tests, only 76.5% were categorized as luminal A by PAM50. In this population-based cohort, African-American women were more likely to have basal-like tumors (OR: 4.4; 2.3–8.4), and Asian and Pacific Islander women had reduced odds of the basal-like subtype (OR: 0.5; 0.3–0.9). In another population-based cohort of 1691 patients, early (<5 years) and late (>5 years) risks of recurrence were determined based on the PAM50 risk of recurrence score according to intrinsic subtypes of breast cancer [70]. The risks of disease recurrence and death were lower in patients with luminal A tumors compared with luminal B, HER2-enriched, and basal-like subgroups of breast cancer at 2, 5, and 10 years. In that study, PAM50 better defined the patients with lower risk from the higher risk of recurrence tumors than the standard immunohistochemistry or tumor grade.

Prediction of Response to Treatment

In a study of 104 postmenopausal ER-positive breast cancer patients, tumor biopsies were taken before and 2 weeks after the beginning of treatment with anastrozole [71]. The risk of recurrence was calculated by PAM50 for luminal A and B tumors. Among the pretreatment samples, all intrinsic subtypes were present, but the luminal subgroups were most

highly represented. The decrease in Ki67 levels was evaluated between subgroups according to PAM50, and there was a similar proportionate decrease in Ki67 levels in the luminal A and B subgroups (mean suppression: 75% for both), which suggests that patients in the luminal A and B subgroups derive similar benefit from anastrozole treatment. However, in the subgroups of basal-like (15%) and HER2-enriched (50%) cancers, Ki67 reductions were low under treatment with anastrozole. The normal breast-like subgroup showed the greatest reduction of Ki67 (83%) with anastrozole treatment. Residual Ki67 staining remained high after 2 weeks of anastrozole treatment in the luminal B subgroup. The PAM50 risk of recurrence score was significantly associated with clinical outcome ($p = 0.03$) and antiproliferative response to anastrozole treatment ($p = 0.0019$). These data show that the short-term response to anastrozole treatment may be similar between luminal subgroups and that higher residual Ki67 levels might show poor response to anastrozole treatment in the luminal B subgroup.

Another validation study was the MA.5 NCIC CTG trial, which randomized early premenopausal, lymph node-positive breast cancer patients into CMF versus CEF (cyclophosphamide, epirubicin, and 5-fluorouracil) chemotherapy. PAM50 classified 467 patients into intrinsic subtypes, and the HER2-enriched subtype strongly predicted anthracycline sensitivity [72].

A subset of patients from the GEICAM/9906 phase III trial who were identified by PAM50 as having low proliferation status derived a larger benefit from weekly paclitaxel [52]. The original GEICAM/9916 study tested FEC versus FEC followed by weekly paclitaxel in node-positive early breast cancer patients. The PAM50 risk of recurrence was studied in 820 patients, and the median follow-up was 8.7 years. The median OS was higher in the FEC plus weekly paclitaxel arm compared with the FEC arm (HR, 0.693; $p = 0.013$). A benefit from weekly paclitaxel treatment was achieved only in patients with a low PAM50 risk of recurrence score (HR, 0.23; $p < 0.001$).

The NCIC CTG MA.21 trial was a phase III study of 2104 patients who were ≤61 years old and had high-risk node-negative or node-positive disease [73]. It tested different anthracycline and taxane combinations for the adjuvant treatment of breast cancer. Randomization was performed among doxorubicin plus cyclophosphamide and paclitaxel (AC/T), dose-dense CEF, and dose-dense, intense epirubicin, cyclophosphamide, and paclitaxel (EC/T) groups. Intrinsic subtyping was performed in 1094 available patients with the PAM50 assay [74]. Of these patients, 27% were in the luminal A subgroup, 23% were in the luminal B subgroup, 18% were in the HER2-enriched subgroup, and 32% were in the basal-like subgroup. Dose-dense CEF and dose-dense, intense EC/T treatments were superior to AC/T treatment ($p = 0.01$). In the multivariate analysis, a high risk of recurrence was associated with worse RFS ($p = 0.03$). However, categorical risk of recurrence was neither prognostic nor predictive for any treatment. In the multivariate analysis, intrinsic subtyping with PAM50 had a significant prognostic effect on RFS ($p = 0.002$). Compared with luminal A, the hazard ratios were 1.48 (95% CI 0.92–2.37) for luminal B, 2.68 (95% CI 1.6–4.48) for HER2-enriched, and 1.97 (95% CI 1.1–3.53) for basal-like. Intrinsic subtypes were not predictive of treatment benefit (AC/T vs. EC/T-CEF). However, subgroup analysis showed that the nonluminal subtype was predictive for taxane benefit compared with the luminal subtype ($p = 0.05$).

Based on the results of these trials, PAM50 provides valuable information for intrinsic subtyping and also distant relapse-free survival and likelihood of recurrence at 10 years, at least for ER-positive and tamoxifen-treated breast cancer patients. PAM50 subtype classification is superior to IHC for both prognosis and predicting the benefit of tamoxifen. The risk of recurrence score gives an individual risk assessment in early-stage, hormone receptor-positive, pre- and postmenopausal breast cancer patients and allows subtype classification. The risk of recurrence score estimates the probability of disease recurrence in pre- and postmenopausal, hormone receptor-positive early breast cancer patients treated with ET. The HER2-enriched subtype, as defined by PAM50, was a strong predictor of adjuvant anthracycline treatment. In light of these data, the US FDA approved PAM50 in Europe in 2012 and in 2013.

Other Genomic Tests

BluePrint® is an 80-gene microarray that was specifically designed for molecular subclassification in early-stage (stage I and II), lymph node-negative or node-positive and ER-positive or ER-negative breast cancer patients [75]. In a neoadjuvant chemotherapy study of 426 breast cancer patients, the BluePrint® assay reassigned 22% of the previously classified patients with IHC/FISH [76]. Of 211 patients classified as hormone receptor-positive/HER2-negative according to IHC/FISH, 37 were reclassified into the basal ($N = 35$) and HER2 ($N = 2$) subtypes by the BluePrint® assay. The pathological complete response rate was higher in the HER2 subgroup defined by BluePrint® (53%) than in the HER2 subgroup defined by IHC/FISH (38%) ($p = 0.047$).

Endopredict® (Sividon Diagnostics GmbH, Germany) is an 11-gene assay that was introduced to predict disease recurrence risk in ER-positive and HER2-negative early breast cancers treated with adjuvant ET alone [77]. Eight selected genes and three additional control genes were used for quantification of mRNA levels by RT-PCR. FFPE can be used for Endopredict® analysis. The test can be performed in specialized molecular pathological laboratories instead of

central laboratories [78]. The assay result is expressed as the Endopredict® (EP) score and is used in combination with nodal status and tumor size to calculate a clinical risk score (EPclin). EP and EPclin scores are dichotomized to high- and low-risk groups. EPclin was used in two validation cohorts of 378 and 1324 patients from the ABCSG-6 and ABCSG-8 studies, respectively [77, 79]. Overall, 63% of the patients were in the low-risk group according to EPclin scores. Distant recurrence rates at 10 years were 4% in the low and 22–28% in the high-risk groups. The 11-gene EP risk score was an independent predictor of disease recurrence in a multivariate analysis, and the EPclin score outperformed conventional clinicopathological risk factors. Performance of EP was also assessed in patients with node-positive disease who were treated with chemotherapy [80]. The study cohort included 555 patients from GEICAM 9906 trial. According to EP scores, 25% of the patients were assigned to low-risk group, and 10-year metastasis-free survival rates were 93% and 70% in low- and high-risk groups, respectively. This test is used in laboratories in several centers in Germany, Austria, and Switzerland.

Breast cancer index (BCI) is a continuous risk model based on algorithmic combination of two independent prognostic markers: the expression ratio of *HOXB13* and *IL17BR* genes (H/I) and molecular grade index (MGI). MGI is a gene expression assay, which evaluates five genes associated with histological grade and tumor progression. Both H/I and MGI assays are performed using RT-PCR. BCI was developed in a cohort of ER-positive, node-negative patients from the Stockholm trial, who were treated with tamoxifen or received no treatment [81]. BCI assigned a risk score on a scale of 0–10 to each patient, with higher scores indicating higher risk of recurrence. BCI was further categorized into three risk groups: low risk (BCI <5), intermediate risk (BCI 5–6.4), and high risk (BCI $\geq$ 6.4). Among 314 tamoxifen-treated patients, 60% had low risk. Ten-year distant metastasis rates were 1.1, 17.8, and 20%, in the low-, intermediate-, and high-risk groups, respectively. The BCI was also prognostic for recurrence and survival in the untreated patients. In another validation study involving an ER-positive node-negative cohort of patients from TransATAC trial, who were treated with ET, BCI successfully categorized patients into low-, intermediate-, and high-risk groups with increasing risk of distant recurrence at 5 years [82]. Additionally, BCI was also significantly associated with risk of late (5–10 years) recurrence, with late distant recurrence rates of 3.5, 13.4, and 13.3% in low-, intermediate-, and high-risk patients. Prognostic utility of BCI was also demonstrated in a cohort of patients from MA.14 trial, which included ER-positive patients treated with tamoxifen with or without octreotide LAR [83]. BCI was shown to have a prognostic effect on recurrence-free survival at 5 and 10 years, in both node-negative and node-positive groups of patients. In addition to providing prognostic information, H/I may be useful in predicting benefit of extended adjuvant endocrine therapy. Based on a case-control study of patients who were randomized to letrozole or placebo after 5 years of tamoxifen in MA.17 trial, those with high H/L had higher risk of late recurrence and were less likely to have recurrence when treated with extended adjuvant letrozole [84].

Genomic grade index (GGI) is another microarray-based assay, which was aimed to determine histological grade more accurately based on gene expression levels. The GGI assay included 97 genes that were differentially expressed between histologic grade 1 and grade 3 tumors, developed in a training set of 64 ER-positive tumor samples [85]. The assay was validated in a series of 597 tumor samples, which were categorized into gene expression grade (GG) 1 if GGI score was negative and GG 3 if GGI score was zero or positive. Patients with GG 1 tumors had superior RFS than those with GG 3. GGI was a better predictor of recurrence than histologic grade. Additionally, in subset of patients with histologic grade 2 tumors, GGI was able to categorize patients into prognostic groups, i.e., those with high GGI had worse RFS. GGI was also shown to predict response to neoadjuvant chemotherapy in patients with HER2-negative tumors regardless of ER expression status [86]. Pathologic complete response or minimal residual disease rates were higher (40% vs. 12%) in the high GGI risk group than the low GGI risk group.

MapQuantDx® (Genomic Grade, Ipsogen, France) is a 97-gene histologic grade predictor that was developed in recent years. It is a microarray-based assay that calculates the genomic grade index (GGI). It provides prognostic information in addition to standard clinicopathological variables and had significant impact on treatment decisions in several retrospective studies [87, 88]. Fresh or frozen tissue is needed for this 97-gene signature. However, a 6-gene PCR genomic grade was developed from the initial 97 genes for FFPE tissue. A high correlation was shown between the microarray and RT-PCR assays studied in frozen and FFPE tissues. The prognostic value of PCR-GGI was confirmed on FFPE samples [89].

The Rotterdam 76-gene signature was developed at the Erasmus University Cancer Center in Rotterdam (the Netherlands) [90, 91]. It was introduced to the market by Veridex (Raritan, USA). This 76-gene assay includes proliferation genes, none of which overlap with MammaPrint® or Oncotype DX®. Fresh or frozen tissue is needed for this assay. Validation was performed in patients with node-negative breast cancer that was either positive or negative for hormone receptors. The 76-gene assay was validated in a group of 198 node-negative, systemically untreated breast cancer patients, and a high-risk group was defined for early disease recurrence in this study [92]. A very-low-risk subgroup was also defined in another validation study in breast cancer patients treated with adjuvant ET [93].

Conclusion

Numerous multigene assays have been developed based on multigene profiling assays to avoid over- and undertreatment and to better define the prognosis and predictive markers in early-stage breast cancer. Some of these assays provide subclassifications based on gene expression profiling. The main limitation for many gene profiling assays is the lack of "level of evidence I" due to the need for crucial prospective data and sufficient numbers of robust retrospective studies. Very few assays are suitable for use in common clinical practice due to technical and clinical validity. Clinicians must carefully consider the indications of these gene array-based assays regarding differences in technical prerequisites, reproducibility, clinical validity, underlying evidence, and clinical impact on the special patient populations. Among the most widely used multigene assays are Oncotype DX™, MammaPrint™, and PAM50™. Less expensive and more feasible assays are needed for decision-making about adjuvant or neoadjuvant treatment of breast cancer. Avoiding chemotherapy for a group of patients who would not benefit is very important for acute and long-term toxicities, quality-of-life issues, and cost. Based on numerous retrospective studies, ongoing prospective studies will provide important information about the treatment prediction in early breast cancer. Technical ease is a major concern for the general implementation of these tests in clinics. The availability of the test in a local lab is an advantage. However, the need for special equipment and trained personnel is critical for the local facility. Several other assays are undergoing validation studies or are otherwise in the process of development.

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Bone Marrow Micrometastases and Circulating Tumor Cells

13

Saadettin Kilickap, Burak Yasin Aktas, and Y. Yavuz Ozisik

Introduction

Breast cancer is the worldwide leading cause of cancer death in women. Breast cancer-related mortality is associated with distant metastases. The 5-year relative survival rate is 22% for stage IV breast cancer [1]. The response to treatment and survival outcomes of patients with breast cancer differ among individuals. The aim of treatment in patients with advanced stage breast cancer is palliative. By contrast, surgery is an essential treatment for early-stage breast cancer, and local or distant recurrence is the most important issue at follow-up in these patients. Among patients with favorable prognoses, approximately 20% develop recurrence during the follow-up period, whereas other patients have long-term recurrence-free survival despite unfavorable prognoses [2, 3]. The aim of treatment in early-stage breast cancer is to achieve a cure or longer disease-free and overall survival. Adjuvant therapy with chemotherapy and/or radiotherapy following curative surgery aims to eradicate residual microscopic tumors. The traditional approach in the treatment of breast cancer is based on preventing and identifying the development of metastatic disease in early-stage breast cancer. At this stage, the presence of minimal residual disease or metastatic tumor cells is presumed but not certain. Clinical and pathological features associated with distant metastasis, such as high tumor grade, large tumor size, lymph node involvement, lack of hormone receptor expression, and overexpression of HER2, determine the treatment strategies. In clinical practice, while local recurrence or distant metastasis develops in some individuals who were assessed as low risk despite treatment, some individuals with high-risk disease do not relapse after systemic and local therapy. Therefore, oncologists must establish objective prognostic factors for identifying early recurrence and metastasis in patients with breast cancer.

S. Kilickap (✉) · B. Y. Aktas · Y. Y. Ozisik
Department of Medical Oncology, Hacettepe University Cancer Institute, Ankara, Turkey
e-mail: yozisik@hacettepe.edu.tr

The risk of recurrence after curative surgery in early-stage breast cancer is estimated with the 21-gene Oncotype DX and the 70-gene MammaPrint in patients with hormone receptor-positive cancers [4, 5]. After risk estimation, patients with a high recurrence score are treated with chemotherapy and hormone therapy, whereas women with a low recurrence score are treated only with endocrine therapy. Despite classification as "high risk" for recurrence, some patients do not develop relapses within their lifetimes. Because of the presumption of residual disease, clinicians have recently tried to identify micrometastases based on disseminated tumor cells (DTCs) in the bone marrow and circulating tumor cells (CTCs) from the peripheral blood. These cells can be detected with immunohistochemistry and molecular techniques. However, the detection of DTCs and CTCs requires experienced staff and specialized laboratories.

Detection of Bone Marrow Micrometastases

In the bone marrow, DTCs can be identified as epithelial cells and are also recognized as micrometastases. Immunohistochemistry is the most common method used to detect micrometastases in bone marrow aspirates. The aspirates obtained from bone marrow are stained for epithelial cells. DTCs are observed in approximately 30% of early-stage breast cancer patients [2]. The morphological characteristics of DTCs include large cells with a large nucleus, nuclear granulation or stippling, strong or irregular staining for cytokeratin, and cytokeratin filaments. The presence of at least one CK+ cell in the bone marrow is defined as DTC [6]. DTC may be established using immunohistochemistry and molecular techniques. The terms DTC or micrometastasis have been recently used in guidelines such as those of the American Society of Clinical Oncology and the San Gallen Consensus [7, 8].

The prognostic value of bone marrow micrometastases in breast cancer is illustrated in Table 13.1. The value of prognostic DTCs detected in the bone marrow after adjuvant

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Table 13.1 Prognostic significance of bone marrow micrometastases in selected important breast cancer trials

	Year	*N*	Method	Positivity rate (%)	Prognostic value	Outcome
Braun et al. [2]	2005	4703	IHC	31	DFS, DDFS, OS	Prognostic
Bidard et al. [12]	2008	621	IHC	15	DMFS, OS	Prognostic
Molino et al. [13]	2008	125	IHC	31	RFS, OS	Non-prognostic
Falck et al. [14]	2012	401	IF, IHC	38	DDFS, OS	Non-prognostic
Hartkopf et al. [15]	2014	3141	IHC	26	DFS, OS	Prognostic

IHC immunohistochemistry, *IF* immunofluorescence, *DFS* disease-free survival, *DDFS* distant disease-free survival, *OS* overall survival, *DMFS* distant metastasis-free survival, *RFS* relapse-free survival

chemotherapy is associated with poor prognosis [9]. In a study evaluating whether DTCs represented a prognostic factor in patients with breast cancer, the authors reported DTCs in 15% of these patients [9]. The presence of DTCs in the bone marrow of breast cancer patients was negatively correlated with survival. Those results have been supported by studies using adjuvant and neoadjuvant chemotherapy [10, 11].

The detection of DTCs before surgery for early-stage breast cancer is an independent prognostic factor and is associated with shorter survival [2, 12, 16–18]. Most of these studies have shown a correlation between DTCs and other prognostic factors, including tumor size, histologic grade, and lymph node status.

In a meta-analysis that included individual data from 4703 patients, the frequency of DTCs was approximately 31% [2]. These patients tended to have larger tumors, hormone receptor-negative cancers, and lymph node metastases. The presence of micrometastases in the bone marrow of patients with early-stage breast cancer before the initiation of primary therapy was an independent unfavorable prognostic factor for disease-free, distant-free, and overall survival in breast cancer. The mortality rate in patients with micrometastases is 2.4 times higher than in those without micrometastases. Additionally, persistent DTCs at follow-up after therapy in these patients predicted the risk of relapse and death and were associated with shortened overall survival [19]. Moreover, the results were consistent with those of several small studies in which the presence of DTCs in the bone marrow was a prognostic factor for survival [17, 18].

Molino et al. investigated the prognostic value of epithelial tumor cells in bone marrow in patients with breast cancer [13]. The authors found that the 10-year probability of relapse-free and overall survival rates in the patients in whom epithelial cells were observed in the first bone marrow sample was similar with those without epithelial cells in the bone marrow. In Cox's model, the rates of relapse and death were different between patients with and without micrometastases in the first bone marrow samples. In a prospective observational study from Sweden, DTCs in the bone marrow were found in 38% of 401 patients with breast cancer [14]. Tumor characteristics were similar between patients with and without detected DTCs. Detection of DTCs was not a prognostic factor for distant disease-free survival (DFS) or overall survival. These results did not support the results of previous published studies. Recently, a single-center study from Germany addressed whether the presence of DTCs had prognostic value in early-stage breast cancer patients [15]. In this study, which had a large sample size, data from 3141 patients were analyzed. DTCs were detected in 26% of these patients. Patients who were DTC positive in the bone marrow tended to have larger tumor sizes as well as lymph node-positive and HER2-positive cancer. The presence of DTCs before surgery was associated with shortened disease-free and overall survival. The detection of DTCs in early-stage breast cancer is an independent predictor of disease-free and overall survival. However, DTCs are not detected in approximately half of patients with early breast cancer, and relapse can occur in patients without detectable DTCs [2].

The association between subtypes of breast cancer and the presence of DTCs in the bone marrow was examined [20]. In the luminal A group, DTC detection was associated with a significantly poorer prognosis for relapse and death compared with the other groups. The lowest rate was observed in the basal-like group. However, for all subtypes, the relapse risk was significantly higher in DTC-positive patients than in DTC-negative patients.

Despite data suggesting that micrometastases in the bone marrow are an independent prognostic factor for survival, there are concerns regarding its validation. Thus, the clinical utility of DTC detection remains unclear.

DTCS and CTCS

Bone marrow aspiration is an invasive procedure and is often painful, and standardizing its quality is particularly difficult. Therefore, effort has been focused on CTCs in the peripheral blood. However, it is unclear whether DTCs are the same cells as CTCs. To date, the accuracy of this hypothesis has been evaluated in clinical studies showing a positive correlation between DTCs and CTCs [21, 22]. DTCs were detected at a higher rate than CTCs.

Furthermore, these results were confirmed by a recent study performed using AdnaTest for the detection of CTCs [23]. Although these results support the possibility that DTC and CTC may be the same cells, the evidence remains insufficient.

CTCS and Breast Cancer

Tumor cells circulating in the peripheral blood of patients with cancer are called CTCs. CTCs are cells that have entered the peripheral blood circulation after having detached from an existing primary tumor or its metastases. The presence of CTCs in the peripheral blood of patients with cancer was first described by Thomas Ashworth in 1869 [24]. However, cancer investigators have more recently focused on the molecular characterization and prognostic value of CTCs in many tumors, including breast, prostate, colorectal, ovarian, and pancreatic cancers [25]. Additionally, detecting CTCs can help to better understand the biology of tumors and their metastasis in cancer patients [26, 27]. In many solid tumors, baseline CTC detection can provide useful information for estimating the prognosis and efficacy of treatment. The potential benefits of CTC detection in cancer patients are shown in Table 13.2. However, many unanswered questions remain, including the optimal method for quantifying and characterizing CTCs [28]. Another problem is the high heterogeneity of CTCs. Another problem is the high heterogeneity of CTCs. Thus, an ideal CTC detection platform would be able to isolate and detect all heterogeneous CTCs while discarding the very large amount of normal blood cells in circulation [29].

The CellSearch (Veridex LLC, Raritan, NJ, USA) system, a semi-automated methodology to detect and count CTCs in breast, colorectal, and prostate cancers, has been approved by the US Food and Drug Administration [30–32]. To detect CTCs, a 7.5 ml sample of peripheral blood is often sufficient. CTCs are analyzed using antibodies targeting three proteins: cytokeratin (CK), epithelial cell adhesion molecule (EpCAM), and CD45. Additionally, cell nuclei are fluorescently labeled with the DAPI (4′,6-diamidino-2-phenylindole) nuclear dye. CTCs are defined as positive for nuclei and for epithelial cell adhesion molecule (EpCAM) and CK expression but negative for expression of the common leukocyte antigen CD45. The detection of at least one CTC per 7.5 ml of blood sample is considered positive. The advantages and disadvantages of CTC analysis are summarized in Table 13.3.

Recently, several technologies have been used to isolate CTCs, but they have not been approved by the FDA. These technologies include AdnaTest® (AdnaGen AG, Langerhagen, Germany), MACS (magnetic activated cell sorting system), and MagSweeper [33, 34]. Using AdnaTest, CTCs are isolated with antibodies against EpCAM and MUC1 [35]. The results of studies comparing CellSearch with other methods in patients with advanced cancer are conflicting. Muller et al. found that the CellSearch system is superior to the AdnaTest in metastatic breast cancer [36]. In contrast, another study demonstrated that CellSearch® and CTC-Chip systems were similarly effective [37]. However, at present, only the CellSearch system has been approved by the FDA.

Table 13.2 The potential benefits of CTC detection

Estimation of the risk of metastasis or tumor progression
Monitoring of treatment efficacy
Identification of resistance mechanisms
Understanding of the biology of metastasis
Estimation of prognosis

Table 13.3 Advantages and disadvantages of CTC analysis

Advantages	Disadvantages
Minimally invasive	Expensive
Monitoring of treatment response	Isolation can be difficult
May be a prognostic marker	Requires experienced staff and specialized equipment
	Cut-off value uncertain

In breast cancer patients, the hormone receptor and HER2 status are key determinants for both histological classification of the tumor and treatment decisions. Although the use of CTCs to characterize these markers in disease progression or metastatic tissue is theoretically rational, emerging data are not convincing. In a prospective study, investigators demonstrated that molecular detection of overexpression in CTCs can predict the HER2 profile of metastases, but not the hormone receptor status [38]. The same group obtained similar results in a previous study. Primary tumors and CTCs displayed concordant ER and PR statuses in only 41% and 45% of cases, respectively, in their preliminary study in 2011 [39]. Beije et al. demonstrated 25% discordance in ER status between primary tumors and CTCs in their study, but the group emphasized that the discordances in ER status between CTCs and the primary tumor had no prognostic impact in their metastatic breast cancer cohort [40]. Tumor heterogeneity, loss of receptors in CTCs, or technical issues may cause discordance of marker expression among primary tumors, metastases, and CTCs [41]. Although CTCs can provide prognostic information in breast cancer, their utility as predictive markers is less certain. Studies on the prognostic significance of CTCs in breast cancer have focused on both early-stage and metastatic disease. CTCs can be detected in the blood of many patients with breast cancer, even those without an established metastasis. The prognostic significance of the detection of CTCs in breast cancer trials is presented in Table 13.4.

