The Pathology Report

16

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16.1 Introduction

An accurate pathological assessment of core biopsies or resection specimens provides important information on the major features of breast cancer, such as tumor type, size, biological characteristics, lymph node status, stage, and extent of residual disease in case of neoadjuvant chemotherapy, and is crucial for ensuring an appropriate patient management. In the era of molecular medicine and tailored therapies, the pathologic assessment of primary tumor still represents an essential guide for oncologists and surgeons to inform the choice of the best treatment options available for individual patients. Therefore, the management of patients with breast cancer detected through imaging or symptomatic presentation depends heavily on the quality of the pathology service.

Pathologists deal routinely with breast cancer samples, either as surgical resection specimens (both intraoperatively and after fixation and embedding) or as core biopsies and fine