Monitoring treatment efficacy is considered another remarkable potential benefit of measuring CTCs in peripheral blood samples. Numerous studies have evaluated the relation between the CTC count and treatment response in breast cancer patients. A large meta-analysis of 50 studies showed a significant reduction of the CTC-positive rate

Table 13.4 Prognostic significance of the detection of circulating tumor cells in selected important breast cancer trials

	Year	*N*	Method	Positivity rate (%)	Prognostic value	Outcome
Non-metastatic breast cancer						
Xenidis et al. [42]	2006	167	RT-PCR	22	DFS, OS	Predictive and prognostic
Ignatiadis et al. [43]	2007	444	RT-PCR	41	DFS, OS	Predictive
Pierga et al. [10]	2008	118	CellSearch	23	DFS	Prognostic for early relapse
Rack et al. [44]	2010	2026	CellSearch	22	DFS, OS	Prognostic
Bidard et al. [45]	2010	115	CellSearch	23	DFS, OS	Predict overall survival
Franken et al. [46]	2012	404	CellSearch	19	DFS	Prognostic
Lucci et al. [47]	2012	302	CellSearch	24	PFS, OS	Prognostic
Hall et al. [48]	2016	509	CellSearch	24	RFS, OS	Predictive and prognostic
Riethdorf et al. [49]	2017	213	CellSearch	22	DFS, OS	Prognostic
Metastatic breast cancer						
Cristofanilli et al. [30]	2004	177	CellSearch	49	PFS, OS	Predictive
Hayes et al. [50]	2006	177	CellSearch	54	PFS, OS	Predictive for PFS but not OS
Giuliano et al. [51]	2011	235	CellSearch	40	PFS, OS	Prognostic
Müller et al. [36]	2012	254	CellSearch	50	PFS, OS	Predictive
			AdnaTest	40		
Giordano et al. [52]	2012	517	CellSearch	40	PFS, OS	Predictive
Pierga et al. [53]	2012	267	CellSearch	44	PFS, OS	Predictive
Wallwiener et al. [54]	2013	468	CellSearch	42	PFS, OS	Prognostic
Jiang et al. [55]	2013	294	CellSearch	77	PFS, OS	Prognostic
Smerage et al. [56]	2014	595	CellSearch	54	PFS, OS	Prognostic
Wallwiener et al. [57]	2014	393	CellSearch	34	PFS, OS	Predictive
Giuliano et al. [58]	2014	492	CellSearch	62	OS	Predictive
Bidard et al. [59]	2014	1944	CellSearch	47	PFS, OS	Prognostic

RT-PCR real-time polymerase chain reaction, *DFS* disease-free survival, *PFS* progression-free survival, *OS* overall survival

(RR = 0.68, 95% CI 0.61–0.76, $P < 0.00001$) after treatment. Reduction of the CTC-positive rate was associated with a lower probability of disease progression (OR = 0.54, 95% CI 0.33–0.89, $P = 0.01$), longer overall survival period (mean difference = 11.61 months, 95% CI 8.63–14.59, $P < 0.00001$), and longer progression-free survival period (mean difference = 5.07 months, 95% CI 2.70–7.44, $P < 0.0001$). Subgroup analyses indicated that a reduction was found in HER2+ or HER2− patients, but not in triple-negative patients [60].

Early-Stage Breast Cancer

CTCs can be detected in early-stage breast cancer and are associated with a high risk of relapse [44, 61, 62]. Many clinical studies have evaluated the prognostic value of CTCs in early-stage breast cancer. These studies include patients treated with both neoadjuvant and adjuvant chemotherapy. In these studies, the presence of CTCs in the peripheral blood has been associated with an increased risk of relapse and reduced disease-free and overall survival [42, 63–65]. In a phase II trial including patients who were treated with neoadjuvant chemotherapy, ≥ 1 CTC was a predictive surrogate marker in predicting overall survival [10]. In the adjuvant setting, the presence of ≥5 CTCs/7.5 ml of peripheral blood was predictive of decreased survival in patients with HER2-positive but hormone receptor-negative cancer [43]. Furthermore, the presence of one or more CTC was associated with early recurrence and decreased overall survival in chemo-naive operable breast cancer patients [47].

Rack et al. investigated the prognostic value of CTCs in early breast cancer [11]. In a large, multicenter, prospective, randomized trial, called the SUCCESS study, investigators used the CellSearch system to analyze CTCs at baseline and after the completion of chemotherapy. At baseline, CTCs were found in 21.5% of 2026 patients who were diagnosed with early-stage breast cancer, and they were detected in 22.1% of 1492 patients after the chemotherapy. The authors concluded that there was no association between the presence of CTCs and tumor characteristics, including size, grade, and hormone receptor status. However, they showed that the presence of CTCs before systemic chemotherapy was an independent prognostic factor for DFS and overall survival and that CTCs were associated with poor prognosis. In patients who were positive for CTCs, DFS at 36 months was 88.1%, whereas it was 93.7% in CTC-negative patients. Similarly, the breast cancer-related mortality rate was significantly higher in CTC-positive patients than in CTC-negative patients (40.9% vs. 20.8%, respectively).

Additionally, authors also analyzed the prognostic value of CTCs in early breast cancer patients. The DFS and overall survival after the completion of chemotherapy were significantly lower in persistently CTC-positive patients than in persistently CTC-negative patients (85.9% vs. 93.9% for DFS and 92.8% vs. 97.6% for OS, respectively).

Moreover, the prognostic impact of the presence of CTCs in early-stage breast cancer patients was preoperatively evaluated in three different studies [46–48]. In one study including 404 patients with stage I–III breast cancer, CTCs were detected in approximately 20% of cases at the time of primary surgery [46]. Lucci et al. found that CTCs were detected preoperatively in 24% of patients [47]. Hall et al. identified CTCs in 124 of 509 nonmetastatic breast cancer patients (24.3%) in a prospective study [48].These studies suggested that the presence of CTCs before primary surgery was associated with early recurrence and an increased risk of breast cancer-related death, and these results have been confirmed in many small studies. CTCs were detected in 18–30% of cases with early-stage breast cancer [45, 62, 66, 67]. The frequency of CTCs in early-stage breast cancer is lower than in metastatic patients [11, 30, 68].

The presence of CTCs and its prognostic impact were also evaluated in patients with locally advanced breast cancer in the neoadjuvant setting (Table 13.3). Two phase III trials, GeparQuattro and GeparQuinto, examined the relationship between tumor response and the presence of CTCs in breast cancer patients in the neoadjuvant setting [49, 68, 69]. In both of these trials, CTCs were present in approximately 22% of patients. CTC detection was similar across different tumor characteristics. There was no association between CTC detection and pathologic complete response in these neoadjuvant chemotherapy studies. In a French study, REMAGUS02, the presence of CTCs at baseline was inversely correlated with survival in patients treated with neoadjuvant chemotherapy [70].

Finally, in two different meta-analyses including locally advanced stage breast cancer patients treated with neoadjuvant chemotherapy, the change in the number of CTCs was not associated with the pathologic response rate [59, 71]. However, the presence of CTCs was associated with shorter DFS and overall survival.

Metastatic Breast Cancer

Metastatic disease is the most common cause of breast cancer-related mortality. The traditional prognostic factors affect treatment strategies and prognosis in breast cancer. However, new treatment strategies and prognostic factors are needed in patients with asymptomatic visceral metastases and in those with the disease confined to non-visceral organs.

The prognostic role of CTCs in metastatic breast cancer has been investigated in many trials. Most of these studies have shown that the presence of CTCs is a prognostic factor for both disease-free and overall survival in the metastatic setting. Cristofanilli et al. showed that CTCs with a cutoff of 5 CTCs/7.5 ml of peripheral blood in patients with breast cancer represented an independent prognostic factor for progression-free and overall survival in the metastatic setting [30]. Elevated CTC counts were associated with significantly shorter progression-free and overall survival compared with the rates in patients with < 5 CTCs per 7.5 ml of blood. The outcomes were highly predictive. In a pooled analysis of 1944 metastatic breast cancer patients from 20 different studies, the authors found that patients with a CTC count of 5 per 7.5 ml or higher at baseline showed decreased progression-free and overall survival [59]. Despite debates about the appropriate cutoff value for CTCs, the same value was supported in additional studies [50, 72, 73]. Whether CTCs act as a prognostic and predictive surrogate marker in many solid tumors has been evaluated by multiple investigators [36, 52–54, 74]. Additionally, the CTC count may predict the response to treatment in breast cancer patients. The lack of a CTC response after chemotherapy is associated with a worse prognosis and shorter progression-free survival and overall survival [75, 76].

The CTC count at baseline is an independent prognostic factor for progression-free survival and overall survival in metastatic breast cancer [55]. The effect is very prominent in the HER2-positive histologic subtype of breast cancer, regardless of treatment. The presence of CTCs in peripheral blood may also be a potential marker of micrometastatic disease in patients with breast cancer. The prognostic value of elevated CTCs may not only correlate with higher metastatic tumor burden and metastatic sites but also predict micrometastatic disease [51, 56–58, 74, 77]. The results of a retrospective study including 492 advanced stage breast cancer patients suggested that the number of metastatic sites increased with a pretreatment level of ≥5 CTCs/7.5 ml compared with <5 CTCs/7.5 ml [51]. The authors showed that the development of new metastatic lesions and metastatic sites increased in patients with higher baseline CTC counts and that CTCs could be used as an indicator of metastatic potential in patients with limited metastatic dissemination.

However, whether the detection of CTCs using only the baseline measurement correctly identifies the prognosis remains unclear. Some clinical studies indicate that serial enumeration of CTCs should be performed to predict prognosis rather than a single measurement [50, 53, 57, 58, 78, 79]. Wallwiener et al. showed that serial enumeration of CTCs was more effective as a prognostic indicator and was useful for therapeutic monitoring of metastatic breast cancer [57]. The authors found that progression-free survival and overall survival were significantly higher in patients with negative CTC status compared with CTC-positive patients after one cycle of treatment. They recommended that monitoring of CTCs should be performed at baseline and after one

cycle of treatment. They also suggested that changes in CTC status from baseline to completion of one treatment cycle were predictive for progression-free survival and overall survival.

In the SWOG S0500 trial, the presence of CTCs before first-line chemotherapy in metastatic breast cancer patients was strongly prognostic and was associated with decreased survival [56]. The worst survival was prominent in patients who did not show reduced numbers of CTCs after chemotherapy. In a phase II study, the authors evaluated the prognostic role of CTCs in HER2-positive metastatic breast cancer patients with brain metastasis; CTCs were detected at baseline and at the third week after chemotherapy with lapatinib and capecitabine [80]. The proportion of patients with detectable CTCs decreased from 49% to 18% after only one cycle of chemotherapy. The response was significantly higher in patients who did not show CTCs at the third week, and the 1-year overall survival rate in these patients (84%) was significantly higher than for those with ≥1 CTCs (43%).

CTCs and Breast Cancer Subtypes

The authors of the SWOG S0500 trial analyzed all patients with metastatic breast cancer according to three biologic subtypes [56]. The first group consisted of patients who were negative for CTCs at baseline (group A), and group B was defined as patients who were initially positive for CTCs but who showed decreased CTCs after only one cycle of chemotherapy. In contrast, group C consisted of patients who showed increased CTCs after the first cycle of chemotherapy. In subtype analysis, the median overall survival was significantly lower in group C than in the other groups. The median overall survival rates were 35 months, 23 months, and 13 months in groups A, B, and C, respectively. Additionally, within each subgroup, the median overall survival was higher in patients with HER2-negative but hormone receptor-positive cancers than in those with hormone receptor-negative cancer.

Although within group A, the worst prognosis was found in triple-negative patients, and the median overall survival was still higher than for HER2-negative but hormone receptor-positive patients (22 vs. 15 months, respectively). Furthermore, the lowest median overall survival was observed in patients who showed increases in CTCs from the baseline (9.5 months), regardless of biologic subtype. In this group, 75% of patients died within approximately 15 months. This study also showed that the quantification of CTCs after chemotherapy provided additional information regarding survival.

In their study, Rack et al. showed no association between the presence of CTCs and histological subtypes, including luminal, basal-like, and HER2-positive cancers [79]. However, the presence of CTCs at baseline was an independent prognostic factor for reduced DFS. In all subtypes, DFS was significantly reduced in node-positive patients compared with those with lymph node-negative cancer.

Lymph node positivity at the time of initial diagnosis is also a prognostic marker for survival. In the SUCCESS and EUDRA-CT trials, CTC detection was higher in patients with lymph node-positive cancer [81]. These trials showed that lymph node metastasis is an independent prognostic factor for survival in multivariate analysis.

Circulating Tumor DNA and Breast Cancer

Tumors shed fragments into circulation, which represents one of the cornerstones of metastasis. This phenomenon led to the notion of investigating tumor-related fragments in the bloodstream. Analyses of CTCs, circulating tumor DNA (ctDNA), and tumor-derived exosomes are often referred to as liquid biopsies.

The role of CTCs in breast cancer has been discussed in detail above. Another rapid, cost-effective, and noninvasive alternative to surgical biopsies of solid tissues is interrogation of ctDNA during the course of disease [82, 83]. Various technologies are used to evaluate the circulating tumor DNA (ctDNA). In a large preclinical study, ctDNA was detectable in >75% of advanced solid tumors [84].

Dawson et al. detected ctDNA in 29 of 30 women (97%) with metastatic breast cancer [85]. Investigators also noted that ctDNA levels showed a greater correlation with changes in tumor burden than CTCs and that ctDNA provided the earliest measure of treatment response. Although the number of patients in the study was low, the results are promising. Madhavan et al. evaluated the integrity of ctDNA in breast cancer patients (n = 383) and a set of healthy controls (n = 100) [86]. An increase in the ctDNA concentration from healthy controls to patients with localized disease to metastatic breast cancer patients was observed. In a recent meta-analysis of 11 publications involving 1467 patients, ctDNA was shown to be significantly associated with progression-free survival and overall survival [87]. Emerging data have demonstrated the diagnostic and prognostic significance of ctDNA.

Conclusion

Whether the presence of tumor cells in the bone marrow and peripheral blood is sufficient to detect micrometastases and to predict survival remains unclear. In the absence of overt metastases, the detection of DTCs in the bone marrow or CTCs in the peripheral blood is a comparable factor to predictors such as tumor size, differentiation, lymphatic involvement, and HER2 and hormonal status. Evidence suggests that CTCs represent residual disease and are associated with

disease-free, recurrence-free, and overall survival. Further large studies and scientific evidence are needed to support whether DTCs and/or CTCs reflect patient outcomes.

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Pathology of Breast Cancer

14

Sitki Tuzlali

Pathology of Breast Cancer

Histopathologically, breast carcinoma is simply divided into two major categories with respect to its confinement to the ductal-lobular system of the breast or not:

1. In situ
2. Invasive

Carcinoma In Situ

Ductal carcinoma in situ
Lobular carcinoma in situ

Ductal Carcinoma In Situ (DCIS)

DCIS is characterized by neoplastic proliferation of epithelial cells that is confined to the ductal-lobular system of the breast without evidence of invasion through the basement membrane into the surrounding stroma. DCIS encompasses a heterogeneous group of lesions that differ in regard to their presentation, histopathological features, biological markers, and risk for progression to invasive cancer [1]. In areas where mammographic breast screening is not performed, DCIS constitutes approximately 5% of breast cancers, but within screening programs, it comprises approximately 20–25% of these tumors [2]. Approximately 10–20% of DCIS cases are bilateral.

These tumors are traditionally classified according to their architecture and are divided into comedo and non-comedo subtypes. The non-comedo subtype is further subdivided into solid, cribriform, micropapillary, and papillary types. Recent grading systems use the nuclear grade alone or in combination with necrosis [3].

S. Tuzlali (✉)
Tuzlali Private Pathology Laboratory, Istanbul, Turkey

Department of Pathology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

DCIS is generally divided into three grades according to nuclear features [3, 4]:

High-nuclear-grade DCIS: The tumor is composed of large, pleomorphic cells, often with prominent nucleoli. The nuclei are more than 2.5 times the diameter of red blood cells. Chromatin is coarse and clumped, and its distribution is irregular. Comedo necrosis with or without microcalcification is frequent but not necessary. Polarization toward the luminal surface is usually lost. Mitoses may be frequent (Fig. 14.1).

Low-nuclear-grade DCIS: The cells are small, monotonous cells that form arcades, micropapillae, and cribriform and solid patterns. Their nuclei are uniform and 1.5–2.5 times the size of normal red blood cells. Nuclei are usually but not invariably small [1]. The chromatin is finely dispersed. Nucleoli are inconspicuous. Mitoses are sparse, and the cells are polarized toward the luminal spaces (Fig. 14.2).

Intermediate-grade DCIS: When the lesion cannot be assigned easily to the high- or low-grade DCIS categories, it is diagnosed as intermediate grade. The features mentioned above are usually intermediate between low- and high-nuclear-grade DCIS.

In the presence of foci of different grades, the case should be graded according to the highest grade.

Pathology Reporting for DCIS

A pathology report for DCIS should include the following [3–6]:

- *Size/extent of the lesion*: Precisely measuring the extent of DCIS is often not possible. The volume of the breast tissue that is involved by DCIS is estimated by the pathologist based on the preferred sampling method. Mammographic correlation is also necessary, and this information should be provided by the clinician.
- *Nuclear grade*

A. Aydiner et al. (eds.), *Breast Disease*, https://doi.org/10.1007/978-3-030-04606-4_14

- *The presence or absence of necrosis and its type*: The type of necrosis can be classified as punctate or comedo. Comedo necrosis is the classic central necrosis in the duct lumina with karyorrhectic debris. This form of necrosis is associated with mammographic microcalcifications. Punctate necrosis presents small foci or single-cell necrosis that is indistinct at low magnification.
- *Architectural pattern*(*s*): The comedo, solid, cribriform, micropapillary, and papillary patterns are considered in the traditional classification schemes.
- *Cell polarization*: The presence or loss of polarization toward the luminal surfaces is considered in some grading schemes [7].
- *Location of microcalcifications*: When microcalcifications are present, their localization should be reported (in DCIS alone, in benign breast tissue, or in both). This information provides the correlation with mammographic findings.
- *Surgical margin status*: The surgeon provides the orientation using sutures or clips. In the presence of microcalcifications, specimen mammography should be provided. The surface of the specimen should be inked by the pathologist, and sampling is performed using any of several methods, depending upon the pathologist's choice.

Necrosis and polarization appear to have secondary importance compared with the nuclear grade.

Sampling the whole lesion is mandatory to exclude any minute foci of invasion before giving a diagnosis of DCIS.

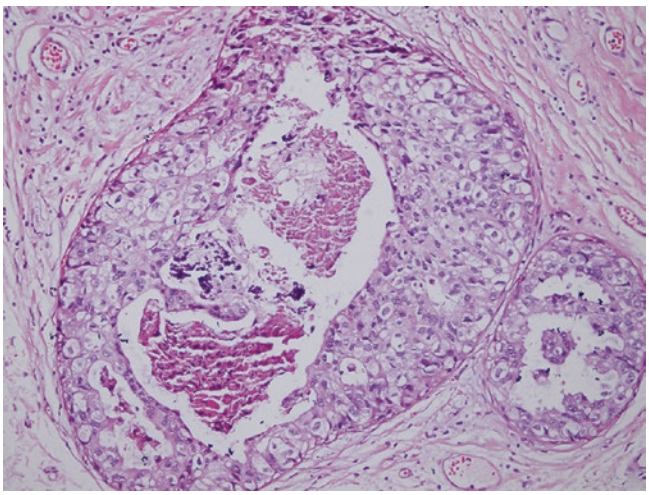

Fig. 14.1 DCIS of high nuclear grade. Proliferation of pleomorphic cells in two spaces in the center, and comedo necrosis is apparent

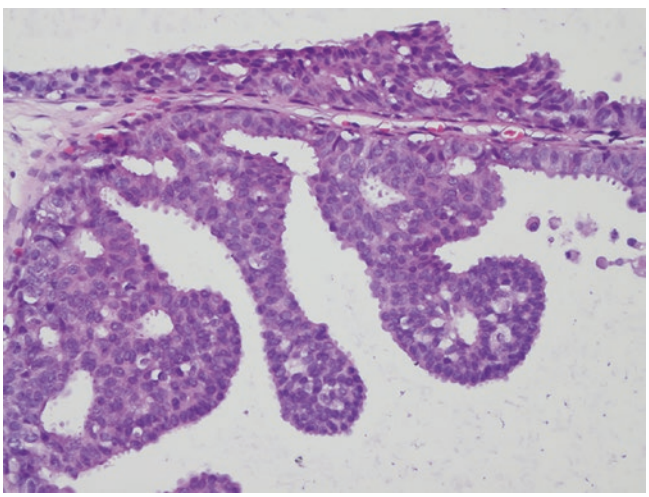

Fig. 14.2 DCIS of low nuclear grade. Proliferation of monotonous, uniform cells forming a micropapillary and cribriform architecture

Differential Diagnosis

- *Lobular carcinoma* in situ (*LCIS*) versus *DCIS*: DCIS with a solid pattern must occasionally be distinguished from LCIS. This distinction may be difficult on a morphological basis, especially in pleomorphic LCIS and LCIS with central necrosis. Immunohistochemically, the presence of E-cadherin is helpful in categorizing an individual case in favor of DCIS. LCIS is characterized by the loss of E-cadherin and cytoplasmic localization of *p120* [8].
- *Usual ductal hyperplasia* (*UDH*) *and atypical ductal hyperplasia* (*ADH*) versus *low-grade DCIS*: The difference between ADH and low-grade DCIS lies in the extent of the involvement of the duct system. In ADH, there is partial involvement of multiple spaces, whereas low-grade DCIS involves the entire duct space. Page and Tavassoli have proposed that to describe a lesion as low-grade DCIS, complete involvement should include at least two sites or be larger than 2 mm [9, 10]. Lesions occupying fewer than two sites or a total area smaller than 2 mm are called ADH (Fig. 14.3). This distinction is

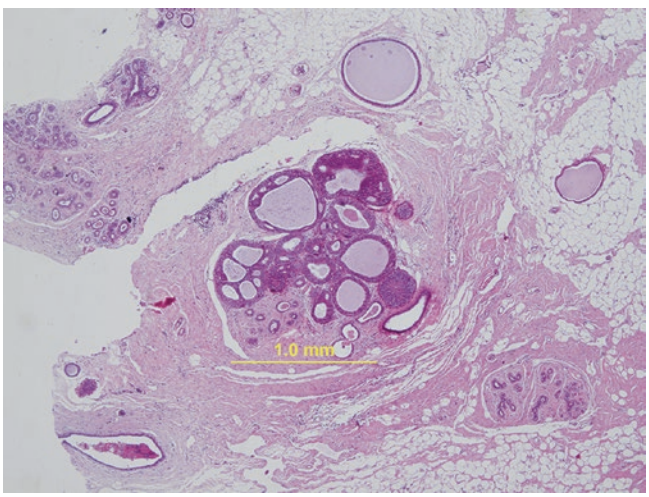

Fig. 14.3 Atypical ductal hyperplasia (ADH). Architectural and cellular features of a low-grade DCIS but covering less than 2 mm, in a terminal ductal-lobular unit (TDLU)

imperfect, and the levels of concordance and consistency in their diagnosis are low [3].

- *Foci of microinvasion*: DCIS extending into a terminal ductal-lobular unit (TDLU) or an adjacent benign proliferative lesion, such as sclerosing adenosis (SA) or a radial scar, may create the impression of microinvasion. The absence of invasive foci can be confirmed demonstrating the presence of myoepithelial cells (using antibodies against smooth muscle actin, p63, CD10, calponin, etc.) or the basement membrane (using antibodies against collagen type IV or laminin) by immunohistochemistry.
- *Invasive cribriform carcinoma* (*ICC*): This unusual invasive carcinoma can be mistaken as DCIS of the cribriform type. The diagnosis of ICC is based on the recognition of the infiltrative pattern and the absence of myoepithelial markers by immunohistochemistry.
- *Receptor status*: Most cases of DCIS are positive for estrogen receptor (ER). Positivity (defined as ≥1% of tumor cells) is observed in 70–85% of cases [3, 4]. Expression correlates with the grade of DCIS. Almost all cases of ER-negative DCIS are of high nuclear grade. Progesterone receptor (PR) expression is lower than ER expression.

Columnar Cell Lesions and Flat Epithelial Atypia

Lesions lacking intraluminal proliferation have long been recognized, and they have been given a variety of names with regard to cell morphology and the presence or absence of atypia. In 2003, Schnitt et al. classified these lesions as follows [11]:

Columnar cell change (CCC)
Columnar cell hyperplasia (CCH)
CCC with atypia
CCH with atypia

A simplified terminology combining the latter two under the term flat epithelial atypia (FEA) has now become widely used [3, 12]:

CCC
CCH
FEA

CCC and CCH are lesions in the TDLU that are characterized by enlarged, variably dilated acini lined by columnar epithelial cells [3]. These lesions are microscopic in size and are increasingly detected because of mammographic microcalcifications. The cells have ovoid nuclei that are oriented perpendicularly to the basement membrane and have evenly dispersed, fine chromatin and inconspicuous nucleoli. The lesions are frequently associated with intraluminal secretion and microcalcification. Lesions in which the epithelial lining is composed of one or two cell layers are categorized as CCC. If there is cellular stratification of more than two layers and piling up of several layers, the term CCH is used.

Columnar cell lesions are associated with a very low risk for subsequent development of invasive breast cancer, and these lesions do not increase this risk independent of concurrent proliferative changes [13].

FEA: Lesions exhibiting cellular atypia in addition to the architectural patterns described for CCC and CCH are categorized as FEA. FEA is characterized by the replacement of native epithelial cells with one to several layers of monotonous, cuboidal to columnar cells with low-grade cytologic atypia. The cells often have apical snouts. Well-developed bridges or arcades are absent (Fig. 14.4).

FEA corresponds to Azzopardi's "clinging carcinoma, monomorphic type." Other flat proliferations corresponding to the "clinging carcinoma, high-grade polymorphous type" should be categorized as DCIS. If a lesion with low-grade nuclear features has well-developed bridges, arcades, or bulbous micropapillae, it should be diagnosed as ADH or low-grade DCIS depending on the quantity of lesions (see above). The risk of subsequent invasive breast cancer in FEA is low and is substantially lower than the risk associated with established forms of ADH [3]. FEA is often associated with ADH, low-grade DCIS, lobular neoplasia (LN), and tubular carcinoma (TC).

In contrast to the normal breast and UDH, where ER and PR immunostaining are heterogeneous and limited to approximately 10–15% of cells, CCL and FEA exhibit diffuse and homogenous staining in all lesional cells. Most cells show immunostaining for low-molecular-weight cytokeratins and are negative for CK5/6.

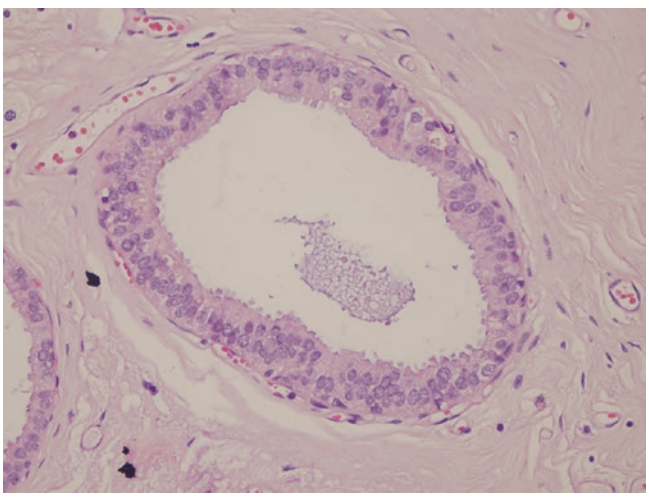

Fig. 14.4 Flat epithelial atypia. The duct is lined by one to three layers of cells with low-grade atypia and apical snouts. There is no bridge or arcade formation

Lobular Neoplasia: Lobular Carcinoma In Situ (LCIS)

The entire spectrum of atypical epithelial lesions originating in the TDLU of the breast, which is characterized by the proliferation of generally small, non-cohesive cells, is called LN. The terms atypical lobular hyperplasia (ALH) and LCIS reflect the extent of the lesion. In both types of lesions, proliferating cells are cuboidal or polygonal, monotonous and poorly cohesive cells with clear or light cytoplasm. Pagetoid spread of these cells between the surface epithelial cells and basement membrane is a common finding. When more than half of the acini of a lobular unit are distended and distorted, the lesion is called LCIS (Fig. 14.5). Lesser involvement with cells showing the same characteristics is called ALH [8] (Fig. 14.6). The differentiation of these two is occasionally subjective. Thus, the term LN, which does not differentiate between LCIS and ALH, was introduced [3].

LCIS occurs predominantly in premenopausal patients and is multicentric in 60–80% of patients and bilateral in 20–60% [14]. Classic LCIS is usually an incidental finding detected in surgical or core biopsies targeting another lesion. The variant types *pleomorphic LCIS* and *LCIS with necrosis* (*florid LCIS*) are usually present as mammographically detected pleomorphic microcalcifications or as mass lesions with or without calcifications [14]. These variant types are more common in older women as compared to classic LCIS.

Pleomorphic LCIS The cells are markedly pleomorphic with large nuclei. Central necrosis and microcalcifications may be present [15] (Fig. 14.7).

LCIS with Comedo Necrosis In addition to the classic, small, monotonous cells of LCIS, there is comedo-type necrosis in the central portion of cellular spaces [16]. This lesion is also referred to as *florid LCIS*.

Differential Diagnosis The morphological distinction from solid-type DCIS is discussed above. It should be kept in mind that some LCIS cases may have aberrant E-cadherin expression. The immunohistochemical findings should always be interpreted in light of morphological findings [14]. p120 immunohistochemistry may be used in combination with E-cadherin in ambiguous cases. Sarrio et al. [8] found that 90% of ALH cases and 100% of LCIS cases had diffuse cytoplasmic staining for p120, in contrast to DCIS cases, which had reduced membranous staining without any cytoplasmic staining.

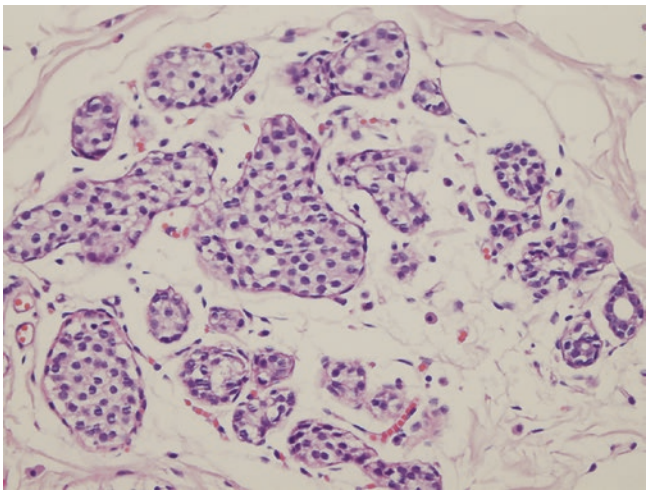

Fig. 14.6 Atypical lobular hyperplasia (ALH). Proliferation of small, uniform cells that slightly distend the acini of the lobule

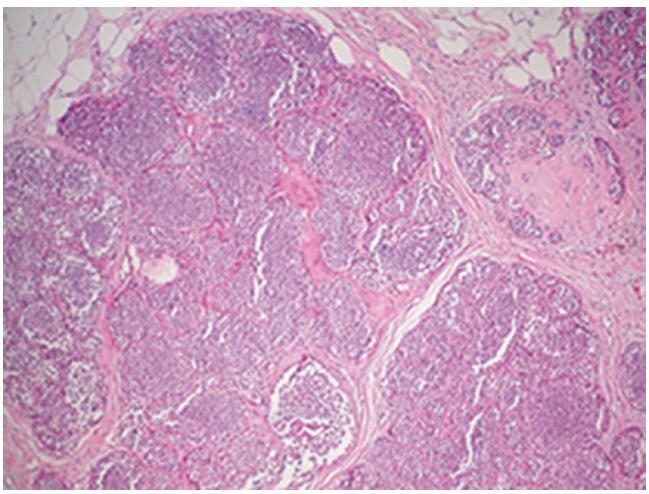

Fig. 14.5 Lobular carcinoma in situ (LCIS). Proliferation of small, uniform, discohesive cells that completely fill and distend the TDLUs

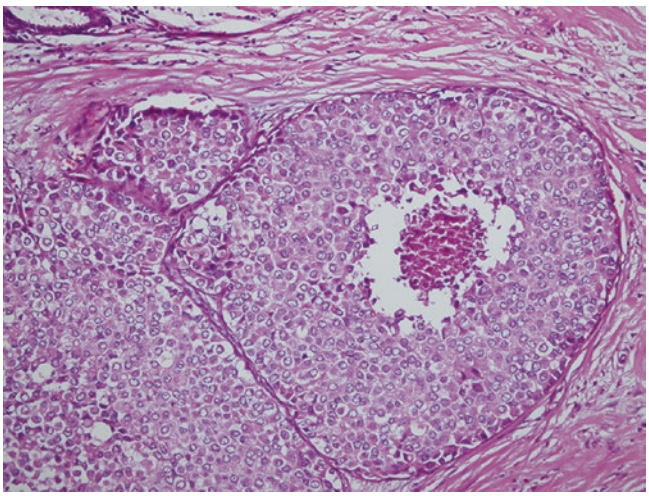

Fig. 14.7 LCIS of pleomorphic type with necrosis. The cells were E-cadherin negative. There is necrosis in the center of one of the spaces

LN/LCIS is almost uniformly positive for ER and PR and negative for E-cadherin. Classic LCIS and LCIS with comedo necrosis are negative for Her2 and p53 and have a low Ki-67 index. However, pleomorphic LCIS may have Her2 and p53 overexpression and moderate to high Ki-67 [17].

LN is classically accepted as a risk indicator of breast cancer development for both breasts; however, recent, carefully conducted cohort studies suggest that the risk is higher in the ipsilateral breast (68% versus 24%) [18]. The available clinical and molecular evidence suggests that ALH and LCIS are clonal and neoplastic and that these lesions are both risk indicators and non-obligate precursors of breast cancer [19]. However, LCIS is currently managed as a benign lesion with an associated risk for developing carcinoma and does not require complete removal and/or evaluation of margin status [20, 21].

In the eighth edition of the TNM staging by the American Joint Committee on Cancer (AJCC), LCIS is no longer staged as Tis [20, 21]. The expert panel of AJCC also does not categorize pleomorphic LCIS in the Tis category because of the insufficient data regarding outcomes and the absence of reproducibility in diagnosis. Observation with interval breast imaging is a reasonable alternative for most cases instead of surgery, after careful radiologic/pathologic correlation is given to exclude discordant cases [22]. However, in cases diagnosed as pleomorphic LCIS alone on core needle biopsy (CNB), the upgrade rate to invasive carcinoma or DCIS after final surgical excision is 18–30% [23]. The florid form of LCIS is more frequently associated with an invasive component than the nonflorid form (87% versus 73%, respectively). The invasive component is lobular in 100% of florid LCIS lesions but only 82% of nonflorid LCIS lesions [24]. Recent evidence also suggests that the florid form of LCIS is genetically more advanced than the indolent phenotype of classic LCIS [25]. This difference may explain the greater frequency of concurrent invasive carcinoma in florid LCIS compared with that of classic LCIS [25].

Microinvasive Carcinoma

This lesion is characterized by one or more clearly separate microscopic foci of tumor cells that infiltrated the mammary stroma, each less than or equal to 1 mm in size, and is most commonly observed in a background of high-grade DCIS [3, 26].

Microinvasive carcinoma is most commonly observed in a background of extensive high-grade DCIS with prominent inflammatory infiltration [3] accompanied by stromal edema and desmoplasia. This entity is commonly overdiagnosed. Central consultation usually downgrades the lesion. Even in cases initially suspected or diagnosed as microinvasion, subsequent review downgrades the diagnosis in 80% of cases [27]. Differentiation from DCIS is described above. These foci are commonly lost in serial sections performed for immunostaining.

The prognosis is not clearly different from that of patients with DCIS of equivalent grade [3].

Invasive Carcinomas

Invasive carcinomas can broadly be divided into two categories: invasive carcinoma of no special type (NST) and special subtypes [3]. Invasive carcinoma NST and invasive lobular carcinoma (ILC) constitute the major types of breast carcinoma. The cytoarchitectural and spread patterns of some carcinomas are sufficiently distinctive to be recognized as special subtypes, especially when associated with a particular behavior [28].

According to the recent WHO classification, invasive breast carcinomas are classified as indicated in Table 14.1 [3].

Invasive Carcinoma of No Special Type (NST)

This carcinoma is the most common type of invasive breast cancer and represents up to 75% of cases in published series. Terms such as infiltrating ductal carcinoma and invasive ductal carcinoma, not otherwise specified (NOS), are also used. There is a wide range in the frequency of invasive carcinoma NST because there are no strict criteria for inclusion of these tumors in the special types and because different centers have varying attitudes in categorizing cases with different amounts of NST and special types. A tumor should be called invasive ductal carcinoma (IDC) NST if it cannot be categorized as one of the special or rare types. There is great variation in their appearance.

Gross Features IDC NST has no specific gross features and also shows a great variation in size, ranging from a few millimeters to huge masses. In typical cases, these tumors have irregular, stellate borders (Fig. 14.8). These tumors have a firm consistency, and their cut surface is generally gray-white with a gritty sensation. Less frequently, the tumor may have a nodular configuration with circumscribed margins and relatively softer consistency.

Microscopic Features The tumor cells are arranged in sheets, clusters, cords, trabeculae, and glands/tubules or occasionally in a solid pattern with no or little intervening stroma. Cellular features also show great variability. Nuclei may be uniform and regular or highly pleomorphic with very prominent and multiple nucleoli. Mitotic activity is also highly variable (Fig. 14.9). IDC NST may have histopathological characteristics of special types, but less than 50% of

Table 14.1 WHO classification of breast cancer

Invasive carcinoma of no special type
Pleomorphic carcinoma
Carcinoma with osteoclast-like stromal giant cells
Carcinoma with choriocarcinomatous features
Carcinoma with melanocytic features
Special types:
Invasive lobular carcinoma
Classical lobular carcinoma
Solid lobular carcinoma
Alveolar lobular carcinoma
Pleomorphic lobular carcinoma
Tubulolobular carcinoma
Mixed lobular carcinoma
Tubular carcinoma
Cribriform carcinoma
Mucinous carcinoma
Carcinoma with medullary features
Medullary carcinoma
Atypical medullary carcinoma
Invasive carcinoma NST with medullary features
Carcinoma with apocrine differentiation
Carcinoma with signet ring cell differentiation
Invasive micropapillary carcinoma
Metaplastic carcinoma of no special type
Low-grade adenosquamous carcinoma
Fibromatosis-like metaplastic carcinoma
Squamous cell carcinoma
Spindle cell carcinoma
Metaplastic carcinoma with mesenchymal differentiation
Chondroid differentiation
Osseous differentiation
Other types of mesenchymal differentiation
Mixed metaplastic carcinoma
Myoepithelial carcinoma
Rare types
Carcinoma with neuroendocrine features
Neuroendocrine tumor, well differentiated
Neuroendocrine carcinoma, poorly differentiated (small-cell carcinoma)
Secretory carcinoma
Invasive papillary carcinoma
Acinic cell carcinoma
Mucoepidermoid carcinoma
Polymorphous carcinoma
Oncocytic carcinoma
Lipid-rich carcinoma
Glycogen-rich clear cell carcinoma
Sebaceous carcinoma
Salivary gland/skin adnexal-type tumors
Cylindroma
Clear cell hidradenoma
Epithelial-myoepithelial tumors
Pleomorphic adenoma
Adenomyoepithelioma
Adenomyoepithelioma with carcinoma
Adenoid cystic carcinoma

Modified from Lakhani et al. [3]

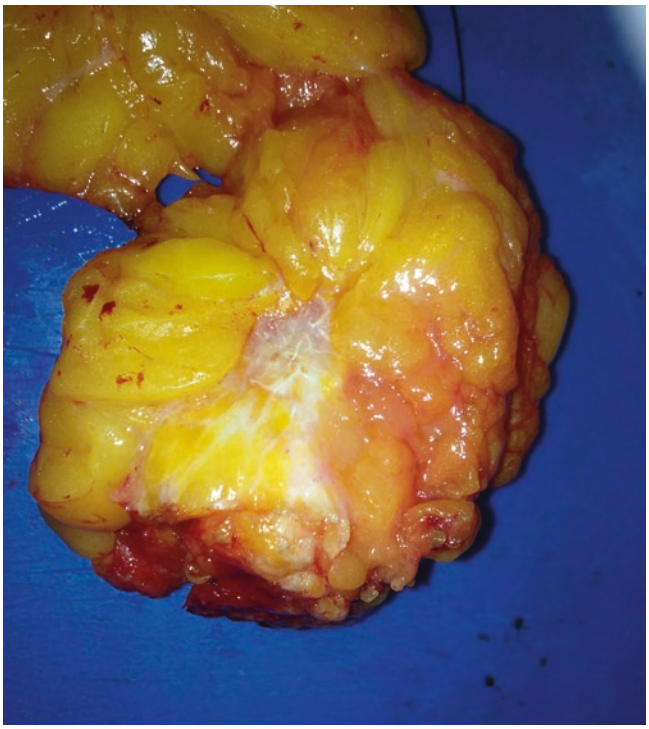

Fig. 14.8 Typical gross appearance of an invasive carcinoma located in the center of the specimen. Grayish-white tumor with ill-defined borders. Fibrocystic changes below the tumor and fatty appearance in the rest of the excision

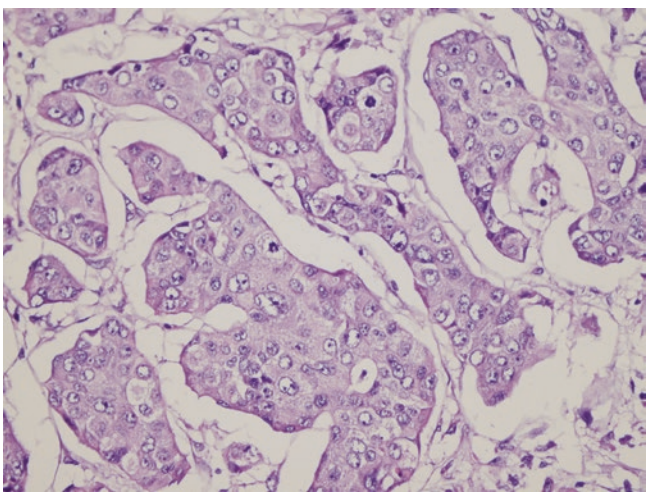

Fig. 14.9 Invasive ductal carcinoma NOS. Grade 3 carcinoma with sheets of cells with pleomorphic nuclei and frequent mitosis

the tumor will fall into this category. In other words, in IDC NST, at least 50% of the tumor should be composed of a nonspecialized type. The tumor stroma may be abundant.

When a proportion of specialized histopathological forms accompany the IDC NST, these carcinomas are described as "mixed type" [3].

Pleomorphic carcinoma, carcinoma with osteoclast-like stromal giant cells, carcinoma with choriocarcinomatous features, and carcinoma with melanocytic features are not recognized as distinct special types but as variants of IDC NST [3]. The latter two are exceptionally rare.

Pleomorphic Carcinoma

Pleomorphic carcinoma is characterized by the proliferation of bizarre, highly anaplastic, and occasionally multinucleated cells. Approximately one third of the cases have a metaplastic spindle cell component [29, 30]. Spindle cell components and tumor size (>5 cm) are associated with poor clinical outcome [30].

This prognostically unfavorable tumor represents the extreme end of the morphological spectrum of grade III infiltrating ductal carcinoma [29].

Carcinoma with Osteoclast-Like Stromal Giant Cells

The distinctive feature is the presence of osteoclastic giant cells (OGCs). Grossly, they have a striking red-brown cut section with a hemorrhagic appearance, especially in cases with numerous OGCs. These cells are generally associated with a fibroblastic and hypervascular stroma that contains inflammatory cells, erythrocytes, and hemosiderin as evidence of recent and past hemorrhages. OGCs are usually close to the edges of carcinomatous glands or in the intervening stroma and are occasionally present in the glandular lumens. OGCs may appear to be fused with the glandular component and may be difficult to discern (Fig. 14.10). Associated carcinomas are mostly well to moderately differentiated, showing a relatively more common cribriform pattern. OGCs are positive for CD 68, acid phosphatase, and lysozyme but negative for cytokeratin and alkaline phosphatase. OGCs are negative for ER, PR, cytokeratin, epithelial membrane antigen (EMA), actin, and S-100 protein [31–33]. This immunohistochemical profile, along with the absence of any epithelial features in ultrastructural examination, supports the histiocytic origin of these cells [33]. OGCs also express the osteoclast markers MMP-9, TRAP, and cathepsin K, and these markers appear to form in response to the specific hypervascular stroma, which secretes cytokines, such as VEGF and MMP-12 [34, 35].

Axillary lymph node involvement has been reported in one third of cases [32, 33]. Distant metastasis to a variety of sites has also been reported [32, 33]. The 5-year survival rate is approximately 70%, which is similar to or slightly better than that of patients with ordinary invasive ductal carcinoma [3]. In a series of 42 patients with a mean follow-up time of 46.4 months by Zhou et al. [36], lung metastasis was observed in 2 patients (5%) at 7 and 11 years after operation, respectively. All of their cases for which immunohistochemistry was available were luminal type, of which 89% had a luminal A phenotype.

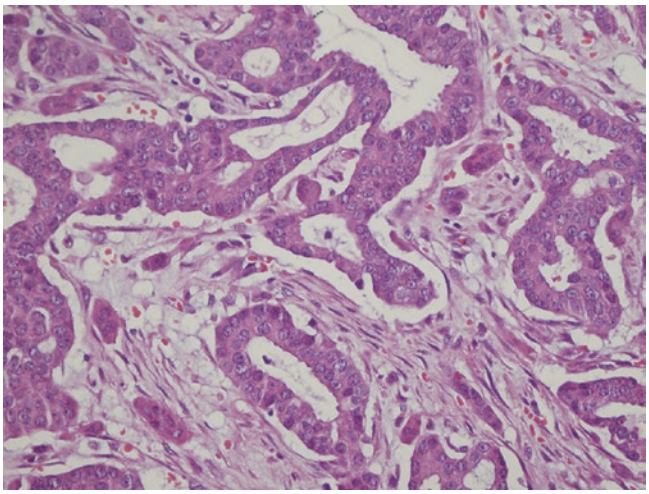

Fig. 14.10 Carcinoma with osteoclast-like giant cells. The cribriform architecture is formed by the tumor cells. Multinucleated giant cells resemble osteoclasts in the fibroblastic stroma

Invasive Lobular Carcinoma

ILC is a carcinoma composed of non-cohesive cells that are individually dispersed or arranged in a single-file linear pattern in fibrous stroma [3]. ILC represents 5–15% of invasive breast carcinomas [3]. In most series, its incidence is approximately 10% [33].

ILC frequently presents as a mass with irregular borders that occasionally cannot be defined macroscopically, and the breast tissue appears normal, with only a firm consistency by palpation [32]. The size ranges from occult, microscopic lesions to tumors that diffusely involve the entire breast [32]. ILC may form numerous, fine, hard nodules that grossly and microscopically mimic the benign lesion "sclerosing adenosis."

The incidence of synchronous or metachronous bilateral carcinoma in ILCs is nearly twice that observed in IDCs [37, 38].

Classic ILC

ILC is characterized by the proliferation of small, uniform cells that lack cohesion and are dispersed individually in a fibrous stroma or arranged in linear cords. These cords usually present a concentric pattern around nonneoplastic ducts, forming the "targetoid pattern" (Fig. 14.11). The tumor cells are bland or monotonous and have round to ovoid nuclei. Mitoses are uncommon. Most ILCs are of low-to-intermediate histologic grade.

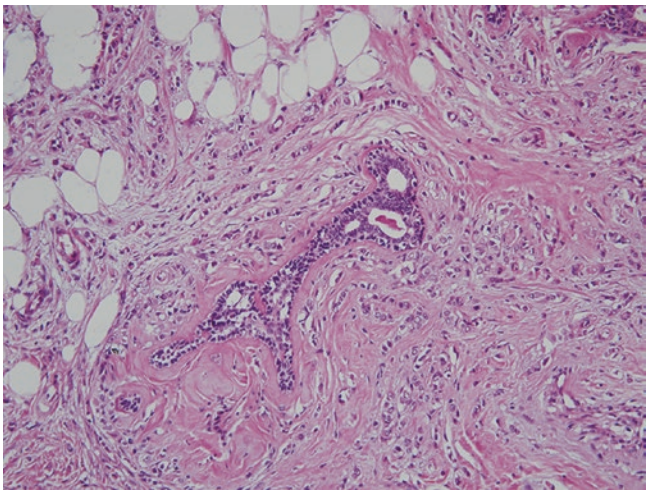

Fig. 14.11 Invasive lobular carcinoma. Small, discohesive tumor cells form the single-file pattern around the intact duct

ILC has some histologic variants that differ from the classic type in terms of their histological growth or cytological patterns but still lack cellular cohesion.

Solid Variant

Uniform, small, and non-cohesive cells are arranged in sheets. There is little stroma. These tumors are often more pleomorphic and have a higher mitotic rate compared with that of classic ILC [37–39].

Alveolar Variant

Alveolar variant exhibits small, globular aggregates of 20 or more cells [40].

Tubulolobular Variant

Tubulolobular variant ILC exhibits small tubules and cords of neoplastic cells in a lobular configuration reminiscent of ILC [41]. *Tubulolobular variant ILC* has many features that are intermediate between tubular carcinoma and classic ILC [41].

This tumor is morphologically different from tumors showing a mixture of tubular carcinoma and classic ILC, which should be categorized in the mixed category.

Pleomorphic Variant

Pleomorphic ILC exhibits the growth pattern of classic ILC but a greater degree of cellular atypia and pleomorphism and a higher mitotic rate than classic ILC. These cells retain their lobular characteristics with their single-file and/or targetoid arrangement and non-cohesive appearance. LCIS is present in 45–60% of cases [14, 42, 43] and is frequently of the pleomorphic type [43]. Pleomorphic ILC may show apocrine [15] or histiocytoid [44, 45] differentiation and may be composed of signet ring cells [3].

Mixed Type

These cases exhibit mixtures of the abovementioned variants and were described by Dixon [44] as "none of these patterns are prominent." Lobular differentiation accompanying IDC NST is observed in approximately 5% of invasive breast cancers [3].

ILC is almost invariably ER positive. PR positivity is present in approximately 70–80% of cases. Her-2 positivity by immunohistochemistry or in situ hybridization is very rare and generally limited to pleomorphic ILC. Immunohistochemically, E-cadherin is absent or reduced in ILC compared with that in IDC. However, a subset of ILCs express E-cadherin, ranging from 10% to 16% of ILCs [46, 47], and this subset is described as being aberrant without any significance or any correlation with known prognostic parameters [47, 48].

Most ILCs also show loss of membrane-specific catenin immunoreactivity in parallel with E-cadherin loss [46] and mislocalization of catenin p120 in the cytoplasm [49].

In general, ILCs have more favorable prognostic features than IDC NST. A higher frequency of ILC was placed in the good Nottingham Prognostic Index group (40% compared with 21% for IDC) [50] and has a better or similar outcome in the short-term period (first 6–10 years). However, the long-term outcome for ILC is worse than that for IDC NST [50, 51].

A more favorable outcome is reported for the classic type than the pleomorphic type [32]. The differences in outcome between variant forms and classic ILC have not been statistically significant [32]. Rakha et al. found that survival in patients with pleomorphic lobular carcinoma was associated with mitotic score but not with nuclear pleomorphism [52].

Distinctive patterns of metastases are associated with ILC. ILC shows a higher frequency of metastases in the intra-abdominal serosal surfaces and retroperitoneum, leptomeninges, gastrointestinal tract, and gynecologic organs and a lower frequency of pulmonary metastases [3, 31–33].

Tubular Carcinoma

Tubular carcinoma (TC) is a low-grade (grade I) carcinoma with a particularly favorable prognosis. TC is composed of well-differentiated tubular structures lined by a single layer of cells and has open lumina. Pure TC accounts for approximately 2% of invasive breast cancers. Its frequency is higher in populations where screening mammography is used. TC is more likely to be smaller lesions with less frequent nodal involvement and a better outcome than IDC NST [3].

TC often presents as an ill-defined, gray-white, firm-to-hard, stellate mass with an average size of 1.3 cm (0.2–5 cm). The cut surfaces frequently show elastotic, yellow streaks.

Microscopically, the tubules are haphazardly arranged in a typical desmoplastic stroma. The lumina of the tubules are oval or rounded with angulated ends. The single cells lining these tubules have little nuclear pleomorphism with inconspicuous nucleoli, and they exhibit very few mitoses. The cells may have apical snouts, but this characteristic has no diagnostic significance (Fig. 14.12). The myoepithelial cell layer and basal membrane are lacking in contrast to the nonneoplastic proliferations. TC occurs in association with FEA and low-grade DCIS.

There is a lack of consensus regarding the proportion of tubules necessary to establish a diagnosis of TC. In several studies, the threshold for tubule formation was set between 75% and 100%. However, a cutoff of 90% is more widely accepted [53]. Patients diagnosed with TC with this cutoff and small lesions have the same overall survival as the age-matched general population [53, 54].

Tubule formation in less than 90% of the tumor should be regarded as mixed type. One exception that should be considered is the cribriform pattern. In the presence of invasive cribriform carcinoma (ICC) intermingled with TC, these areas are also regarded as tubule formation.

Differential Diagnosis

- *Microglandular adenosis* (*MGA*): TC is occasionally composed of small, round tubules of relatively uniform caliber that are irregularly dispersed in a fibrofatty stroma resembling MGA. Glands in MGA are more rounded and regular and contain secretory material [3, 33]. The myoepithelium is lacking in both types of lesions, and immunostaining reveals no staining for calponin, p63, CD10, or cytokeratin 5. The basement membrane is lacking in TC, which can be demonstrated around the glands of MGA by periodic acid-Schiff (PAS) staining and immunostaining for collagen IV and laminin [3, 31–33]. Epithelial membrane antigen (EMA), which is present in TC, is absent in MGA [33].
- *Sclerosing adenosis* (*SA*): SA is a lobulocentric proliferation with a compressed and distorted appearance of the tubular structures. Myoepithelial cells and basement membrane are always present in SA and can be highlighted with the immunostaining described above. TC does not have a lobulocentric growth pattern and does not contain myoepithelial cells or a basement membrane.
- *Complex sclerosing lesion* (*radial scar*): The central fibroelastotic core of this lesion may have a few, distorted, entrapped, pseudoinfiltrative glands, creating diagnostic difficulty due to its resemblance to TC. The glands at the periphery of the core are hyperplastic and dilated. This zoning phenomenon is lacking in TC. The glands in CSL also contain myoepithelial cells and a basement membrane.

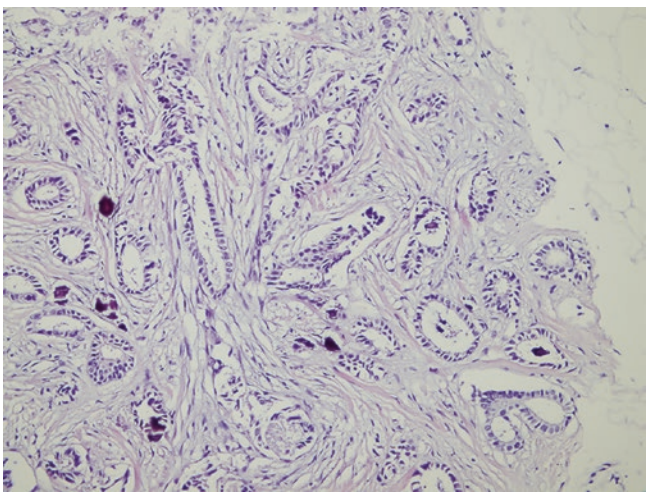

Fig. 14.12 Tubular carcinoma. Tumor cells form tubules, some of which have microcalcifications in their lumens

Women with "pure" TC have an excellent prognosis. The frequency of axillary lymph node metastasis is approximately 10%. TC has a better prognosis than grade I IDC or tubular mixed carcinomas, independent of other prognostic factors [53, 54]. At a follow-up of 127 months (4–217 months), recurrent disease was found in 13.2% of patients with TCs, with no cancer-specific deaths, in contrast to 29.4% of patients with grade I IDCs and a cancer-specific death rate of 9% [53].

Invasive Cribriform Carcinoma

ICC is a low-grade carcinoma with excellent prognosis in which the majority of the invasive component shows a cribriform pattern of growth, similar to intraductal cribriform carcinoma. Pure ICC consists of an invasive cribriform pattern in more than 90% of the tumor [55, 56]. The tumor cells, which have mild to moderate pleomorphism, are arranged to form cribriform spaces (sievelike pattern). Mitoses are rare. There are no specific gross features of this tumor.

Differential Diagnosis

Adenoid cystic carcinoma (*ACC*): ICC most closely resembles ACC. ICC is composed of one cell type and lacks the basal-myoepithelial type. There is mucinous material in the cribriform spaces, and tumor cells are diffusely positive for ER. In ACC, there are two cell types, basal-myoepithelial and luminal, and secretory and basement membrane-like material is present in the glandular spaces. ACC also shows a triple-negative immunoprofile.

Cribriform DCIS: ICC has a more irregular and angular cribriform pattern with a more haphazard distribution compared with that of cribriform DCIS. Cribriform DCIS has a myoepithelial cell layer around the cribriform structures.

In cribriform DCIS, 100% of cases are ER positive, 69% of cases are PR positive [50], and HER2 expression is absent [33].

The prognosis of ICC is favorable [56] and similar to TC [55]. The 10-year overall survival is 90–100% [56, 57].

Carcinoma with Medullary Features

These tumors exhibit some or all of the following features: a circumscribed or pushing border, syncytial growth pattern, cells with high-grade nuclei, and prominent lymphoid infiltration. According to the 2003 WHO classification [58], tumors that fulfill all of these criteria are called medullary carcinoma (MC), and tumors that fulfill most but not all of these criteria are called atypical MC. In the more recent classification, there has been an attempt to categorize these tumors into three groups under the heading "Carcinomas with Medullary Features" as follows [3]:

- MC
- Atypical MC
- IDC NST with medullary features

The criteria that distinguish these groups are vague and have poor interobserver reproducibility. Distinguishing between the latter two groups is particularly difficult. In our institutional practice, we prefer reserving the term MC for tumors exhibiting all of the features described above, using very strict criteria, and calling the tumors exhibiting some of these features atypical MC.

Despite poor clinicopathologic features, patients with medullary histology demonstrate favorable long-term distant relapse-free survival compared with that of patients with IDC NST. Local control rates of MC and IDC are comparable [59]. In a retrospective study of 165 cases of basal-like carcinomas, the Nottingham group found that prominent inflammation and anastomosing sheets in at least 30% of the tumor were associated with a better prognosis in a univariate analysis [60]. The combination of these two features was present in 17% of tumors and was an independent prognostic factor in a multivariate analysis. The authors also proposed a simplified definition of medullary-like type based on these two features [60].

Mucinous Carcinoma

Mucinous carcinoma is characterized by the production of extracellular and/or intracellular mucinous material. Clusters of generally small and uniform cells are seen as floating in large amounts of mucin. A lesion is called pure mucinous carcinoma if the mucinous component constitutes more than 90% of the lesion [61]. Mucinous carcinoma is also observed as part of a mixed carcinoma with IDC NST. The axillary lymph nodes are rarely involved.

Gross examination of mucinous carcinomas reveals a circumscribed, gelatinous mass with pushing margins and soft consistency. The cut surface has a glistening appearance. Confluent hemorrhagic areas are frequent [33] (Fig. 14.13). The tumor size ranges from 0.5 to 20 cm. Despite these large diameters, axillary nodal involvement is infrequent.

Microscopically, there are clusters of tumor cells floating in mucin lakes separated by delicate fibrovascular septae. The clusters are variable in size. Nuclear atypia is generally low. Mucinous carcinoma can be divided into two categories, types A and B [62]:

Type A mucinous carcinoma: This is the classic or non-endocrine variant and is characterized by larger quantities of mucin. Mucin is always extracellular [33] (Fig. 14.14).

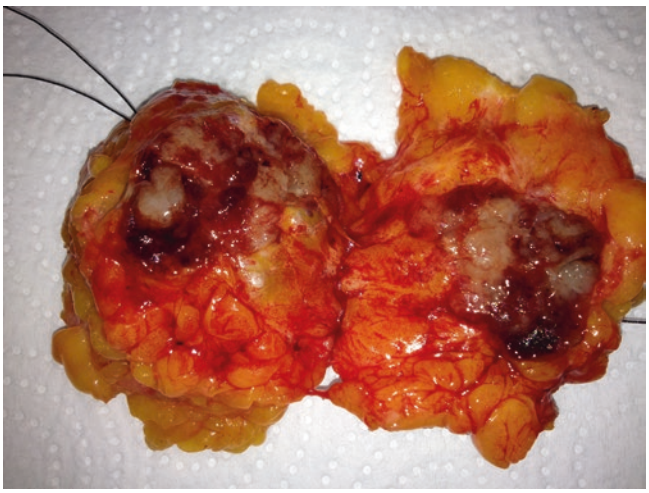

Fig. 14.13 Mucinous carcinoma. The tumor has well-defined borders and a lobulated appearance with a glistening and partially hemorrhagic cut surface

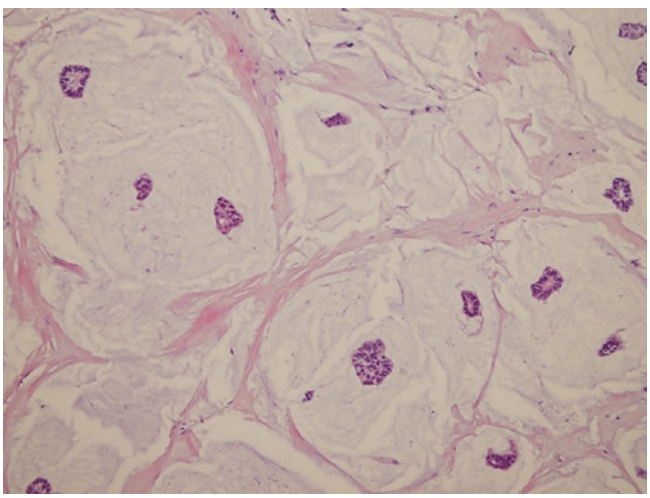

Fig. 14.14 Mucinous carcinoma type A with a huge amount of extracellular mucin and low-grade cellular atypia

Type B mucinous carcinoma: This type is more cellular with large clusters and has frequent neuroendocrine differentiation. Intracytoplasmic mucin is abundant in type B lesions (Fig. 14.15).

Mucinous carcinoma is usually positive for ER and PR and negative for HER2.

Type AB mucinous carcinoma constitutes 20% of cases and is an intermediate lesion that has features of both types.

The most important entity in the differential diagnosis is the "mucocele-like lesion" [61]. Mucinous carcinoma should also be distinguished from myxoid fibroadenomas, especially in fine-needle aspiration biopsies.

Mucinous carcinomas have a favorable outcome [54]. In a follow-up series of 11,400 cases of pure mucinous carcinoma, the 5-, 10-, 15-, and 20-year survival rates were 94, 89, 85, and 81%, respectively [63]. Nodal involvement was associated with significant disease-free survival and overall survival [54]. The separation of cases as types A and B has no clinical significance.

Mucinous Carcinoma with Micropapillary Pattern (MCMP)

MCMP is an otherwise pure mucinous carcinoma with a component of micropapillary architecture, similar to that of an invasive micropapillary carcinoma (see below). MCMP is a variant of mucinous carcinoma with intermediate- to high-grade nuclei, a hobnail pattern, and micropapillary architecture. MCMP pursues a more aggressive clinical course than pure mucinous carcinoma [64–66].

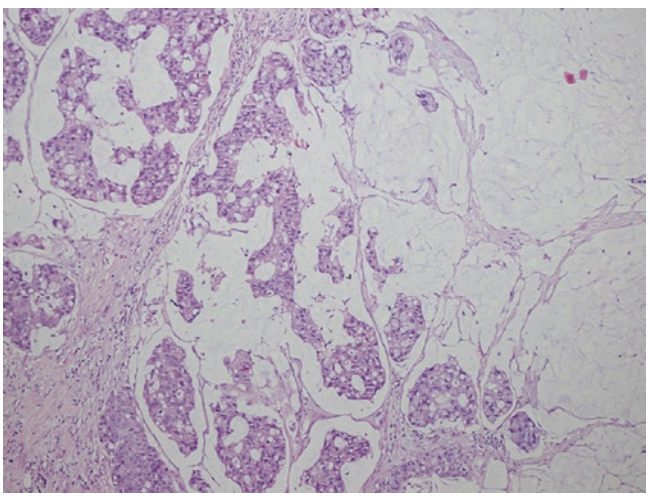

Fig. 14.15 Mucinous carcinoma type B with a more cellular appearance and a higher grade of cellular atypia

Carcinomas with Signet Ring Cell Differentiation

Cells with signet ring cell differentiation have abundant mucin in their cytoplasm, which pushes the nucleus to one side, creating the typical signet ring cell appearance (Fig. 14.16). Carcinomas with extensive signet ring cell differentiation are rare. Focal signet ring cell differentiation is more commonly observed.

Prominent signet ring cell differentiation is most common in ILC.

Pathologists must occasionally distinguish these cases from gastrointestinal metastasis. The presence of an in situ component suggests primary breast cancer. In difficult cases, steroid receptor expression and antibodies specific to breast carcinoma, such as GCDFP or mammaglobin, are helpful.

The prognostic importance of signet ring cell differentiation is uncertain [3].

Carcinoma with Apocrine Differentiation

This class includes any invasive carcinoma containing cells with cytological features of apocrine differentiation. These cells have abundant, eosinophilic, granular cytoplasm and large nuclei with prominent nucleoli. There is a transition to cells with foamy cytoplasm resembling sebaceous cells, which may occasionally dominate the histology. Focal apocrine differentiation is not very rare. However, a tumor is called "pure" apocrine carcinoma if 90% of the lesion is composed of these cells. ER and PR expression are usually negative. Androgen receptor (AR) positivity is encountered

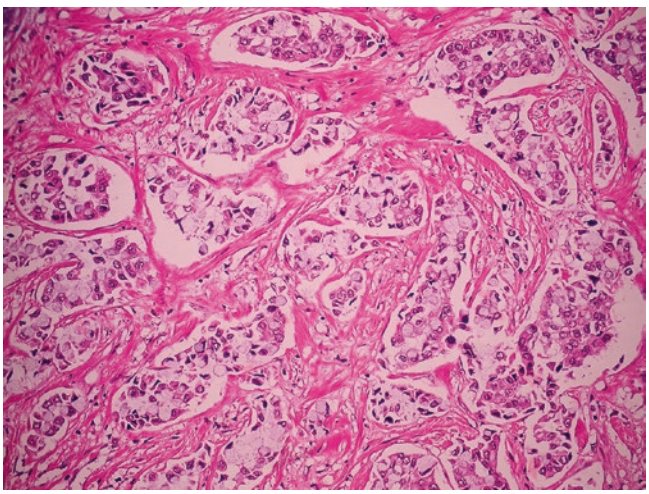

Fig. 14.16 Carcinoma with signet ring cell differentiation. Nuclei of most of the cells are pushed to one side by mucous, creating the "signet ring" appearance

in more than 70% of apocrine carcinomas. GCDFP-15 is characteristic but not specific for apocrine cells [33, 67]. From a practical perspective, we do not call tumors pure apocrine carcinoma if there is ER or PR expression. The expression of AR in ER-/PR-/HER2+ tumors, which commonly show apocrine differentiation, and a subset of triple-negative apocrine tumors suggests that these tumors together form a molecular apocrine group [68].

A study with long-term follow-up revealed that patients with pure apocrine carcinomas (negative for ER and PR and positive for AR) have shorter disease-free survival than patients with IDC NST and apocrine-like IDC (ER or PR positive and AR negative) [69]. Recently, Meattini et al. [70] found that triple-negative apocrine carcinomas had a favorable long-term outcome compared to that of triple-negative non-apocrine carcinomas (83% versus 63% 10-year overall survival).

Invasive Micropapillary Carcinoma (IMPC)

This type of tumor, which was first described by Tavassoli and her colleagues [71], is now regarded as a distinct entity. IMPC accounts for 0.9–1.7% of invasive breast carcinomas when occurring in pure form and up to 7.6% when admixed with other types of mammary carcinoma [72]. Most patients present with a palpable mass [72].

The tumor is composed of small, hollow, or morula-like clusters of tumor cells that lack fibrovascular cores and are surrounded by clear stromal spaces (Fig. 14.17). The "reverse polarity" of cancer cells is typical and can be facilitated by immunohistochemical demonstration of MUC1 in the stroma-facing surface. This reaction may also be used to differentiate these spaces from lymphovascular invasion or retraction artifacts [3, 72]. The presence of an in situ component is helpful in excluding rare cases of metastatic ovarian serous papillary carcinoma to the breast.

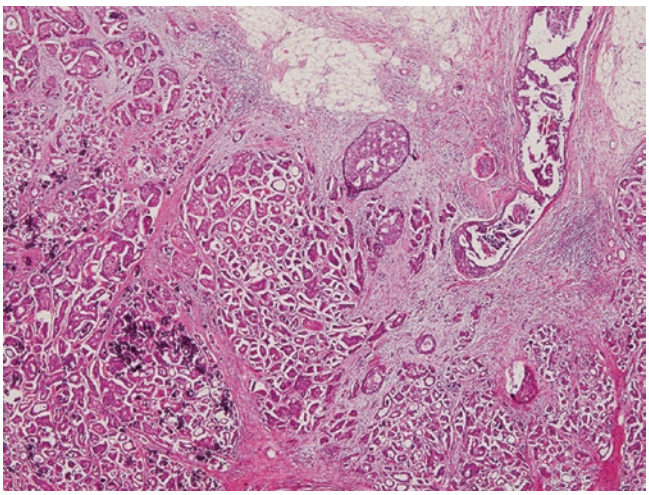

Fig. 14.17 Invasive micropapillary carcinoma. Lobular configuration of the invasive tumor. The clusters of tumor cells are surrounded by clear spaces. DCIS is evident on the upper right, and microcalcification is visible in the lower left

Most cases are grade 2 or 3 carcinomas, and the majority are ER and PR positive. HER2 overexpression is present in less than 10–35% of cases [3].

High-resolution microarray comparative genomic hybridization has revealed that high cyclin D1 expression, high proliferation rates, and MYC (8q24) amplification are significantly associated with IMPCs [73].

IMPCs present more frequently with lymphovascular invasion and lymph node metastasis compared with those in IDC NST [74]. The Ki-67 proliferative index is significantly higher in IMP carcinomas with p63 expression (nuclear or cytoplasmic) than in those without and is also higher in cases with lymph node metastasis than in cases without [75]. However, the association of this histology with survival remains unclear. In a recent series of 49 patients, IMPC histology did not add any independent information to the risk of locoregional or distant relapse or to overall survival [76].

Metaplastic Carcinoma

Metaplastic carcinoma encompasses a group of neoplasms that are characterized by the differentiation of the neoplastic epithelium into squamous and/or mesenchymal-looking elements, including but not restricted to spindle, chondroid, osseous, and rhabdomyoid cells [3]. The tumor may be entirely composed of metaplastic elements or may include a mixture of carcinoma and metaplastic elements. Its incidence is less than 1% [77, 78]. Metaplastic carcinoma is usually diagnosed as T2 disease, and the mean size is 3.4–4.4 cm [77].

These tumors can present either as a circumscribed nodule or as a mass with indistinct borders. Cystic changes can occur, especially in cases that are accompanied by squamous cell carcinoma (SCC).

The recent WHO classification [3] categorizes metaplastic carcinomas in a descriptive manner.

Low-Grade Adenosquamous Carcinoma (LGASC)

This tumor is similar to the infiltrating syringomatous tumors of the salivary glands and microcystic adnexal carcinomas of the skin of the lip [79]. Patients present with a palpable mass [80], and grossly, the tumors are smaller than other forms of metaplastic carcinoma [32]. The tumors have a hard consistency and ill-defined borders [80]. Microscopically, there are well-defined tubules and glands in a spindle cell background.

Squamous differentiation is observed as solid nests, syringoma-like areas, and isolated inconspicuous foci in glandular structures and solid cords. Squamous differentiation may be extensive with large keratinizing cyst formations. In our experience, this rare tumor is an underdiagnosed entity and therefore may be left untreated; during their long evolution, they recur and metastasize.

Fibromatosis-Like Metaplastic Carcinoma

This tumor is characterized by bland spindle cells having slender nuclei with tapered ends. Nuclear atypia is mild or absent. These cells are arranged in wavy, interlacing fascicles. Focal squamous differentiation is observed. Because of the bland appearance of tumor cells, this tumor may be underdiagnosed as benign [81]. The tumor is always positive for keratins [81] and p63 [3].

Squamous Cell Carcinoma

Grossly, it is often a cystic lesion [3]. The cavity is lined by squamous cells, often with bland nuclear features. The infiltrating squamous cells form sheets and nests with varying degrees of differentiation. A combination of patterns with a transition to spindle cells or to less differentiated forms may occur. A rare variant is the acantholytic type of SCC, which may be confused with angiosarcoma. The irregular spaces lined by atypical squamous cells create a pseudoglandular and/or pseudovascular appearance. SCC can be easily discriminated by the positive staining of these cells for keratin and the absence of FVIII and CD34 [82].

An origin from the overlying skin should also be excluded.

SCC may be mixed with an invasive ductal carcinoma NST. Focal squamous differentiation can also be found in IDC NST and may accompany carcinomas with medullary features.

Spindle Cell Carcinoma

This tumor is characterized by the pseudosarcomatous growth pattern of its neoplastic spindle cells. The distinction between spindle cell carcinoma and primary sarcomas of the breast, including fibrosarcoma and malignant fibrous histiocytoma, may be problematic. The presence of focal squamous differentiation and small clusters of spindle cells with more epithelioid histology are clues for differentiating these lesions. Epithelial differentiation can be demonstrated by immunohistochemistry using a panel of antibodies (high-molecular-weight cytokeratins). P63 staining is also very common [83].

Metaplastic Carcinoma with Mesenchymal Differentiation

These tumors display an admixture of carcinomatous and mesenchymal elements. Mesenchymal components include chondroid, osseous, and rhabdomyoid elements with varying degrees of differentiation.

Metaplastic carcinomas often contain a mixture of different elements (Fig. 14.18).

Matrix-Producing Carcinoma This is a subgroup of metaplastic carcinomas that show an abrupt transition from epithelial to mesenchymal elements without intervening spindle cells.

More than 90% of metaplastic carcinomas are triple-negative cancers and express keratins 5/6 and 14 and EGFR [3]. Immunohistochemically, they have a basal-like phenotype, regardless of the types of metaplastic elements. Metaplastic carcinomas also overexpress EGFR in more than half of cases [84, 85].

MBCs are molecularly distinct from other breast cancers and are molecularly heterogeneous [86]. The vast majority of metaplastic carcinomas are of the claudin-low subtype [87]. Weigelt et al. [88] found that all metaplastic breast carcinomas with spindle cell metaplasia were of claudin-low subtype in their series, whereas those with squamous or chondroid metaplasia were more heterogenous and preferentially of the basal-like subtype. The claudin-low intrinsic subtype has been shown to have a lower pathologic complete response rate (38.9%) after neoadjuvant chemotherapy than basal-like cancers [87].

Lymph node metastases are less frequent in metaplastic carcinomas than in IDC NST. However, distant metastasis

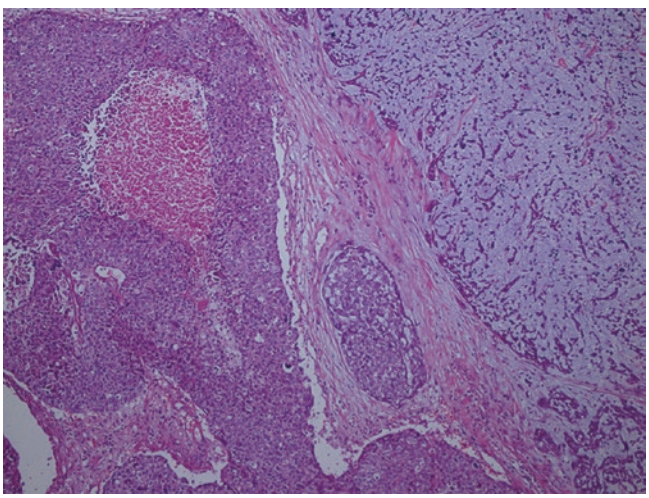

Fig. 14.18 Metaplastic carcinoma. The tumor has squamous (*left*) and chondromyxoid components

can occur in the absence of lymph node metastasis, as observed in other triple-negative breast cancers [3]. Metaplastic carcinoma is significantly correlated with worse progression-free survival and overall survival compared with those of triple-negative carcinomas [89, 90].

Carcinomas with Neuroendocrine Features

These carcinomas exhibit the morphological and immunohistochemical features of endocrine tumors, similar to those observed in the GI tract and lung, with the formation of solid, trabecular, glandular, and organoid structures. In the recent WHO classification [3], neuroendocrine breast carcinomas are categorized as follows:

- Neuroendocrine tumor, well differentiated
- Neuroendocrine carcinoma, poorly differentiated/small-cell carcinoma
- Invasive breast carcinoma with neuroendocrine differentiation

Invasive cancers of NST and other special types may show endocrine differentiation.

These tumors do not have any specific clinical presentation.

Well-Differentiated Neuroendocrine Tumor

The tumor consists of densely cellular, solid nests and trabeculae of cells separated by a thin fibrovascular stroma [91]. These tumors are of a low or intermediate grade [3]. There is chromogranin positivity in more than 50% of cases [92]. Other endocrine markers, such as synaptophysin and CD56, are also positive. These tumors are typically positive for ER and PR and negative for HER2.

Neuroendocrine Carcinoma

The tumor is composed of highly atypical cells with hyperchromatic nuclei and scant cytoplasm. Mitotic figures are frequent, and necrosis may accompany the lesion. The tumor should be distinguished from metastatic small-cell carcinoma of the lung; this distinction cannot be made on the sole basis of morphology. The presence of an in situ component supports the diagnosis of the breast as the primary cancer. Monoclonal NSE is positive in all cases of small-cell carcinomas, and other neuroendocrine markers are positive in approximately 50% of the cases [3]. ER and PR expression may also be observed in more than 50% of cases and is generally correlated with the degree of differentiation. Small cell carcinoma is negative for HER2 expression [92, 93].

Invasive Breast Carcinoma with Neuroendocrine Differentiation

Mucinous carcinoma of type B and solid papillary carcinoma (SPC) are the two tumors representing the most frequent examples of this category [3, 91].

Neuroendocrine breast carcinomas show a distinctive repertoire of somatic mutations compared with those in common forms of luminal breast carcinomas. These carcinomas have lower frequencies of *TP53* and *PIK3CA* mutations, are enriched for *FOXA1* and *TBX3* mutations, and have *ARID1A* mutations, similar to neuroendocrine tumors of other sites [94].

A recent study of a series of 47 patients with neuroendocrine breast carcinomas revealed that all tumors were estrogen receptor positive and the large majority expressed progesterone receptor (89%), GATA3 (98%), FOXA1 (96%), and CK8/18 (98%). There was an almost equal distribution of luminal A (52%) and B (48%) carcinomas. Patients with a neuroendocrine carcinoma had shorter disease-free survival compared with those with carcinomas of no special type when matched for age, size, grade, and estrogen receptor status. However, no significant differences were observed in terms of overall survival. No statistically significant differences were observed among the distinct categories (well-differentiated neuroendocrine tumors, poorly differentiated neuroendocrine carcinomas, and invasive breast carcinomas with neuroendocrine differentiation) of the WHO 2102 classification in terms of either progression-free or overall survival. The authors concluded that neuroendocrine breast carcinoma is a distinct subtype of luminal carcinoma with a low rate (7%) of *PIK3CA* mutations and with an aggressive clinical behavior [95]. Another group also observed poor local control and worse overall survival of breast neuroendocrine carcinoma despite its association with apparently indolent prognostic factors [96].

Secretory Carcinoma

Secretory carcinoma is an exceptionally rare variant representing 0.02% of all breast cancer [3]. Secretory carcinoma presents as a well-circumscribed mobile mass. The median age of presentation is 25 years. Microscopically, tumors show microcystic, tubular, and solid patterns, frequently in combination with each other. The characteristic finding is the presence of intracellular and extracellular secretory material showing positive staining with PAS. ER, PR, and HER2 are absent. EMA, alpha-lactalbumin, and S-100 protein are frequently present. There is a high expression rate of basal-like markers (CK5/6 or epidermal growth factor receptor) in

secretory carcinomas [97]. Tognon et al. [98] showed that 12 of 13 of their cases of secretory breast carcinoma expressed the *ETV6-NTRK3* gene fusion. Laé et al. [99] demonstrated that secretory breast carcinoma with the *ETV6-NTRK3* fusion gene belongs to the phenotypic spectrum of basal-like breast carcinomas and that the immunohistochemical and genetic features of secretory breast carcinomas distinguish them from other basal-like breast cancers. Secretory carcinoma has an indolent clinical behavior, especially in children and young adults [97, 100]. The *ETV6-NTRK3* translocation may serve as a potential therapeutic target for more aggressive cases of either breast or salivary gland secretory carcinoma, which is named "mammary analogue secretory carcinoma" [101].

Papillary Lesions

These lesions, especially from the clinical perspective, are often confused with each other. For this reason, all will be discussed consecutively under the title "papillary lesions."

Intraductal Papillary Carcinoma (IDPC)

IPDC is a malignant, noninvasive neoplastic epithelial proliferation with papillary architectural features that occurs in the lumen of the ductal-lobular system [3]. Two types of IDPC exist:

- Central, solitary: Presentation may include nipple discharge.
- Peripheral, multifocal: Presentation may be as a mass.

Microscopically, ducts or TDLU are filled and dilated with slender, branching fibrovascular stalks, lined by a single layer or several layers of a monomorphic epithelial cell population. Tumor cells have a bland appearance. High-grade nuclear features are rare. Solid, cribriform, and micropapillary patterns also exist. There is complete or near-complete (90%) absence of myoepithelial cells in the fibrovascular cores. However, there are myoepithelial cells at the periphery of the involved duct [102, 103].

Encapsulated Papillary Carcinoma (EPC)

This lesion has a fibrous capsule, and its size ranges between 0.5 and 8 cm [84]. EPC frequently occurs in elderly patients, with an average age of 65 years [3], and is also called intracystic papillary carcinoma. All papillary intraductal carcinomas arise in a background of a variably cystically dilated duct. The main difference in EPC is the loss of myoepithelial cells at the periphery of the lesion. EPC lacks these cells both in the fibrovascular cores and at the periphery [103, 104]. The absence of these cells and the reported cases of metastatic cases raise the possibility that these tumors represent low-grade invasive carcinomas with an expansile growth pattern [105]. However, the presence of continuous and intense collagen IV expression at the periphery is regarded as highly suggestive of a noninvasive carcinoma that is confined within an intact basement membrane [105].

EPC without an adjacent DCIS or any invasive component has a very favorable prognosis with adequate local therapy. The presence of associated DCIS confers a higher risk of local recurrence, and meticulous radiologic examination before surgery is necessary.

Solid Papillary Carcinoma

SPC is a variant of papillary carcinoma that is characterized by compact cellular growth within multiple nodules representing dilated ducts [72]. SPC presents in older women [102]. The neoplastic cells are ovoid or spindle cells of low-to-intermediate grade and have a streaming pattern. These cells are homogeneous and do not form papillary or cribriform patterns. The most important indicator of the papillary nature of the lesion is the presence of thin, fibrovascular cores that are inconspicuous at low magnification. Neuroendocrine differentiation is frequent. Mucin production is common, and invasive mucinous carcinoma may coexist. Other types of invasive carcinoma may also be observed [106]. The distinction between in situ and invasive disease in SPC is difficult. Some authors regard this entity as an expansile variant of invasive carcinoma [106, 107]. SPC has an indolent clinical course even in cases with obvious invasion [106].

In the papillary lesions mentioned above, the lesion is called in situ if there is any doubt about the invasion. If there is obvious invasion, the staging should be conducted according to the measurement of the invasive component.

Invasive Papillary Carcinoma

Invasive papillary carcinoma (IPC) is a carcinoma with a predominantly papillary morphology in its "invasive" component. IPC is a rare lesion, and there are no specific clinical and macroscopic features of this tumor. IPC should be distinguished from invasive carcinomas arising from EPC and SPC. Many cases in older series may have included such cases in this category [72].

The genomic profiles of encapsulated, solid, and invasive papillary carcinomas are similar, and these tumors are characterized by consistent ER expression, a high prevalence of *PIK3CA* mutations, and relatively low rates of p53 expression and gene copy number aberrations [108].

Solid Papillary Carcinoma with Reverse Polarity

Solid papillary carcinoma with reverse polarity (SPCRP) is a recently described entity that was initially named "breast tumor resembling the tall cell variant of papillary

thyroid carcinoma" and "solid papillary carcinoma resembling the tall cell variant of papillary thyroid carcinoma" because of its morphologic overlap with papillary thyroid carcinoma [109, 110]. SPCRP is seen primarily in older women, with a median age of 64 years [111]. These tumors also tend to be small, with a median reported tumor size of 1.5 cm [111]. SPCRP is characterized by solid, circumscribed nodules of columnar epithelial cells, often with a rounded contour but occasionally exhibiting a geographic, jigsaw-like growth pattern. These nodules, many of which contain fibrovascular cores, are distributed haphazardly throughout the breast stroma. The cells in many nodules appear back-to-back, and their nuclei are often present at the apical rather than basal pole of the cells, creating the impression of reverse polarity. Tumor nodules invariably lack a surrounding myoepithelial cell layer, supporting the invasive nature of these lesions [111]. Tumor cells in SPCRP are usually negative or weakly positive for ER and positive for CK5/6, whereas tumor cells in solid papillary carcinoma are strongly positive for ER and negative for CK5/6. SPCRP has a generally favorable prognosis, with only a few cases containing regional nodal involvement or distant metastases [111]. *IDH2* mutations are frequent and *PIK3CA* mutations are found in some of these tumors [111].

Adenoid Cystic Carcinoma

ACC is a carcinoma of low-grade malignant potential that is histologically similar to its counterpart in the salivary gland. ACC is a rare tumor. Approximately half of the cases arise from the subareolar region [112]. ACC is usually a circumscribed tumor.

Histologically, the tumor has the following basic patterns: tubular, cribriform, trabecular, and solid [113]. The dual population of neoplastic cells, namely, epithelial and myoepithelial (basal), are arranged to form glandular spaces and pseudolumina [33]. The pseudolumina contain a myxoid acidic substance that is surrounded by myoepithelial cells. In smaller spaces, small spherules or cylinders of hyaline material are formed. True glandular spaces are surrounded by luminal cells and contain neutral mucosubstances. Luminal epithelial cells are often positive for CK7 and CD117 (c-kit) and negative for p63, whereas myoepithelial cells are positive for p63 but negative for CK7 and CD117 [111]. With occasional exceptions, ACC is triple negative [33].

Breast ACC rarely involves the axillary lymph nodes, and survival is excellent [112, 114]. A solid variant with basaloid features has a higher frequency of axillary lymph node metastasis [115].

Glycogen-Rich Clear Cell Carcinoma

Glycogen-rich clear cell carcinoma is a carcinoma in which 90% or more of the tumor cells have abundant clear cytoplasm containing glycogen [3] and accounts for 1–3% of breast carcinomas.

Histologically, the tumor cells have polygonal, sharply defined contours. The clear or finely granular cytoplasm contains PAS-positive diastase-labile glycogen. ER is present in 50% of the cases, and PR is absent [32].

This tumor should be distinguished from lipid-rich carcinoma, histiocytoid carcinoma, and metastatic renal cell carcinoma [33].

There are conflicting reports regarding the prognosis of these tumors [116, 117]. The prognosis of this tumor has been found to be significantly related to the number of positive lymph nodes. There is no significant difference in overall survival and disease-free survival compared to those of the usual invasive ductal carcinomas [118].

Inflammatory Carcinoma

Inflammatory carcinoma (IC) is an aggressive form of breast carcinoma with distinct clinical features. Clinically, there is rapid breast enlargement with edema and erythema of the skin (orange peel skin). Currently, there are no definitive molecular or pathological diagnostic criteria for IC. Therefore, the diagnosis is based on the clinical findings described above [119]. The signs and symptoms required for a diagnosis of IC include erythema occupation of at least one third of the breast, edema and/or peau d'orange of the breast, and/or a warm breast, without an underlying palpable mass in the majority of cases [119, 120]. The onset of these signs and symptoms should be rapid; the duration of signs and symptoms at initial presentation should be $\leq$3 months [119].

IC is not considered a specific histological subtype of breast carcinoma, and there are no special pathological diagnostic criteria for IC [119, 120]. The underlying carcinoma is most often IDC NST of high grade; there may or may not be a distinct mass.

The pathognomonic histopathologic finding in IC is the presence of many lymphovascular tumor emboli in the papillary and reticular dermis overlying the breast. Although skin emboli are occasionally noted in the skin of patients with non-IC, emboli in patients with non-IC are usually less numerous and smaller than the skin emboli in patients with IBC [119]. The absence of tumor emboli in skin punch biopsies should not negate the diagnosis of IC because dermal tumor emboli can be detected in no more than 75% of the cases despite meticulous sampling and sectioning [120]. Approximately 55% of the cases are negative for ER and PR, 45% are HER2 positive, and 33% are triple negative [120].

Molecular profiling has revealed that 75% of IC samples belong to the classically more aggressive basal-like, HER2-enriched, claudin-low, or luminal B subtypes, whereas these subtypes account for 54% of noninflammatory breast carcinomas. Luminal A subtype represents 19% of IC samples, whereas in noninflammatory breast carcinoma, this subgroup represents 42% [121].

Survival is worse than in patients with locally advanced breast cancer without IC [3].

Neither molecular subtypes nor the gene expression profile patterns of IC differ from noninflammatory carcinomas with respect to pathologic complete response to chemotherapy and distant metastasis-free survival [122].

Mucoepidermoid carcinoma, polymorphous carcinoma, oncocytic carcinoma, sebaceous carcinoma, lipid-rich carcinoma, and acinic cell carcinoma are very rare tumors and beyond the scope of this chapter.

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Intraoperative Pathological Examination of Breast Lesions

15

Ekrem Yavuz

Introduction

The intraoperative pathological examination of resected breast tissue is performed for a number of reasons, including microscopic diagnosis of the breast lesion, assessment of surgical margins and sentinel lymph node status, and, rarely, determination of tissue adequacy for subsequent paraffin-block examination. The type of method used for the intraoperative pathological examination depends on the experience and circumstances of the pathologist and varies from a simple gross examination to complex molecular techniques. However, the most commonly used methods are cytological and frozen section (FS) examinations in addition to gross analysis. Regardless of the intraoperative pathological method used, indefinite results may be obtained.

Intraoperative Pathological Diagnosis of Breast Lesions

Surgeons prefer a definite preoperative diagnosis to develop a better strategy for the operation. Thus, the majority of breast lesions are diagnosed preoperatively by core biopsy or fine-needle aspiration cytology. Consequently, FS examination of a primary breast tumor is requested only when these preoperative procedures are not diagnostic, have failed to determine the presence of an invasion, or have been omitted. Routine FS examination is not recommended for grossly indistinct and "possibly benign" breast lesions, although a small percentage of these specimens may contain grossly undetectable in situ or invasive breast carcinoma. Many pathologists are reluctant to perform FS analysis on breast lesions with a diameter of less than 1 cm because the frozen tissue remaining from the intraoperative examination is not ideal for determining prognostic and predictive parameters of the breast carcinoma. However, in our opinion, careful gross slicing of a breast lesion even with a diameter of 5 mm provides a sufficient amount of tissue for subsequent paraffin-block examination [1–3].

There is also a debate on the use of FS for mammographically detected non-palpable breast lesions. Some authors have reported little difficulty in using FS in this setting, unless the lesion was only a mammographic calcification [4–6]. Some authors have also proposed to perform FS in cases of ductal carcinoma in situ that was previously diagnosed by core biopsy to select cases with invasion in which sentinel lymph node biopsy should be performed [7]. However, we agree with the recommendation that FS not be routinely performed for breast lesions without a grossly detectable mass because of the difficulty of subsequent gross and microscopic examinations. The tissue may be distorted by freezing artifacts. Furthermore, some portion of the tissue may be lost, and tissue orientation may be very difficult [8].

The FS diagnosis of a grossly detectable breast lump is straightforward and has high specificity, sensitivity, and accuracy rates close to 100% [9, 10]. However, because urgent intraoperative diagnosis of a primary breast lump is rarely requested, young pathologists should be aware of the possible diagnostic pitfalls in this setting. Intraoperative diagnosis of a primary breast lump by touch print cytology can be performed accurately and can be used as an adjunct method to FS but requires experience in the cytological features of breast lesions [11–13]. Furthermore, because rapid preoperative cytological analysis of a breast lump can be performed in more appropriate circumstances, pathologists deserve the right to refuse to employ only cytological methods to diagnose a malignancy during the operation.

Intraoperative Pathological Assessment of Surgical Margins

The intraoperative pathological assessment of surgical margins plays a critical role in breast-conserving surgery (BCS)

E. Yavuz (✉)
Department of Pathology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey
e-mail: yavuze@istanbul.edu.tr

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because surgical margin negativity is the most important relevant parameter for local recurrence after BCS for breast cancer. However, there are many issues limiting the success rates of this procedure that a pathologist must address. Due to the lipid-rich characteristics of breast tissue, the surface of the excised tissue is frequently irregular and contains crevasses and defects. Tissue flattening, which is referred to as the "pancake phenomenon", after the removal of the breast tissue also contributes to inaccurate results [14–16].

It is the surgeon's responsibility to submit an intact specimen and include appropriate orientation markings. The pathologist can easily be oriented to the specimen with a short suture at the superior margin and a long suture at the lateral margin placed by the surgeon (Fig. 15.1). Upon receipt, the pathologist should approach the specimen as if it contains a malignant tumor. The surface of the specimen should be marked using India ink or another dye that is resistant to processing solutions and remains visible at the edge of the sample during the microscopic examination. If a cotton applicator is used and the ink is rapidly fixed in Bouin's or a similar solution, the ink will not permeate into the tissue crevasses. A breast excision specimen sliced by the surgeon may be reassembled but will not be as accurate as an intact specimen. The most important part of the surgical margin assessment is the gross examination. The pathologist must be informed about the number and size of the lumps and the radiological findings. For this reason, it is preferable to receive the patient's file during the intraoperative pathological examination. After carefully slicing the specimen, the relationship of an apparent tumor to the six margins can be observed and reported grossly (Figs. 15.2 and 15.3). However, the gross impressions may fail to estimate the involvement of a surgical margin adjacent to the microscopic focus of an invasive or in situ carcinoma. Hence, a FS examination should be performed if a margin is grossly close to the tumor. However, the success of surgical margin assessment depends on the pathologist's experience and the methods used. A random sectioning of grossly normal surgical margins is not recommended because it has limited sensitivity [17].

In our opinion, samples for FS should be perpendicular to the inked surface because the true distance between the tumor and the margin can be determined (Figs. 15.4 and 15.5). Samples taken parallel to the surface (enface samples) can

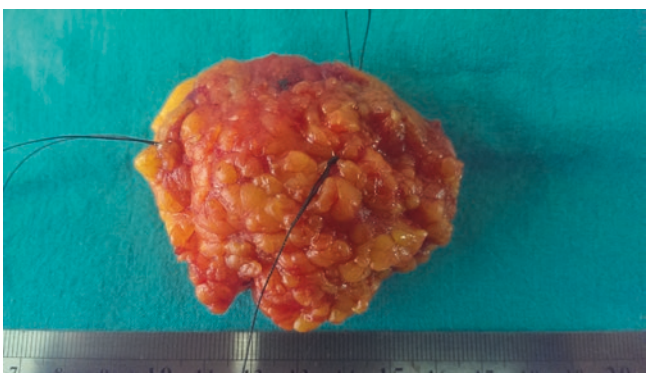

Fig. 15.1 Gross appearance of a resected right breast lump with orientation sutures. A short suture at the superior margin, a long stitch at the lateral margin, and long and short stitches at the anterior margin were placed by the surgeon

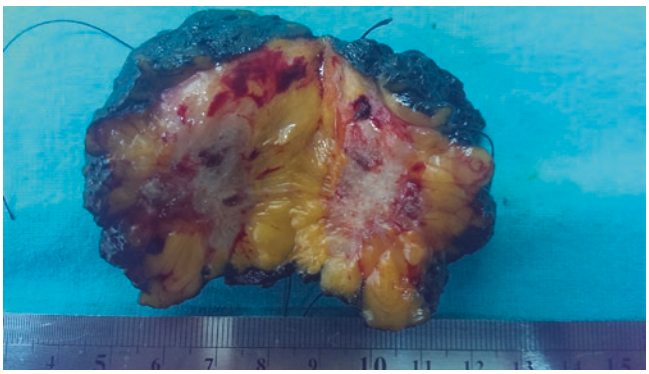

Fig. 15.2 Distance from the irregular breast lump to the superior, inferior, anterior, and posterior margins can be observed after gross slicing of an inked specimen

Fig. 15.3 Surgical margin assessment in a breast lesion containing comedo-type necrotic areas and grossly suggesting a ductal carcinoma in situ

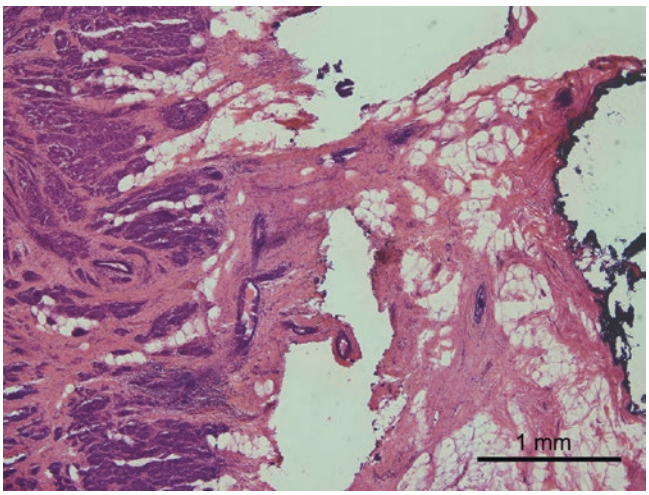

Fig. 15.4 Frozen section appearance of an invasive ductal carcinoma sectioned perpendicular to the margin. The fatty part of the breast tissue has been lost due to difficulties with frozen sectioning (H&E × 4 original magnification)

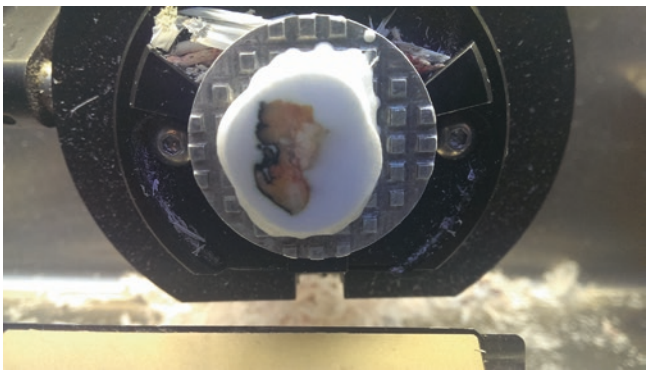

Fig. 15.5 Gross appearance of frozen-sectioned tissue may also facilitate surgical margin assessment because freezing usually highlights neoplastic tissue

also be frozen sectioned for surgical margin assessment. In this setting, any tumor in the section indicates margin positivity. However, if no tumor is detected in the section, we can estimate a tumor-free distance of at least 2 mm between the tumor and the margin because a shaved sample is usually at least 2 mm in thickness. The sampling method, whether perpendicular to the inked surface or a shaved sample, should be clearly stated on the report.

Some surgeons prefer to perform re-excisions by shaving the excision cavity at the initial operation. These specimens should also be marked by suture or ink for orientation by the operator. These re-excisions are helpful in the decision to reoperate if there is a "close" or positive margin in the excision [18–20].

Surgical shavings from the excision cavity have revealed that negative excision margins during the intraoperative pathological examination do not absolutely assure that all the carcinomatous tissue in the region has been successfully removed. The likelihood of the presence of a carcinoma in a shave biopsy of the tumor bed after a negative lumpectomy margin varies from 9% to 39% [20–22]. This variation may reflect the characteristics of either the primary tumor or the surgical team. Invasive lobular carcinomas and similar tumors showing microscopic multifocal growth patterns, an extensive intraductal component and extensive lymphovascular invasion, are more likely to result in a false-negative intraoperative surgical margin assessment. Furthermore, the type and extent of the radiological methods used and the experience of the surgeon and pathologist have a great impact on the success rates of BCS.

Retrospective analyses of the impact of FS on BCS have revealed that during the first operation, additional excisions are performed in 24–27% of cases based on FS results, while 5–9% necessitated a second re-excision due to definitive histopathological examination [23–25].

Cytological methods can also be used for margin assessment. Intraoperative touch preparation cytology (IOTPC) or "imprint cytology" is based on the ability of malignant cells but not benign mammary fat tissue to adhere to glass slides. To assess margin status, glass slides are first brought against the borders of the excised specimen, and then the slides are rapidly fixed and stained. Some studies have reported that IOTPC is inexpensive, accurate, and rapid and conserves tissue for permanent sectioning and histopathological examination [26–28]. Klimberg et al. [25] reported a diagnostic sensitivity and specificity of 96 and 100%, respectively, in a study of 428 patients. Weinberg et al. [29] stated that IOTPC significantly reduced local recurrence (LR) rates compared to other methods. However, there are limitations regarding the use of IOTPC. It necessitates experience in breast cytology, and some pathologists are very reluctant to rely only on cytological methods intraoperatively. In our opinion, a proportion of indefinite results are inevitable when using cytology for margin assessment, particularly for tumors with low nuclear atypia, such as invasive lobular carcinoma. Another disadvantage of IOTPC is that close margins cannot be observed because only superficial tumor cells are detected with this technique. Therefore, no information regarding margin distance, tumor multifocality, or the presence of either in situ or invasive carcinoma can be provided by this method. Cox et al. [17] reported three false-positive interpretations with cytology and no false-positive and five false-negative interpretations with FS. Cytospin preparations can be prepared from BCS specimens by scraping the surface. Veronesi et al. [30] used the monoclonal antibody B72.3 to detect carcinomatous cells in cytospin preparations obtained by scraping. However, they detected immunoreactive cells in 33% of the cytospin specimens, whereas only 12% had definitive margin positivity. We suggest that the addition of rapid immunohistochemistry to the cytological method can

increase the sensitivity of diagnosing positive margins but will not resolve any of the outlined limitations of using cytological methods for margin assessment.

Surgical margin assessment for ductal carcinoma in situ (DCIS) during BCS is usually performed using specimen radiography. We do not recommend performing FS for surgical margin assessment in DCIS cases because it will increase the cost without decreasing reoperation rates [31]. However, some authors have advocated the utilization of FS analysis in selected DCIS cases with close or suspicious margins on specimen radiography and reported conversion from positive to negative margins in one third of cases [32].

Surgical margin status is reported to be a risk factor for local recurrence in patients with breast cancer treated with mastectomy and without adjuvant radiotherapy [33]. Hence, intraoperative surgical margin analysis should be performed for these patients as well.

There are also non-pathological methods for intraoperative surgical margin assessment, including intraoperative ultrasonography, radiofrequency spectroscopy (Marginprobe™), and some other physical methods. However, results with these methods are currently inferior to those obtained using pathological methods for intraoperative surgical margin assessment [34].

Reporting the pathological assessment of excisions is also problematic. The existence of carcinomatous cells on the inked surface should be reported as positive margins, and the extent of the positivity should be detailed if possible. However, the definition of a close margin is debated. The definition "close to the margin" is conventionally used for tumor foci that are less than one high-power field away from the surgical margin, whereas some pathologists use this term for tumors that are less than 3 mm from the surgical margin. We only use the term "positive margin"; otherwise, we give the distance to the inked surface.

Pathological Examination of Sentinel Lymph Nodes

Intraoperative Pathological Diagnosis of Sentinel Lymph Nodes

Intraoperative pathological examination of sentinel lymph nodes (IPESLN) should be performed if the result will influence the type of surgical approach. According to this definition, an axillary lymph node dissection (ALND) should be performed if the intraoperative sentinel lymph node (SLN) diagnosis is metastatic and omitted if the SLN is metastasis-free. However, clinicians should be aware of the limitations of IPESLN. In a number of patients, metastatic foci will not be detected because of inadequate sampling and the difficulty of detecting small metastatic foci in SLNs. Therefore, patients must be informed of the possibility or risk of a second operation for completion of ALND. A previous study by the American College of Surgeons Clinical Oncology Group (ACOSOG) demonstrated that the completion of ALND after a pathological diagnosis of SLN metastasis was not superior to SLN biopsy alone in terms of disease-free and overall survival for early-stage clinically N0 breast cancer patients [35]. Furthermore, the utilization of radiotherapy in select patients with sentinel lymph node positivity resulted in excellent and similar regional control compared with that of those who received ALND [36]. These results caused a decrease in the use of IPESLNs. However, there are some clinical settings without the use of radiotherapy in which pathologists will continue to perform IPESLNs [37].

IPESLNs can be performed either using FS or imprint cytology or a combination of both techniques. Another cytological method for intraoperative SLN analysis is the evaluation of cells scraped from the cut surface of the node.

Cytological methods have some advantages compared to frozen sectioning. Cytology conserves tissue for subsequent permanent histopathological examination. In addition, cytological analysis is not time-consuming, and it permits the analysis of specimens prepared from multiple cut surfaces. Cytological preparations can be rapidly stained with H&E (Fig. 15.6), Giemsa, or other stains depending on the pathologist's preference. However, many pathologists are not skilled at cytological diagnosis and are reluctant to use cytology for IPESLN because observing sparse carcinomatous cells in the highly cellular background of the lymph node imprint may be very difficult. Another disadvantage of cytology is that the detected metastasis cannot be measured, although sparse metastatic cells on imprint preparations usually correspond to either micrometastasis or isolated tumor cells (ITCs). Several studies have reported controversial results with regard to imprint cytology for IPESLNs. In a

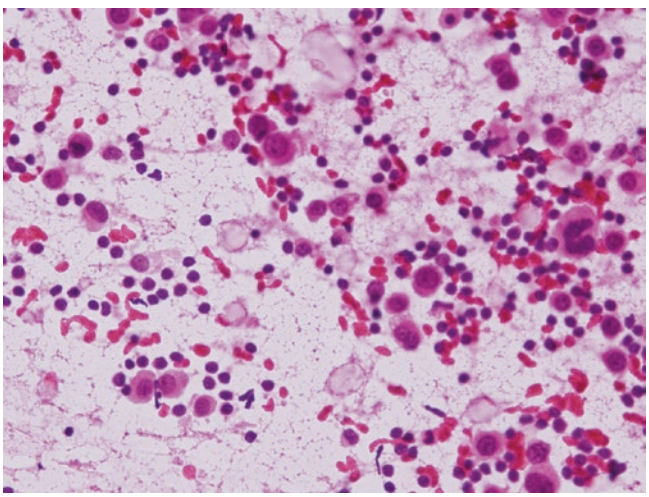

Fig. 15.6 Imprint cytology of the sentinel lymph node showing metastatic breast cancer cells within the lymphoid cellular background (H&E × 60 original magnification)

meta-analysis of 32 studies, Tew et al. [38] concluded that intraoperative cytology (IC) is simple, rapid, and sensitive for macrometastases (MAM) but not micrometastases (MIM). The pooled sensitivity of IC was 63% (81% for MAM and 22% for MIM), and its specificity was 99%. These success rates are slightly worse than those for frozen sectioning reported in another meta-analysis of the use of frozen sectioning for IPESLNs [39].

The FS method for IPESLN (Fig. 15.7) has been evaluated in several studies, and satisfactory results have usually been reported. In a meta-analysis of the use of frozen sectioning for IPESLN, Liu et al. reported that the mean sensitivity was 73% (94% for MAM and 40% for MIM/ITC) and the mean specificity was 100% [39]. In a study directly comparing IC and FS methods for IPESLN, van Diest et al. observed false-negative rates of 13% for FS and 38% for IC, with 88% concordance between FS and IC [40]. The sensitivity and overall accuracy (87% and 95%, respectively) of FS were substantially greater than those of IC (62 and 83%, respectively). In a study by Turner et al. [41], combining IC and FS methods resulted in an overall accuracy of 93.2%. However, the combined intraoperative method detected 87% of MAMs but only 28% of MIMs. At our institution, we perform IC for IPESLNs because we are concerned about tissue loss during IPESLN, and there is a consensus in the multidisciplinary team that the main objective of IPESLNs is to detect all MAMs. As mentioned above, we do not consider either FS or IC reliable for ruling out MIMs and ITCs.

Molecular biology techniques, such as reverse transcriptase-polymerase chain reaction, are under investigation for their potential applicability for the evaluation of SLNs. Although these techniques are highly sensitive and permit the evaluation of large amounts of tissue, the tissues are destroyed during the procedure, and consequently, it is not possible to determine the cell from which the signal originated. Viale et al. compared molecular techniques with FS for IPESLNs using a commercially available molecular assay in a series of 293 SLNs [42]. Using the molecular technique, they correctly detected 51 of 52 MAMS and 5 of 20 MIMs. They concluded that the sensitivity of the molecular assay was comparable to that of histopathological examination of the entire SLN by serial sectioning at 1.5–2 mm. Similarly, many studies using molecular techniques have reported satisfactory results in detecting even small metastases during IPESLN [43–45]. Pathologists generally believe that molecular techniques should be evaluated as research studies and are not ready to use in routine IPESLNs. The American Society of Clinical Oncology (ASCO) has recommended that molecular approaches remain investigational and that tissue potentially required for histological diagnosis should not be utilized for investigational purposes until the diagnosis is secure [46]. However, another molecular method for IPESLN, named one-step nucleic acid amplification (OSNA), has recently been used routinely by some pathologists and has been reported to be a powerful tool with high sensitivity, specificity, and positive and negative prediction rates. A recent meta-analysis supported this positive impression with regard to the use of OSNA for IPESLN. OSNA uses rapid nucleic acid amplification technology to detect the level of expression of the messenger RNA (mRNA) of cytokeratin 19 (CK19), an epithelial cell marker that is normally not present in lymph node tissue. Quantitative measurement of the CK19 indicator not only permits differentiation between a positive and a negative result, but it also provides a clear indication of the size of the metastasis [47].

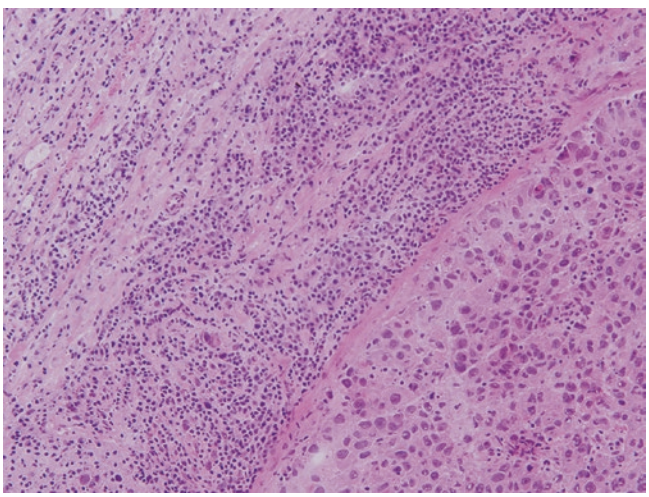

Fig. 15.7 Frozen section of a breast carcinoma metastasis in the sentinel lymph node. A sharp contrast between the carcinoma and lymphoid parenchyma can be observed (H&E × 20 original magnification)

Permanent Pathological Analysis of Sentinel Lymph Nodes

Gross Examination

The submitted tissue may be a single or a few SLNs or axillary fatty tissue containing one or more lymph nodes. Fatty tissue should be carefully dissected to identify all lymph nodes. All lymph nodes should be measured and sliced into 2-mm-thick sections. We prefer producing slices parallel to the longitudinal axis of the SLN (Fig. 15.8). If isosulfan blue dye or methylene blue is used, the afferent lymphatic vessel can be observed, and the section can be made where the afferent lymphatic vessel is connected to the SLN. Such sectioning facilitates the detection of tiny metastases, which are typically located at the marginal sinus into which the lymph fluid first drains. Some pathologists prefer transverse slicing if the SLN is larger than 0.5 cm. All slices should be inspected for changes in color and consistency. The cut surface of a positive lymph node ranges from tan to gray-white in color and from normal to hard in consistency. In cases of partial

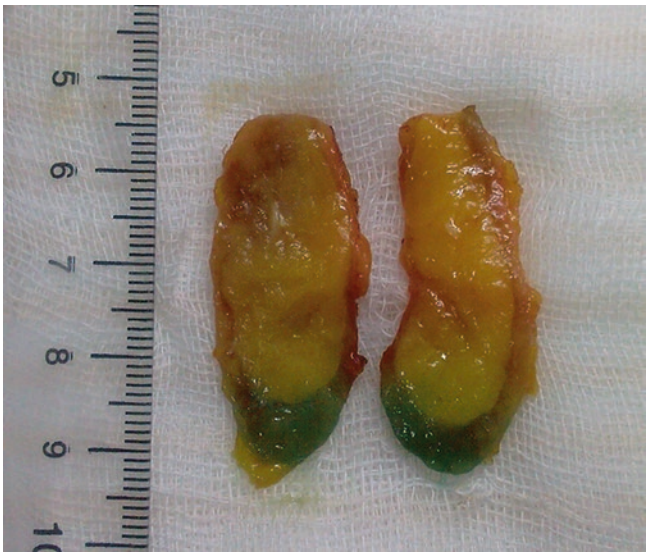

Fig. 15.8 Gross appearance of a sentinel lymph node detected using blue dye and showing fatty changes. Slicing was performed parallel to the longitudinal axis

involvement, a sharp contrast between the metastatic focus and normal lymphoid tissue can easily be observed. However, permeative metastases of lobular carcinomas usually exhibit no or subtle gross findings. Each SLN should be submitted in a separate cassette, and all nodes should be submitted for microscopic examination.

Sectioning

The majority of MAMs and MIMs are easily detected during standard H&E-stained section examination, an approach endorsed by leading surgical pathology organizations [48, 49]. Although superficial serial sectioning limits sampling to the upper limits of the paraffin block, virtually all MAMs can be diagnosed if the gross slicing is performed as recommended [50, 51].

However, if multiple-step sections from different levels of the block are examined, the majority of MIMs can be detected. Various protocols for the pathological examination of SLNs have been reported, but there is no consensus as to which is the most cost-effective. Step sectioning of the block completely at 0.2 mm or smaller intervals enables the detection of all MIMs but will result in too many sections [51–57]. Weaver suggested that "the size of the largest acceptable missed metastasis must be determined prospectively and then the sectioning strategy that will systematically detect metastases larger than the threshold must be employed" [58]. Accordingly, a recent review of the details of IPESLN suggested that this examination should be designed to detect only MAMs [59].

Use of Immunohistochemistry

The use of antibodies to cytokeratin facilitates the detection of small clusters of metastatic carcinomatous cells, particularly in lobular carcinoma metastases that may manifest as dyscohesive cells throughout the sinuses (Fig. 15.9a, b) [60]. Nevertheless, the clinical significance of these small clusters of metastatic cells, which are usually detected by immunohistochemistry, is unknown and the subject of ongoing clinical studies.

Guidelines published by the College of American Pathologists [48] and others [61, 62] state that immunohistochemical analysis is not required for the evaluation of SLN. However, in the latest recommendation paper, a liberal approach was allowed by the American Society of Clinical Oncology [46]. They stated that the decision to utilize immunohistochemical analysis and act on the results remains, for now, a matter of discussion among individual surgeons, oncologists, and pathologists, based on a determination of the best course for their patients as assessed from their own experience and a review of the available literature. A survey of European pathology laboratories identified 123 different protocols described by 240 respondents, 71% of which used immunohistochemistry to evaluate SLNs found to be negative in H&E-stained sections [63]. We prefer using pancytokeratin immunostaining in our routine practice because it accurately determines the extent of metastatic foci. Moreover, most pathologists dealing with SLNs have encountered undetected MAMS identified by immunohistochemistry. Immunohistochemistry is most useful for the discrimination of isolated tumor cells from MIMs.

Histopathology

Breast carcinoma metastases in SLNs usually recapitulate the architectural and cytological patterns of the primary tumor. MAMs usually disrupt the lymph node architecture and can easily be detected at scanning power. Lobular carcinomas may diffusely infiltrate the lymph node parenchyma as single cells or small clusters. A MAM is defined as a lymph node metastasis of breast carcinoma measuring greater than 2 mm in size [64]. A differential diagnosis of MAMs includes other malignancies, such as malignant melanoma, lung carcinoma, and malignant lymphomas.

A MIM is defined as a lymph node metastasis measuring greater than 0.2 mm and/or more than 200 cells in a cross section of a single SLN but not greater than 2.0 mm in size [64]. In patients with MIMs in the SLN, a MAM may exist in the non-sentinel lymph node of the completion ALND in approximately 15% of patients [65]. The majority of MIMs are cohesive malignant cell aggregates without specific patterns and without desmoplasia. Because MIMs are usually found within the subcapsular sinus, careful examination of these

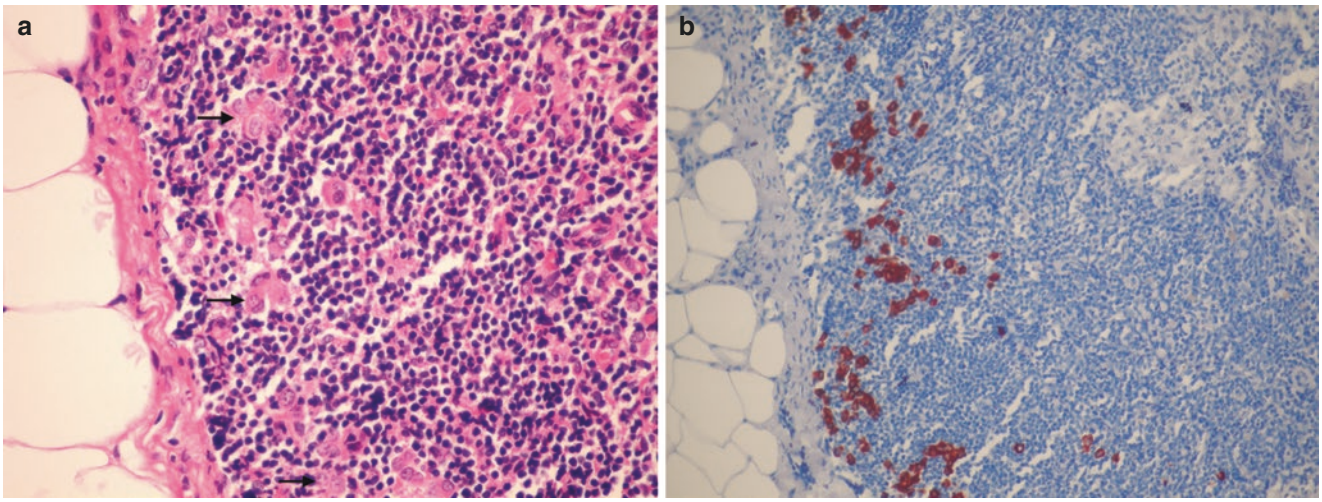

Fig. 15.9 (**a**) Microscopic appearance of an invasive lobular carcinoma in the sentinel lymph node. Observing the neoplastic cells (*arrows*) may be difficult due to the dyshesive nature and the small size of the neoplastic cells (H&E × 40 original magnification). (**b**) Immunohistochemical examination using anti-cytokeratin antibody highlights the metastatic cells dispersed within the parenchyma of the sentinel lymph node (anti-pancytokeratin, Mayer's hematoxylin counterstaining × 20 original magnification)

areas is critical, and pancytokeratin immunostaining is helpful as previously stated. Differential diagnosis of MIMs includes MAMs and ITCs and also some benign lesions, such as nevus cell aggregates, heterotopic glands, and mechanical transport of benign breast epithelium. Multiple MIMs may exist in a SLN, and these should not be diagnosed as MAMs even when the aggregate diameter of the metastatic deposits exceeds 2 mm [66]. Nevus cell aggregates can be found in the lymph node capsule and, occasionally, in capsular invaginations within the lymph node parenchyma. The cells of nevus cell aggregates resemble the cells of cutaneous nevi. They have round to ovoid nuclei with fine chromatin, pale-staining cytoplasm, and indistinct cell borders (Fig. 15.10). Melanin pigment is variably present. The distinction can usually be made by routine microscopic analysis; however, in some lesions, immunohistochemical examination using both pancytokeratin and a melanocytic marker may be necessary. Multiple nevus cell aggregates are possible, and both MIM and nevus cell aggregates may be present in the same SLN [67]. There may be heterotopic glandular epithelia in axillary lymph nodes, which may exhibit ductal, squamous, or salivary gland-type differentiation and even proliferative and metaplastic changes [68]. Development of DCIS from a heterotopic epithelium within a SLN has also been reported [69]. Nevertheless, the diagnosis of heterotopic epithelium during the microscopic examination of a SLN should be made with great caution because some metastatic carcinomas with low nuclear atypia, such as tubular and tubulolobular carcinoma, may resemble nonneoplastic glandular inclusions (Fig. 15.11). For this discrimination, an observation of the myoepithelial cell layer or the low proliferation capacity of the cells using immunohistochemistry may help.

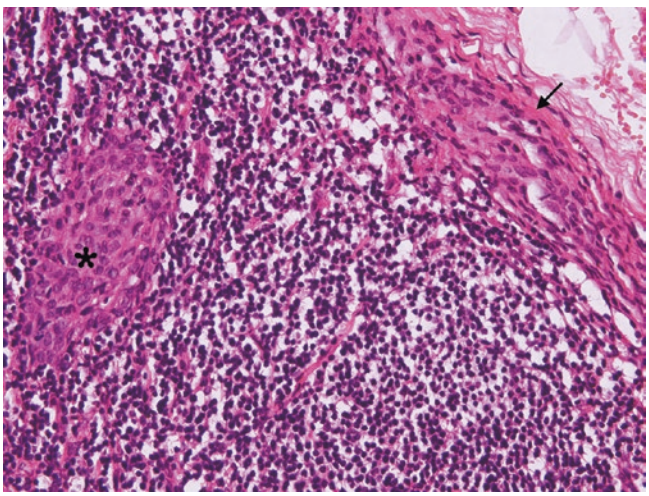

Fig. 15.10 Microscopic appearance of a nevus cell aggregate (*arrow*) at the capsule of the sentinel lymph node. Nevus cells lack nuclear atypia, and their cytoplasmic borders are indistinct, as with dermal nevi (*asterisk*: nevus cells within the capsule invagination through the lymphoid parenchyma) (H&E × 20 original magnification)

ITCs are defined as small tumor-cell clusters with a diameter of less than or equal to 0.2 mm (Fig. 15.12). Another definition of ITCs is the existence of less than 200 tumor cells in a single cross section of a single SLN. The latter definition is particularly useful for differentiating MIMs and ITCs in invasive lobular carcinoma cases [64]. Microscopically, ITCs are usually located within the subcapsular sinus, although they may occasionally be observed within the medullary sinus and lymph node parenchyma. In the case of a few malignant cells within the afferent lymphatics, one should carefully look for a metastatic focus inside

the lymph node. Although the metastatic cells within the afferent lymphatics are traditionally not used for staging purposes, pathologists should note their existence in the report. The differential diagnosis of ITCs includes MIMs, nevus cell aggregates, heterotopic glandular structures, extramedullary hematopoiesis, and benign mechanical transport (BMT) of displaced epithelium. The distinction between ITCs and MIMs in invasive ductal carcinomas is relatively easy and depends on the measurement of the largest focus. However, this is a problematic issue in invasive lobular carcinoma cases because the metastasis is frequently dispersed within the sinuses and lymph node parenchyma as isolated single cells or two- to three-cell groups. In this setting, the alternative size criterion of less than 200 cells in a single cross section of a single lymph node is very useful (Fig. 15.13). Nevertheless, in some cases, the distinction may be extremely difficult, and the pathologist must make a final judgment according to his or her impression. Extramedullary hematopoiesis also occurs in SLNs, as in other organs. The composition of hematopoietic elements may vary. Megakaryocytes and their immature forms are large cells with multiple nuclei that can be misdiagnosed as ITCs (Fig. 15.14).

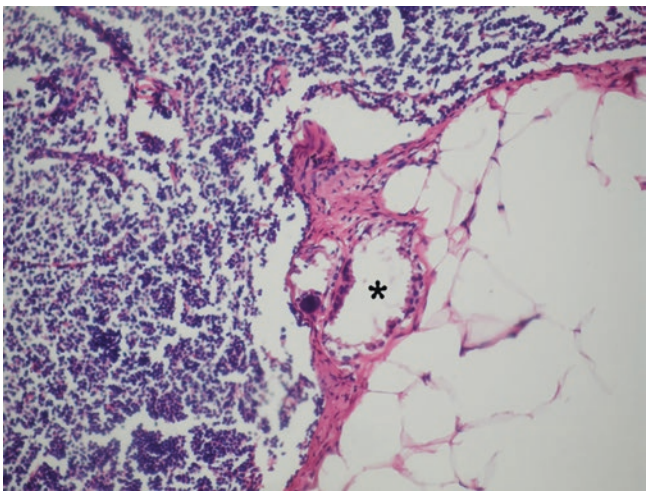

Fig. 15.11 Glandular structure (*asterisk*) simulating a benign heterotopic epithelium within the capsule of a sentinel lymph node that was diagnosed as "isolated tumor cells" resulting from a tubular carcinoma (H&E × 20 original magnification)

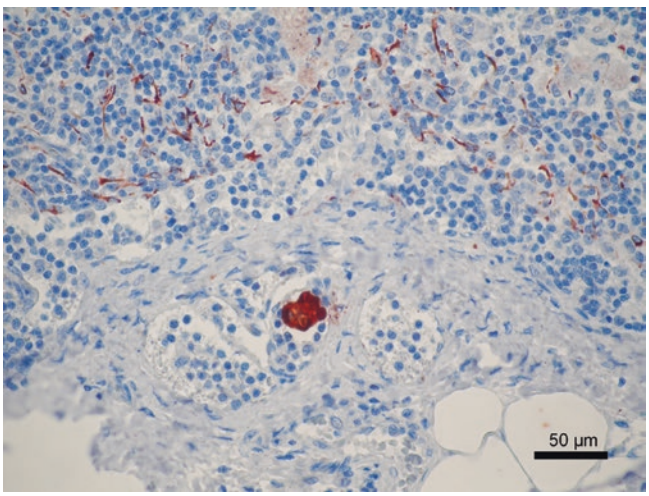

Fig. 15.12 Positive staining for anti-cytokeratin in a small cluster composed of a few metastatic cells from an invasive ductal carcinoma located in the subcapsular sinus of the sentinel lymph node. According to size criteria (less than 0.2 mm), it should have been diagnosed as "isolated tumor cells." Note the slight immune positivity in reticulum cells (anti-pancytokeratin, Mayer's hematoxylin counterstaining × 40 original magnification)

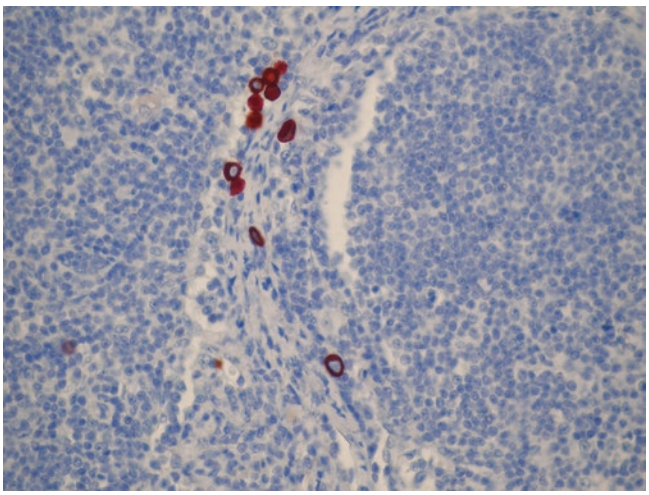

Fig. 15.13 Metastatic cells from an invasive lobular carcinoma within the medullary sinus highlighted with anti-cytokeratin immunostaining. Neoplastic cells are either isolated or in two- to three-cell groups. This case should have been diagnosed as "isolated tumor cells" if less than 200 neoplastic cells were counted in the single cross section (anti-pancytokeratin, Mayer's hematoxylin counterstaining × 40 original magnification)

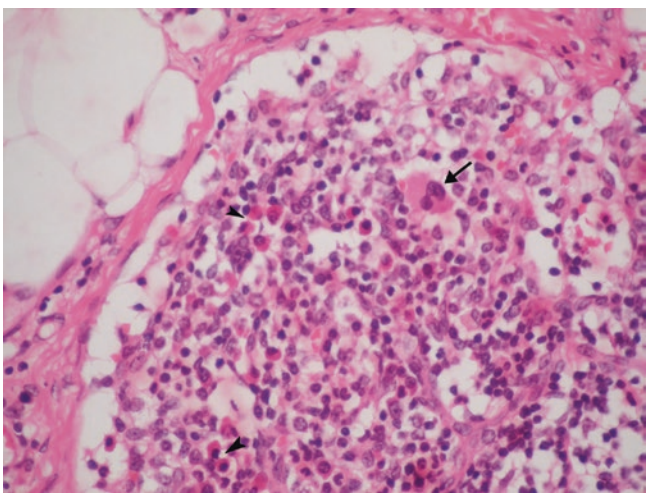

Fig. 15.14 Microscopic appearance of extramedullary hematopoiesis in a sentinel lymph node. A megakaryocyte with nuclear multilobation (*arrow*) and granulocytic precursors with dense eosinophilic cytoplasm (*arrow heads*) are shown (H&E × 40 original magnification)

Immunohistochemical stains are very useful for this discrimination. Both benign and malignant breast tissue may be traumatically displaced by either a biopsy procedure or vigorous breast massage after the injection of radiocolloid or dye tracer for the detection of SLNs [70]. The displaced epithelial cells may also enter the lymphatics and drain into the SLN. Interestingly, fine-needle aspirations produce mechanically transported epithelium within the SLN more commonly than core and excisional biopsy procedures [71]. The mechanical transport of epithelial cells to the SLN is more common in papillary breast lesions because they are usually friable lesions [72]. Some morphological and immunohistochemical details facilitate the identification of BMT. Accompanying hemosiderin-laden histiocytes around epithelial cells, a lack of nuclear atypia (Fig. 15.15a, b) and proliferative activity in epithelial cells, a myoepithelial cell layer at the periphery of the epithelial cell cluster (Fig. 15.15c), a mixture of high- and low-molecular-weight cytokeratin expression (Fig. 15.15d), and entrapped epithelial cells within the granulation tissue at the biopsy site in the breast are helpful morphological and immunohistochemical features for the differential diagnosis of BMT and ITC/MIM. Nevertheless, some pathologists favor the diagnosis of ITC in patients with an

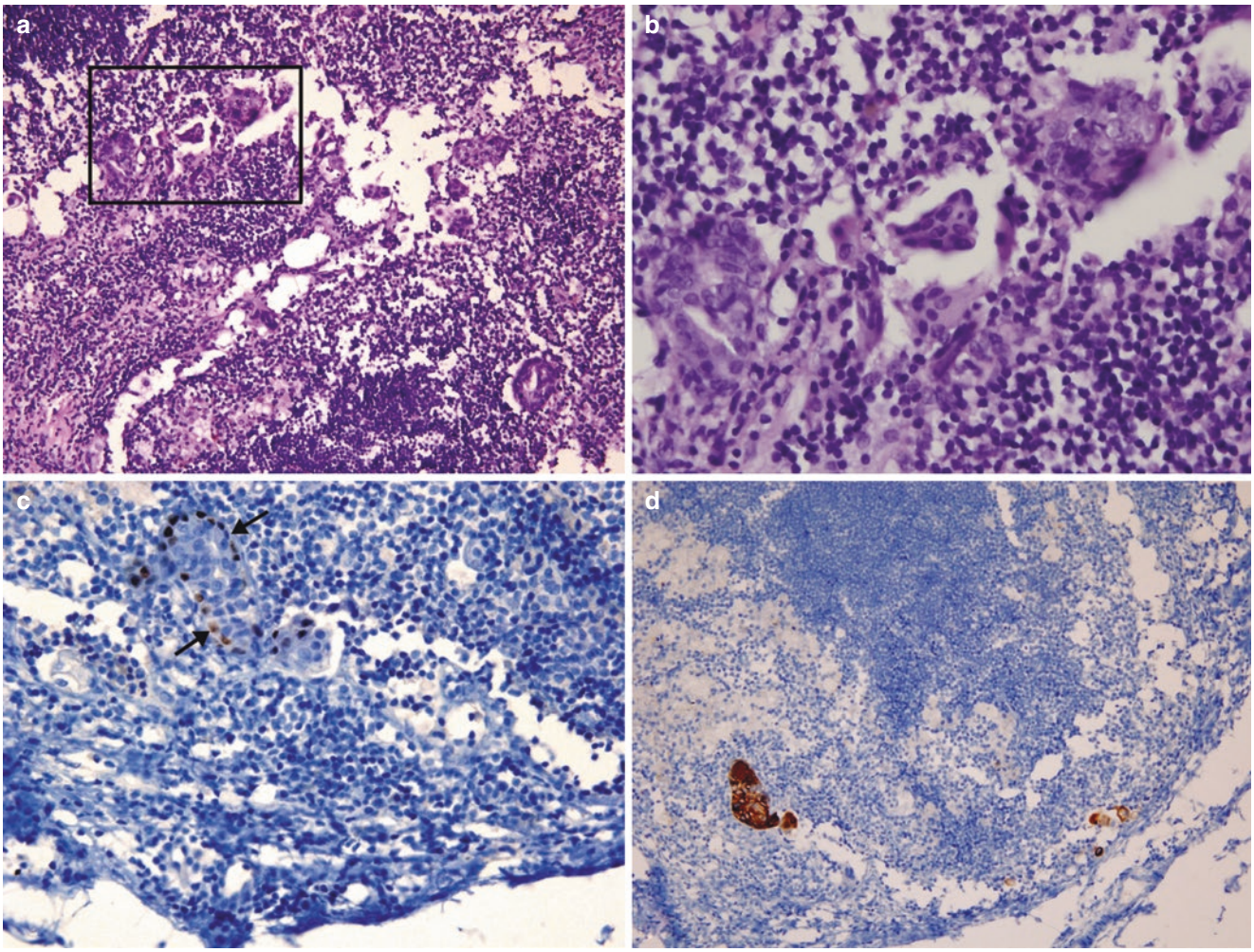

Fig. 15.15 Case of benign mechanical epithelial transport in the sentinel lymph node. The primary breast lesion was misdiagnosed as invasive carcinoma, and the paraffin-block consultation resulted in a diagnosis of sclerosing papilloma. (**a**) Epithelial cell clusters within the lymphoid parenchyma (H&E × 10 original magnification). (**b**) High magnification of the rectangle in "a" showing the lack of obvious nuclear atypia (H&E × 60 original magnification). (**c**) The myoepithelial cell layer at the periphery of the cluster highlighted with anti-p63 immunostaining (*arrows*) (anti-p63, Mayer's hematoxylin counterstaining × 40 original magnification). (**d**) (Anti-cytokeratin 14, Mayer's hematoxylin counterstaining × 4 original magnification)

invasive carcinoma to avoid understaging because it is usually very difficult to diagnose BMT to the SLN.

Role of the Pathological Examination of SLNs in Special Circumstances

Although pathologists usually do not have an active role in selecting patients in which an SLN biopsy (SLNB) will be performed, they should be aware of controversial circumstances. SLNB is currently used in clinically early-stage invasive breast cancer. ASCO recommends against the use of SLNB in patients with T3–T4 breast cancers, inflammatory breast cancer, male breast cancer, prior non-oncological breast surgery, preoperative systemic chemotherapy, and prior axillary surgery and on pregnancy [46]. In patients with DCIS, the frequency of SLNB positivity in previous studies has varied from 1.9% to 9% [73–77]. SLNB should be performed in patients with DCIS when a mastectomy is indicated because performing an SLNB is impossible after finding an invasive tumor during a permanent pathological examination of the mastectomy specimen. SLNB may also be performed in patients with large, high-grade, or palpable DCIS cases to avoid a secondary operation because it is not infrequent to find an invasive focus in these types of DCIS cases [46]. The use of SLNB in breast cancer patients with preoperative chemotherapy (Fig. 15.16) is controversial. In studies with small institutional case series, the false-negative rate ranged from 0% to 33% [78–85]. In cases with neoadjuvant chemotherapy, the success rate of identifying the SLN improves with experience, but the false-negative rate does not change [86]. In the NSABP B-27 trial and SN-FNAC study, the false-negative rates of SLNs were 10.7% and 8.4%, respectively [87, 88]. The utilization of ultrasonography resulted in limited success regarding the estimation of pathologic response in the SLN of patients treated with neoadjuvant chemotherapy for breast carcinoma [89, 90]. The ASCO panel concluded that there are insufficient data to recommend SLNBs in breast cancer with neoadjuvant chemotherapy and that a SLNB should be performed only in clinically negative axillary lymph nodes, whether in the preoperative or postoperative setting [46]. We currently perform IPESLN and use pancytokeratin immunostaining during permanent pathologic analysis of SLNs excised from patients with breast carcinoma treated with neoadjuvant chemotherapy.

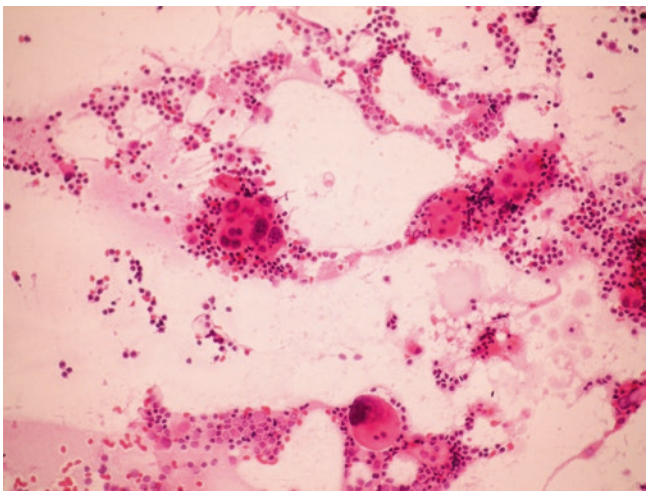

Fig. 15.16 Imprint cytology of a breast carcinoma metastasis in a sentinel lymph node excised after neoadjuvant chemotherapy. Note the cellular enlargement caused by chemotherapy (H&E × 20 original magnification)

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Fibroepithelial Tumors of the Breast

16

Sennur Ilvan

Introduction

Fibroepithelial tumors are characterized by a simultaneous proliferation of both epithelial and mesenchymal elements. Fibroadenomas and phyllodes tumors constitute the major entities. Several breast lesions, such as hamartoma, fibroadenomatoid hyperplasia, and tubular adenoma, are also included in this category.

Fibroadenoma

Fibroadenomas are the most common benign breast tumors in women 20–30 years but may be present at any age. They originate in the terminal duct lobular unit. The initial processes of epithelial and stromal proliferation in multiple lobules either occur spontaneously or are precipitated by hyperestrogenic states. A gradual confluence of the hyperplastic lobules is followed by the formation of fibroadenomatoid nodules. Finally, the fibroadenomatous nodules coalesce to form the fibroadenoma [1].

Fibroadenomas typically present as palpable, slow-growing, firm, mobile masses and are generally less than 3 cm in size. Less frequently, some of these tumors, particularly the juvenile variant, may reach very large dimensions. If rapid growth occurs, the lesion may undergo infarction, particularly in pregnant and lactating women, and may be mistaken for malignancy. Fibroadenomas are usually solitary, but in some patients, bilateral and multiple lesions can be observed [2]. Because fibroadenomas originate in the terminal duct lobular unit, which is absent in the normal male mammary duct system, fibroadenomas are rarely observed in the male breast [3].

Most fibroadenomas are clinically identifiable, but in 25% of cases, they are nonpalpable and can be diagnosed by mammography and ultrasound. Small-sized impalpable fibroadenomas are detected as mammographic nodular densities or as calcified lesions. Ultrasonography shows a well-defined homogeneous hypoechoic mass [2, 4].

Some chromosomal abnormalities have been described in fibroadenomas, such as the deletion of 17p and numerical changes in chromosomes 16, 17, and 21 [5]. Clonality studies have demonstrated the polyclonality of both stroma and epithelium, although monoclonality has been demonstrated in stromal areas of phyllodes tumors [1]. Because the stroma and epithelium of fibroadenomas exhibit estrogen and progesterone receptor expression, it is believed that fibroadenomas develop as a result of unopposed estrogenic influences [1, 6]. Recent studies have identified recurrent mediator complex subunit 12 (MED12) somatic mutations in exon 2 in stromal but not epithelial cells of fibroadenomas [7].

Patients who receive cyclosporin A for immunosuppression after organ transplantation are reported to be predisposed to fibroadenoma development [8]. The risk of developing breast carcinoma is dependent on the presence of proliferative changes in the fibroadenoma itself or in the surrounding breast tissue, as well as a family history of breast carcinoma [4].

Grossly, fibroadenomas are ovoid and well circumscribed. The cut surface is gray or white, solid, rubbery, and often somewhat lobulated. Slit-like spaces corresponding to epithelial structures upon microscopic examination may also be recognized. Rarely, the cut surface may appear myxoid. In older patients, the lesion may be fibrotic and calcified [2].

Microscopically, fibroadenomas consist of an admixture of stromal and epithelial elements. Two growth patterns are recognized. The intracanalicular pattern is characterized by the proliferation of stromal cells around stretched and compressed ducts (Fig. 16.1). In the pericanalicular pattern, stroma surrounds glandular structures with open lumina. These patterns often coexist and are thought to have no clinical significance. The glands have an inner epithelial layer and an outer myoepithelial layer, which are morphologically similar to normal breast ducts. The epithelial component may exhibit typical ductal hyperplasia, sclerosing adenosis,

S. Ilvan (✉)
Department of Pathology, Cerrahpasa School of Medicine, Istanbul University Cerrahpasa, Istanbul, Turkey

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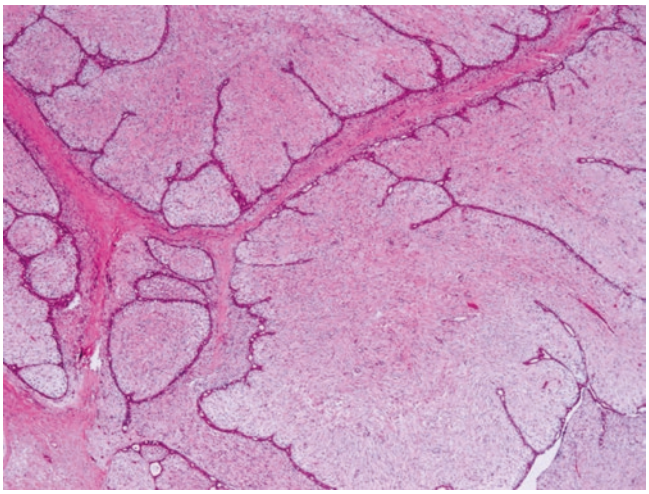

Fig. 16.1 Fibroadenoma, intracanalicular pattern (HE, 40×)

or benign metaplastic changes, such as apocrine or squamous metaplasia. Rarely, lobular or ductal carcinoma in situ and invasive carcinoma may be present within a fibroadenoma [2, 9]. The stroma in fibroadenomas is variable in both appearance and the degree of cellularity; it may be sclerotic, cellular, or myxoid. Usual fibroadenomas exhibit collagenized stroma with low cellularity. Spindle-shaped stromal cells have bland ovoid to elongated nuclei and do not display cytological atypia. Mitotic figures are rare but may be present, particularly in young and pregnant women. A hyalinized, sclerotic stroma is observed more frequently in fibroadenomas in older women. Chondroid, osseous, and smooth muscle metaplasia can very rarely occur in the stroma. Infrequently, scattered enlarged multinucleated stromal giant cells may be present. These cells are benign and should not be misinterpreted as evidence of malignancy [10].

Fibroadenomas with increased stromal cellularity and without leaflike processes are referred to as cellular fibroadenomas. The stroma may display prominent myxoid changes, particularly in younger women. Such myxoid fibroadenomas have been reported in association with Carney syndrome [2]. Juvenile fibroadenoma is observed mostly in patients younger than 20 years of age and is characterized by rapid growth, massive size, cellular stroma, a pericanalicular epithelial growth pattern, and marked epithelial hyperplasia with irregular tufts similar to that observed in gynecomastia [11]. Fibroadenomas that contain cysts greater than 3 mm, sclerosing adenosis, epithelial calcifications, or papillary apocrine hyperplasia are considered complex fibroadenomas. Complex fibroadenomas are associated with a slightly increased risk of subsequent development of breast cancer compared with simple fibroadenomas [12].

An intracanalicular fibroadenoma with increased stromal cellularity can be misinterpreted as a benign phyllodes tumor. The distinction is based on the degree of stromal cellularity and the presence of frond-like projections into cystic spaces (leaflike appearance). The distinction is particularly difficult in core needle biopsy, resulting in poor interobserver agreement [13]. In this situation, a descriptive diagnosis that leads to excision is appropriate.

Fibroadenomatous Change (Fibroadenomatoid Hyperplasia)

Fibroadenomatous change is a term used to describe histological changes in the breast similar to those observed in fibroadenomas but in which no discrete mass is formed.

Tubular Adenoma

Many authorities consider tubular adenoma to be a variant of fibroadenoma. These relatively rare lesions usually occur in young women. On gross examination, tubular adenomas are softer and typically tan-brown in color. They are circumscribed nodular lesions composed of closely packed, small, round acinar structures surrounded by scant, loose cellular stroma [2].

Mammary Hamartoma

These lesions occur at any age but are most commonly observed in premenopausal and perimenopausal women. The clinical features are similar to those of fibroadenoma. Hamartomas are composed of mammary ducts, lobules, collagenous stroma, and adipose tissue. In contrast to normal breast tissue, these structures are disorganized and irregularly dispersed. Because of their similarity to normal breast tissue, histological diagnosis may be impossible. In these situations, mammographic findings are required for correct diagnosis. Smooth muscle may be present, and lesions with marked smooth muscle fibers have been termed myoid hamartomas [10].

Phyllodes Tumor

Phyllodes tumors are uncommon fibroepithelial breast neoplasms that account for 0.3–1.0% of all primary breast tumors. Johannes Mueller, who first described phyllodes tumors in 1838, called them cystosarcoma phyllodes, "sarcoma" for the fleshy nature of the lesion and "phyllodes" for the leaflike appearance [1]. This term is currently considered inappropriate because the tumors are rarely cystic and most of them follow a benign course. The World Health Organization (WHO) currently supports the term "phyllodes tumor" in its

classification of breast tumors. These tumors are classified as benign, borderline, and malignant based on a variety of histopathological variables [10]. Whether phyllodes tumors arise from preexisting fibroadenomas or develop de novo is controversial. It has been speculated that phyllodes tumors begin as fibroadenomas, and subsequently a single stromal cell undergoes mutation and develops into a phyllodes tumor [14]. MED12 is frequently mutated in phyllodes tumors. The mutational spectrum is similar to that of fibroadenomas, with the vast majority of mutations at codon 44, supporting an underlying commonality in pathogenesis [7].

While phyllodes tumors can be observed at any age, including adolescence, they occur most frequently in women 40–50 years of age. Few cases have been reported in the male breast [3]. Phyllodes tumors usually present as unilateral, painless, lobulated, and freely movable breast masses and rarely cause skin changes, such as ulceration, nipple retraction, or nipple discharge. It is generally difficult to clinically differentiate them from fibroadenoma. Patients with phyllodes tumors usually have a history of large, rapidly growing masses [15].

Mammography reveals a rounded or lobulated, sharply defined opaque mass in most cases. It is not possible to reliably distinguish between benign and malignant phyllodes tumors by mammography or ultrasonography [15].

Macroscopically, phyllodes tumors vary in size from a few centimeters to 30 cm in diameter, with an average size of 4–5 cm. Although malignant variants tend to be larger than benign lesions, there is no consistent relationship between tumor size and malignancy. Similar to fibroadenomas, phyllodes tumors have well-delineated borders but appear fleshier, with bulging cut surfaces. Clefts and cystic spaces may be observed, particularly in larger tumors. Malignant lesions may be less well defined, and the clefts may be less obvious. Hemorrhage and necrosis are more common in borderline and malignant tumors [2, 16].

Microscopically, phyllodes tumors are composed of epithelium and cellular stroma arranged in an intracanalicular pattern. Projections of this cellular stroma into cystic spaces form the leaflike pattern characteristic of phyllodes tumors (Fig. 16.2). The morphology of the stromal component varies from only slightly more cellular than that of a fibroadenoma to frankly sarcomatous. In addition, heterogeneity in stromal cellularity within a phyllodes tumor is common. Stromal cellularity and mitotic activity are often increased in periglandular areas. The epithelial component may exhibit usual ductal hyperplasia or metaplastic changes, such as squamous or apocrine [1, 2].

Based on the WHO classification, phyllodes tumors are classified as benign, borderline, and malignant according to stromal cellularity, cellular atypia and pleomorphism, mitotic index, tumor margin, stromal overgrowth, and the presence or absence of heterologous differentiation (Table 16.1) [10].

Mild hypercellularity is characterized by a slight increase in stromal cells compared with normal perilobular stroma cells, with evenly spaced nuclei that are not touching or overlapping. Marked stromal cellularity shows confluent areas of densely overlapping nuclei. Moderate stromal cellularity contains intermediate findings with some overlapping stromal nuclei. Mild stromal atypia shows nuclei with little variation in size and smooth nuclear contours. In moderate atypia, there are variations in nuclear size and wrinkled nuclear membranes. Marked atypia shows marked variation in nuclear size, coarse chromatin, irregular nuclear membranes, and discernible nucleoli [7].

Benign phyllodes tumors typically have pushing borders and mild stromal cellularity, with little or no atypia (Fig. 16.2). The stroma is usually condensed around the epithelial component. Mitotic figures are infrequent; they rarely exceed 1–2 per 10 high-power fields and are generally located in the periductal areas. Stromal overgrowth, defined as at least one microscopic field observed at 4× magnifica-

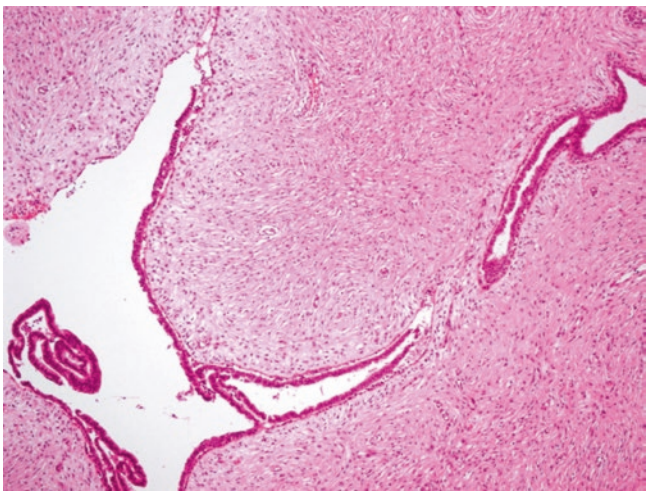

Fig. 16.2 Benign phyllodes tumor with a leaflike structure (HE, 100×)

Table 16.1 Histological features used in the classification of phyllodes tumor subtypes

	Benign	Borderline	Malignant
Tumor border	Well defined	Well defined, focally infiltrative	Infiltrative
Stromal cellularity	Mild	Moderate	Marked
Stromal cell atypia	None to mild	Mild to moderate	Marked
Mitotic activity	<5/10 HPF	5–9/10 HPF	≥10/10 HPF
Stromal overgrowth	Absent	Absent or very focal	Often present
Malignant heterologous elements	Absent	Absent	May be present

Modified from Tan et al. [10]. *HPF* high power fields

tion that contains only stroma without epithelial components, is not present. Benign heterologous stromal elements, such as the skeletal muscle, cartilage, and bone, may be present. The presence of bizarre stromal giant cells should not be taken as a mark of malignancy. Benign phyllodes tumors can be difficult to distinguish from cellular fibroadenoma. Compared to fibroadenoma, phyllodes tumors are characterized in most cases by expansion and the increased cellularity of the stromal component. In addition, numerous leaflike projections into cystic spaces and stromal mitosis favor a diagnosis of phyllodes tumor. When there is histological ambiguity, the WHO Working Group recommends a diagnosis of fibroadenoma instead of phyllodes tumor. Some authors advocate using the term "benign fibroepithelial neoplasm" with an explanation of the diagnostic difficulty [10].

Borderline phyllodes tumors exhibit a relatively expanded stromal component and pronounced intracanalicular growth pattern compared with benign forms. Stromal atypia is mild to moderate, and mitotic figures range from 5 to 9 per 10 high-power fields. Tumor margins may be pushing or focally infiltrative. Stromal overgrowth is absent or very focal [10].

In malignant phyllodes tumors, the stroma is more expanded and highly cellular, and the tumor borders are infiltrative. The stromal cells exhibit marked atypia and brisk mitotic activity (≥10 mitoses/10 high-power fields). Stromal overgrowth is common and may be so prominent that the appearance mimics a primary breast sarcoma. In such cases, extensive sampling is required for correct diagnosis. Although the stroma most often resembles a fibrosarcoma, heterologous stromal differentiation, such as liposarcomatous, chondrosarcomatous, or osteosarcomatous elements, can be observed (Fig. 16.3). The presence of a malignant heterologous component places the phyllodes tumor into the malignant group regardless of other histological features [16].

The local recurrence rates are 10–17%, 14–25%, and 23–30% for benign, borderline, and malignant phyllodes tumors, respectively. Recurrence rates are strongly correlated with the amount of normal breast tissue present at the resection margins. Histological parameters predicting recurrence are stromal overgrowth, atypia, and mitotic activity. Axillary node involvement is rare, but hematogenous spread occurs in the lung, bone, and liver. Metastases generally consist of stromal component alone. Histological type is the most important predictor of metastatic spread, and most distant metastases develop from malignant tumors [10, 15].

The expression levels of many biological markers, such as p53, c-kit, CD10, β-catenin, vascular endothelial growth factor, and CD34, have been explored in phyllodes tumors. Most of them are variably expressed depending on the grade of the tumor. However, the clinical value of these markers is limited in predicting the behavior and classification of phyllodes tumors [17, 18].

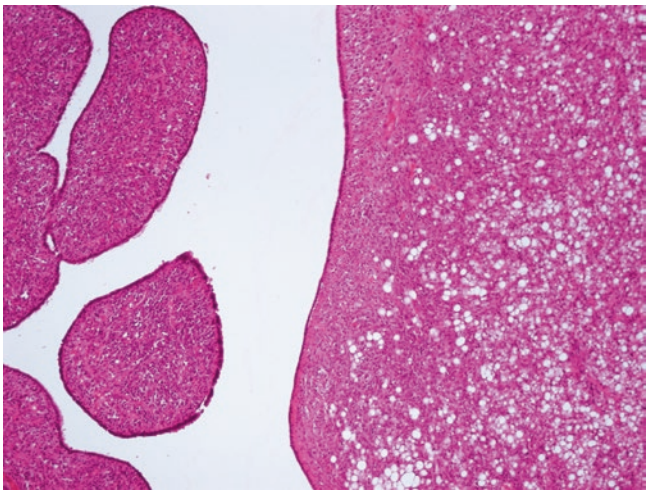

Fig. 16.3 Malignant phyllodes tumor showing liposarcomatous differentiation (HE, 100×)

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Mesenchymal Tumors of the Breast

17

Zerrin Calay

Introduction

Many neoplastic and nonneoplastic mesenchymal lesions have been reported in the breast. In this chapter, only lesions that are observed more frequently or are important for differential diagnosis will be discussed.

Myofibroblastic and Fibroblastic Lesions of the Breast

These are a heterogeneous group that represent the majority of mesenchymal spindle cell proliferations in the breast. Pseudoangiomatous stromal hyperplasia (PASH) and mammary myofibroblastoma are benign lesions with myofibroblastic differentiation in this morphologic spectrum. Myofibroblastic sarcomas and inflammatory myofibroblastic tumors also participate in this spectrum but are very rare. Other mammary lesions with fibroblastic/myofibroblastic differentiation resemble their counterparts at other sites and include nodular fasciitis and fibromatosis, and primary fibrosarcoma may also be rarely seen [1].

Pseudoangiomatous Stromal Hyperplasia (PASH)

PASH is a benign myofibroblastic proliferation that simulates a vascular lesion. This proliferation is thought to represent an exaggerated myofibroblastic response to endogenous or exogenous hormonal stimuli, particularly progesterone [2].

PASH is commonly observed as an incidental microscopic finding in breast biopsies or in association with other breast lesions, such as fibroadenomas, phyllodes tumors, and gynecomastia. Rarely, this proliferation can be florid and form a clinically evident mass mimicking fibroadenoma. In these cases, the terms "nodular or tumorous PASH" have been used [3]. Gross examination of tumorous PASH reveals a round-oval, well-circumscribed, rubbery mass with a homogenous cut surface that is tan to white in color. Typical PASH is characterized by thin, slit-like spaces set in a densely hyalinized stroma. The spaces are often lined by bland spindled myofibroblasts that may mimic the endothelial cells of blood vessels. Occasionally, PASH is more cellular with a fascicular growth pattern that is similar to myofibroblastoma. Multinucleated giant cells have been reported in some examples of PASH [4]. Cytologic atypia and mitotic activity of the myofibroblasts may rarely be observed [5]. The myofibroblasts exhibit staining for CD34 and variable staining for SMA (smooth muscle actin), and desmin stains for CD31 and ERG are negative.

PASH is a benign lesion that is adequately treated by local excision, although recurrences have been reported [2]. The major importance of PASH is that it must be distinguished histologically from a true vascular lesion, namely, low-grade angiosarcoma.

Myofibroblastoma

Myofibroblastoma is an uncommon benign tumor of the breast composed of fibroblasts and myofibroblasts. Clinically, myofibroblastomas are slow-growing, circumscribed, mobile masses that are usually mistaken for fibroadenomas on physical examination and mammography. Myofibroblastoma is now considered part of the same spectrum as spindle cell lipoma [2, 6], with which it shares the same chromosomal rearrangements affecting region 13q [7]. The region of chromosome loss corresponds to deletions in the RB gene, and FISH and immunohistochemistry can be used to assess for loss of Rb.

Gross examination reveals a rubbery-firm, oval-round nodule with a whorled cut surface. A variety of patterns may be observed histologically. Myofibroblastoma is usually

Z. Calay, MD (✉)
Department of Pathology, Cerrahpasa School of Medicine, Istanbul University Cerrahpasa, Istanbul, Turkey

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composed of uniform, oval-spindle cells arranged as short fascicles admixed with hyalinized bands of collagen. Morphological variants are reported as epitheloid, cellular, infiltrative, deciduoid, collagenized, lipomatous, and myxoid types [8]. This morphological variation may cause diagnostic difficulties.

Differential diagnosis includes a variety of reactive and benign spindle cell lesions, spindle cell sarcoma, and carcinoma. Myofibroblastoma cells typically exhibit CD34 and desmin expression, with variable positivity for actin, bcl-2, and CD99. The expression of ER, PR, and androgen receptors is common. Therefore, an epitheloid variant of myofibroblastoma may be confused with invasive lobular carcinoma because both tumors express hormone receptors [9].

Myofibroblastomas are benign and are adequately treated by local excision.

Desmoid-Type Fibromatosis

Mammary fibromatosis (desmoid type) is an infiltrative, locally aggressive proliferation of fibroblasts and myofibroblasts that typically presents as a firm mass that may be mistaken for carcinoma clinically and mammographically. It can occur within the breast parenchyma but frequently arises from the pectoral fascia and extends into the breast. There is an association with previous trauma, particularly surgery and including implants [10]. Desmoid-type fibromatosis occasionally occurs in patients with familial adenomatous polyposis (FAP) [2].

The margins of the lesion are infiltrative and difficult to determine on gross examination. The cut surface reveals a firm consistency with a gray-white to tan appearance. Fibromatosis is characterized by broad sheets of spindle cells producing a storiform or herringbone pattern. The lesion irregularly infiltrates the adjacent breast parenchyma. Nuclear expression of beta-catenin is observed in approximately 80% of cases [2]; however, beta-catenin can also be expressed in spindle cell carcinoma, phyllodes tumors, and fibrosarcoma. CD34 expression is absent.

Scarring from prior trauma, such as surgery, and fibromatosis, such as metaplastic carcinoma (spindle cell carcinoma), are the most important lesions for the differential diagnosis of fibromatosis. An immunohistochemistry panel should be performed using a broad panel of antibodies to keratins, including those of high molecular weight [2].

The major clinical concern in patients with fibromatosis is local recurrence, which is observed in 20–30% of cases. Wide local excision is required to prevent local recurrence.

Nodular Fasciitis

Nodular fasciitis is a self-limited, mass-forming fibroblastic/myofibroblastic proliferation that is clonal [8]. This is further confirmed by the identification of recurrent rearrangements in the USP6 gene locus (2). Nodular fasciitis is very uncommon in the breast but is important to recognize because it may clinically, radiographically, and histologically mimic a malignant tumor. Typically, it grows rapidly and may be tender and painful. These lesions regress spontaneously within a few months [2].

Nodular fasciitis arises in the subcutis or, less often, the mammary parenchyma and is a well-circumscribed grayish nodule that may display central cystic changes. It is composed of short, spindle-shaped cells loosely arranged in a “tissue culture” fashion. Mitoses are typically frequent. They form solid masses and do not infiltrate around ducts and lobules. The cells are negative for CD34 and beta-catenin but positive for actin and focally positive for desmin.

The major differential diagnostic considerations are malignant spindle cell tumors (including spindle cell carcinomas and sarcomas) and fibromatosis. FISH analysis to assess for the presence of USP6 rearrangement is a sensitive and specific modality for diagnosis.

Although nodular fasciitis will spontaneously regress, in general, local excision is performed for definitive diagnosis. Local excision is curative in nearly all cases [2, 8].

Inflammatory Myofibroblastic Tumor

Inflammatory myofibroblastic tumor (IMT) is a low-grade neoplasm consisting of myofibroblastic spindle cells mixed with prominent inflammatory cells, usually plasmocytes. IMT is very rare in the breast [11].

IMT in the breast presents as a painless, circumscribed firm mass. The majority of lesions are benign with a local recurrence rate of 10–25% [2].

Solitary Fibrous Tumor

A solitary fibrous tumor can rarely present as a primary breast tumor. These are circumscribed tumors of variable cellularity and intercellular dense collagen. There is a prominent vasculature ranging from capillaries to staghorn blood vessels. CD34 is usually positive and less commonly positive for actin. Recently, it has been shown that a recurrent fusion gene in chromosome 12, NAB2-STAT6, can be used as a immunohistochemical marker (STAT 6) [12].

Vascular Lesions of the Breast

Vascular lesions of the breast are a heterogeneous group. They can be either benign or malignant; however, vascular lesions with atypical but not frankly malignant features have also been described.

Benign Vascular Lesions

Benign vascular lesions of the mammary parenchyma are relatively uncommon. These include perilobular hemangioma, hemangioma (capillary, cavernous, and venous), and angiomatosis. In addition, there are a variety of benign vascular lesions that involve the subcutaneous tissue [5].

The major clinical importance of these lesions is that they must be distinguished from angiosarcoma. Some angiosarcomas are very well differentiated and simulate benign blood vessels; conversely, some benign vascular lesions may exhibit atypia. Occasionally, differential diagnosis is impossible, particularly if it is a core biopsy; surgical excision should be performed for exclusion.

Atypical Vascular Lesions

Atypical vascular lesions of the breast are angioformative proliferations that can develop in the skin after BCS (breast-conserving surgery) and radiation therapy for breast carcinoma [13]. These lesions typically present as small papules or plaques with pink, red, or brown discoloration. These lesions are characterized by dilated vessels in the dermis that often have complex branching and anastomosing areas. These lesions are positive for the endothelial markers CD31, CD34, and ERG, similar to angiosarcomas. However, in contrast to postradiation (secondary) angiosarcoma, they are negative for MYC by immunohistochemistry and FISH [12]. Although they have a benign clinical course, some may be precursors of angiosarcoma [14].

Angiosarcoma

Angiosarcoma is the most frequent primary sarcoma of the breast, with an incidence of approximately 0.05% of breast malignancies. Angiosarcoma may arise spontaneously (primary angiosarcoma) or following radiation therapy for breast cancer (secondary angiosarcoma). Secondary angiosarcomas show an association with MYC amplification, in contrast to primary angiosarcomas, which can be assessed by immunohistochemistry and FISH [12].

Angiosarcoma of the mammary parenchyma presents as a painless mass, and those that involve the skin may appear as areas of bluish-red discoloration (Fig. 17.1).

Histologically, these lesions are characterized by anastomosing vascular channels that dissect the adipose tissue and mammary stroma (Fig. 17.2). The endothelial cells have atypical hyperchromatic nuclei, but endothelial multilayering and mitoses are often absent. Poorly differentiated angiosarcoma has a more solid, cellular growth pattern, typically with spindled morphology and reduced formation of vascular channels (Fig. 17.3). Angiosarcomas are classified as low, intermediate, and high grades based on a combination of histological features, although grading has no prognostic value [15].

Differential diagnoses for well-differentiated angiosarcoma include PASH, angiolipoma, and benign vascular lesions. Poorly differentiated angiosarcoma must be differentiated from spindle cell carcinoma and other sarcomas.

Angiosarcomas require complete excision and are usually treated by mastectomy. The prognosis is generally poor, particularly for secondary angiosarcomas [5].

Fig. 17.1 Angiosarcoma. In this mastectomy specimen, the skin has areas of bluish-red discoloration

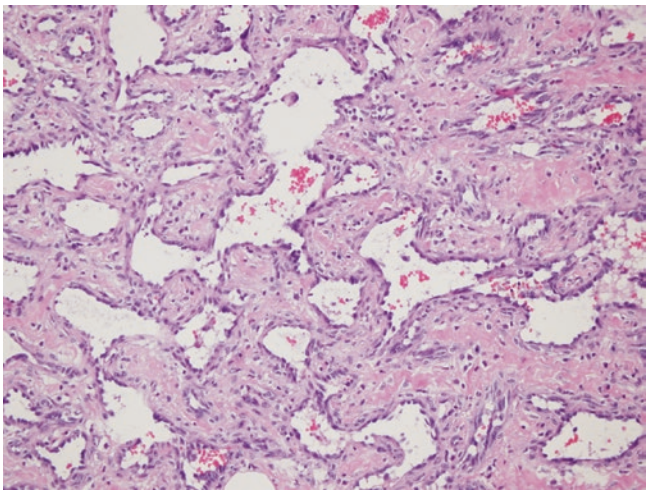

Fig. 17.2 Angiosarcoma, low grade. (Well-formed, interanastomosing vascular spaces are present. The endothelial cells lining the vascular channels show mild nuclear hyperchromasia, but there is no evidence of endothelial cell tufting.) HE, 100×

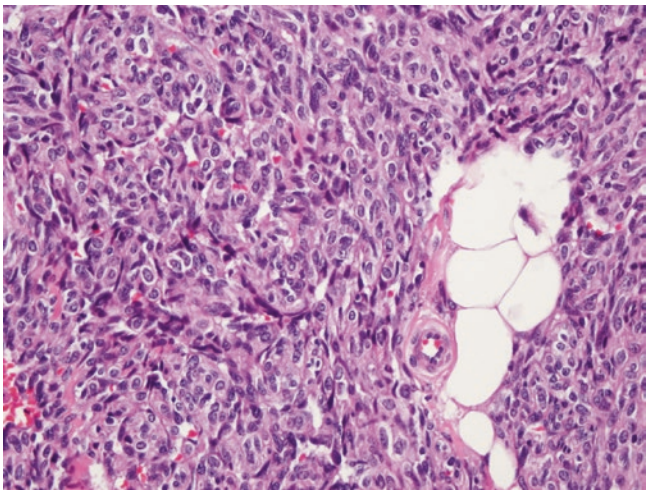

Fig. 17.3 Angiosarcoma, high grade. (This tumor has a more solid, cellular growth pattern, typically with spindled morphology and reduced formation of vascular channels.) HE, 200×

Other Mesenchymal Lesions

Benign mesenchymal tumors that occur elsewhere in the body have been described in the breast, including lipoma, angiolipoma, leiomyoma, neurofibroma, schwannoma, and granular cell tumor of the breast. Granular cell tumor is a benign tumor derived from Schwann cells of peripheral nerves; however, they may appear malignant clinically (irregular and firm mass), radiologically (ill-defined spiculated lesion), and pathologically (infiltrative growth pattern) [2].

Malignant mesenchymal tumors of the breast other than angiosarcomas are extremely rare. After angiosarcoma, the second most common type of primary sarcoma is liposarcoma. However, any type of sarcoma may occur in the breast as a primary lesion, including fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, malignant peripheral nerve sheath tumor, and osteosarcoma.

Mammary mesenchymal lesions require a careful approach, with close attention to morphological clues and consideration of histologic mimics and pitfalls. A sarcomatous-appearing tumor in the breast is far more likely to be a metaplastic carcinoma (spindle cell carcinoma) or a malignant phyllodes tumor rather than a primary sarcoma. Therefore, extensive sampling of the tumor and immunostaining using multiple anti-cytokeratin antibodies are essential for correct diagnosis. Usually, the classification of mammary mesenchymal tumors may be difficult or impossible on core biopsy due to the heterogenous appearance of mimics, and excision may be necessary to make a correct diagnosis. In addition, certain fibroblastic, vascular, and adipocytic tumors show genetic alterations detectable by FISH that may be diagnostically helpful in core biopsy samples.

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Liquid Biopsy in Breast Carcinoma

18

Semen Onder and Ekrem Yavuz

Introduction

Breast cancer remains one of the leading causes of cancer-related deaths in women despite improved treatment options and the development of new targeted therapies [1]. Distant metastasis remains the main cause of death in nearly 20% of patients with breast cancer [2, 3]. To increase the overall survival (OS) and disease-free survival (DFS) by preventing occult micrometastases, adjuvant chemotherapy and/or hormonotherapy are given in the early setting of the disease [3]. There is no acceptable method for detecting and safely monitoring micrometastases that would predict recurrence even in patients without clinical symptoms [3, 4].

Liquid Biopsy

Liquid biopsy is defined as a minimally invasive test for identifying cancer-related factors using a blood sample [3, 5–7]. These factors are circulating tumor cells (CTCs), fragments of tumor DNA, circulating cell-free nucleic acids, and tumor markers. Detection of these circulating biomarkers provides detailed information on the real-time status of the current disease and its heterogeneity. Liquid biopsy is a practical and repeatable method that is driving the development of new, personalized individual therapies [5, 7]. Above all, liquid biopsy is starting to replace conventional tissue biopsies, which is routinely performed when metastasis occurs or any clinical information is needed. Tissue biopsy is an invasive and commonly used method that is certainly not suitable for repetition and provides limited data on the sampled tumoral tissue, without adequately reflecting intratumoral heterogeneity [5, 7–9]. Hence, all clinical targets, including minimal residual disease monitoring, as markers of recurrence as well as response to treatment are potential targets of liquid biopsies [3, 5, 7, 9–14].

Circulating Tumor Cells

Circulating tumor cells are rare malignant cells (estimated at approximately 1 per 10^7 leukocytes) that escape from the primary tumor and migrate through the circulatory system. These cells were first described in 1869 [15]. CTCs are not found in the bloodstream in healthy patients [12, 16] and are generally found in most cancer types, with a lower rate in nonmetastatic cancers [12, 17]. These cells are thought to be the key factors of metastatic dissemination and consist not only of epithelial but also mesenchymal and epithelial-mesenchymal hybrid cells [3]. Epithelial to mesenchymal transition (EMT) has been implicated in cancer progression and metastasis and may also have a role in CTC dissemination and the acquisition of aggressive behavior [18]. Although recent experimental studies do not support such a role, this theory remains open to discussion [3, 19].

Circulating Tumor Cells: Detection Methods

As CTCs are extremely rare in the blood, methods have been developed to enable reliable detection. CTCs are fragile and heterogeneous, and thus every step of detection must be performed carefully to ultimately obtain the correct information. The first step of CTC detection is the cell-enrichment process, which is followed by the detection step (Fig. 18.1) [10–12, 20]. The aim of the cell-enrichment process is to capture CTCs using their biophysical properties, including cell size, density, electrical charge, and positive-negative immunoselection, that differentiate them from leukocytes. Compared to blood cells, CTCs are larger (20–30 μm vs 8–12 μm) [12, 21]. Many filtration methods have been developed depending on the size parameter: dielectrophoretic field-flow fractionation (DEP-FFF), the Metacell filtration device, ScreenCell®, and isolation by size of epithelial tumor cells (ISET®) [22–26]. Most size-based

S. Onder (✉) · E. Yavuz
Department of Pathology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey
e-mail: semen.onder@istanbul.edu.tr; yavuze@istanbul.edu.tr

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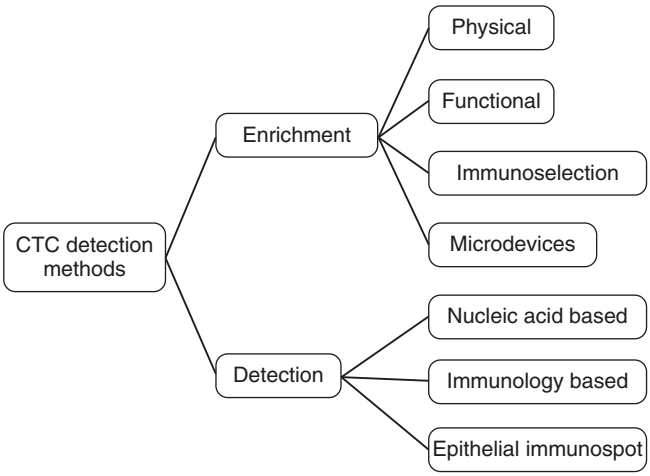

Fig. 18.1 The main methods of CTC detection

methods lack specificity and fail to isolate smaller CTCs and those with shapes similar to leukocytes [24, 27]. Density-based cell-enrichment methods can also easily discriminate CTCs from other types of cells (e.g., AccuCyte®, OncoQuick®) [28, 29].

Another cell-enrichment method uses functional features to detect CD45 protein levels and collagen adhesion matrix (CAM). To remove leukocytes with the CD45 antigen, the EPISPOT® assay is used. The CAM assay collects CTCs using CAM protein labeling [30, 31]. Both assays promise high specificity and sensitivity but are problematic when antigen levels are low [20]. TelomeScan® uses the specific telomerase activity of adenovirus replication in tumor cells [32]. False-positive results have been detected using this method due to the accidental selection of hematopoietic cells with EpCAM (epithelial cell adhesion molecule)-negative tumor cells [20].

The most popular cell-enrichment method is based on immunoselection. This method uses the expression of EpCAM, an epithelial cell-surface antibody, and iron-coated nanoparticles to enrich CTCs. The only FDA-approved CTC detection system is the well-known CellSearch® system, which is a semiautomated system that uses fluorescence imaging and immunohistochemistry to detect CTCs in 7.5 ml of peripheral blood [33]. In this system, for positive discrimination, EpCAM and cytokeratins (CK) (CK8/CK18/CK19) are generally used. In addition, HER-2, mammaglobin, and MUC-1 can be added as positive markers in samples of patients with breast cancer [12, 34]. For negative discrimination, CD45, a leukocyte-specific antigen, and CD61, which is specific to megakaryocytes, can be used [12, 35]. A similar method named IE/FC (immunomagnetic enrichment/flow cytometry) also uses EpCAM antibody labeling but employs flow cytometry instead of CK staining [36]. The last two methods described above show concordant results for CTC detection.

The microdevices used as enrichment tools depend on both physical and immunological methods. Some of these devices are coated with anti-EpCAM bodies (CTC-chip, ^{HB}CTC-Chip, and Ephesia), whereas others use leukocytic antigens (CD45) (CTC-iChip, Cluster-Chip) [37–39].

In general, CTC detection methods use fluorescence microscopy, flow cytometry for immunostaining, and reverse transcription-polymerase chain reaction (RT-PCR) for tumor-related mRNA detection. For CTC detection, there are also nucleic acid-based approaches that utilize mRNA or specific DNA markers [10, 12]. These markers are normally not found in the circulation and can be epithelial specific (CK, EpCAM), tumor specific (HER-2), or organ specific (mammoglobulin, MUC-1) [12]. One of the RT-PCR tests is AdnaTest BreastCancer®, which uses three tumor-associated transcripts (HER2, MUC1, and GA733–2) to detect CTCs in breast cancer [40]. This test is a sensitive and accurate method of CTC detection. AdnaTest BreastCancer® showed a higher detection rate than the CellSearch® system (40% vs 10–23%) in a previous study. However, this result was linked to the false positivity of the former test [41, 42]. The detection rates of these two systems were found to be similar in another head-to-head comparison study, but the CellSearch® system was more successful with regard to clinical results [43, 44].

Circulating Tumor Cells: Clinical Applications

In general, CTC enumeration is used as a prognostic tool, whereas molecular characterization of CTCs is used to identify patients suitable for targeted therapies. The roles of CTCs differ in the settings of early- and advanced-stage breast cancer.

The prognostic and predictive roles of CTCs in the setting of metastatic breast cancer (MBC) have been thoroughly demonstrated in many clinical studies [3, 5, 11, 33, 45]. In 2004, a cutoff value of CTCs of 5 cells/7.5 ml of blood was established for the first time to indicate unfavorable clinical findings (shorter overall survival (OS) and shorter progression-free survival (PFS)) in MBC [45]. Bidard et al. evaluated the clinical effect of CTC enumeration changes in 1944 patients during treatment [46]. They found that patients with <5 CTCs at baseline and no change during treatment had the best prognosis, similar to the results described by Hayes et al. [47].

Early breast cancer can be defined as minimal residual disease with CTCs in the blood that is not detected by standard conventional imaging tools [3, 10]. CTC detection in stage I–II breast cancer was found to be an independent prognostic factor [48]. Detection of at least one CTC in stage I–III breast cancer at the time of surgery was correlated with decreased OS and PFS [49]. This finding was also supported by Franken et al.'s study of CTC analysis before surgery in a series of 602 patients [50]. In operable breast cancer patients,

no prognostic and histopathological findings correlated with CTC presence, other than HER-2 status [51, 52]. CTC positivity after surgery has also been reported to be an independent prognostic factor in operable breast carcinoma [49, 53]. Enumeration of CTCs to predict the response to chemotherapy is another area of research. The presence of at least one CTC before and after adjuvant chemotherapy was found to be correlated with poor DFS [53]. However, patients whose CTC status switched from absent to present after chemotherapy displayed a similar prognosis. In the neoadjuvant setting, enumeration of CTCs is not a strong and convincing tool since suspicious results were found during CTC counts before and after treatment and during follow-up [54–56].

Hormone receptor (ER/PR) and HER2 status in CTCs is also under investigation and is becoming a more important issue for the primary tumor due to its ability to guide targeted therapy [10]. During the course of the disease, modifications in hormone receptor and HER2 status can be detected [57, 58]. Studies using different methods to investigate the concordance of markers in the primary tumor and CTCs have reported different results. There is a wide range of concordance in hormone receptor status between the primary tumor and CTCs of 40–70% [10, 59, 60]. When comparing the HER2 status of CTCs with that of primary tumor, there is also a wide range of 48–93% agreement [42, 44, 61, 62]. Studies comparing HER2 results between the primary tumor and CTCs have used different methods and included small numbers of cases ranging from 8 to 122 (average of 43), primarily cases in the metastatic stage [10]. Different markers, such as EGFR, have also been analyzed using immunohistochemistry in CTCs, with 86% positivity observed in a study group [63]. This finding can be useful for identifying appropriate patients for anti-EGFR-targeted therapy.

Studies of CTC-based biomarker analysis are still ongoing and require more time.

Circulating Tumor DNA

Cell-free DNA (cfDNA) is short DNA fragments (70–200 base pairs in length) circulating in the plasma or serum and was first described in 1948 in a healthy individual's blood [64]. The source of cfDNA in a healthy individual is mostly bone marrow and white blood cells, whereas in cancer patients, the source of cfDNA is dying, necrotic tumoral cells [5, 6, 11, 12]. Circulating tumor DNA (ctDNA) is described as a small fraction of cfDNA originating from the cell death process (necrosis and apoptosis) of tumor cells [5, 6, 9, 11, 12]. The amount of ctDNA, which originates from multiple tumor sites, ranges from 0.001% to 90% of cfDNA [11, 65]. The main parameter controlling the level of ctDNA is tumor burden, although stage, tumor heterogeneity, and the physiological filtering characteristics of the circulation system also have an effect [6, 11]. In the early stages of the disease, the levels of ctDNA are too low to create detection problems compared to those of metastatic or late-stage disease [12, 66].

Circulating Tumor DNA: Detection Methods

The amount of cfDNA, which is a mixture of ctDNA and normal DNA, is higher in serum. Plasma is accepted as the most suitable material for ctDNA analysis due to its low level of normal DNA [6]. There are two main steps for detecting ctDNA: preanalytical and analytical. The preanalytical step is a "before analysis" step that includes specimen handling, storage time and temperature, transport, and DNA-extraction processes [6]. There is a small amount of ctDNA for detection that can be easily fragmented. Therefore, preanalytical variables are very important for the quality of the specimen and the accuracy of the ctDNA analysis [6, 12, 67]. To prevent lysis and overdilution of ctDNA, processing must start within 6 h after collecting the plasma in a K_2EDTA tube [6]. Before storage, the plasma must be purified using filtration or low-speed centrifugation [6, 68]. The storage style, storage temperature, and shipping method also affect ctDNA analysis, but the details remain unclear [6, 68]. There are several extraction methods, including manual and automated systems [6, 7]. The analytical step can be divided into two main categories: small targeted assays and broad-coverage assays [6, 7]. Targeted assays usually depend on polymerase chain reaction (PCR)-based applications to identify point mutations in a small number of genes associated with specific drugs [6]. In addition, there are next-generation sequencing (NGS)-based methods for large-scale mutation analysis of multiple types of tumors.

Circulating Tumor DNA: Clinical Applications

ctDNA is the most promising circulating tumor marker in terms of its potential to provide a true snapshot of the tumor and intratumoral heterogeneity [9]. Many studies have shown a direct connection between the tumor load and ctDNA [9, 11].

Detection of ctDNA in the setting of early breast cancer has high sensitivity and specificity for predicting relapse [6, 11, 69]. Repeating ctDNA monitoring during follow-up improves the sensitivity of prediction [66]. A high level of ctDNA has been shown to correlate with poor prognosis due to the relationship between tumor burden and ctDNA levels [5, 70, 71]. ctDNA is more likely to be detected in metastatic breast cancer than in localized cancer (82% vs 55%) [72]. In the metastatic breast cancer setting, ctDNA detection was found to be more sensitive than Ca 15-3 monitoring for predicting tumor burden [70]. However, tumor progression was recorded in patients with low levels of ctDNA in another study [73], indicating a lack of sensitivity.

By detecting mutations specific to cancer cells, ctDNA can be differentiated from cfDNA and thus used as a cancer biomarker [12]. In breast cancer, PI3K mutation is one of the most frequent mutations, with a rate of 40% [12]. Evidence of PI3KCA mutation in ctDNA specifically suggested metastatic breast carcinoma but did not identify curable patients [12, 74]. Simultaneous analysis of mutations of PI3KCA and TP53 in tissue and plasma samples revealed that these markers were correlated [75, 76]. However, there was no connection between ctDNA levels and prognosis [76]. Some ctDNA mutations have been analyzed to identify specific treatment resistances [5]: mediator complex subunit 1 (MED 1) for trastuzumab resistance, growth arrest-specific 6 (GAS6) for lapatinib resistance, and estrogen receptor 1 for endocrine therapy resistance [5, 77, 78]. ctDNA assessment is a good means of monitoring minimal residual disease or treatment response with the support of digital PCR and NGS technologies [11]. The use of ctDNA for early cancer diagnosis in populations without symptoms has rarely been studied [6]. Some somatic variants of genes are also detected in the cfDNA of healthy people and do not indicate an increased risk of cancer, which is known as "age-related clonal hematopoiesis," especially after the age of 50 [6]. Furthermore, some genomic variants detected in circulation may have no clinical importance and cause overdiagnosis [6]. Thus, evidence for the interpretation of ctDNA assays in healthy people for screening remains insufficient [6].

Liquid Biopsy: Clinical Investigations for Treatment

As CTC counts appear to reflect the response to therapy, the potential use of CTCs for treatment decisions has been questioned. The first study of this subject was the Southwest Oncology Group (SWOG) S0500 trial, which showed no effect of changing therapy based on high CTC levels on prognosis [79]. Another multicenter study (CirCe01) questioning the same issue is ongoing [5]. Regarding the effect of trastuzumab on "HER2-negative" patients, HER2-positive CTCs were detected by RT-PCR analysis in a study that showed a positive effect of trastuzumab on DFS [80]. Another study evaluated the effect of lapatinib, another inhibitor of HER2 and EGFR, in HER2-negative patients with HER2-positive CTCs and showed a decrease in CTCs as a result of therapy [81]. The DETECT studies are the largest studies examining changes in CTCs during therapy in metastatic breast cancer patients. The effect of lapatinib was examined in the DETECT III study, which also had an endpoint of CTC clearance [5].

Liquid Biopsy: Limitations and Future Aspects

The detection of CTCs and ctDNA mostly depends on preanalytical steps, which are not clearly defined. There is a need for increased specificity and sensitivity when performing detection. The literature is confusing due to study results that cannot be compared with each other. Therefore, liquid biopsy analysis is an expensive and risky method due to the detection of very small amounts of fragile material. However, with the development of technologies and awareness, this detection method can become a reliable part of daily practice.

Cancer diagnosis continues to rely on histopathological analysis using tissue biopsies, and liquid biopsies are used for special situations, e.g., drug resistance and treatment monitoring, but mostly for research. Robust and standardized studies targeting large groups of patients are needed to develop clinical practice recommendations. In the future, liquid biopsy is expected to become part of routine practice with all of its benefits, but more time is needed.

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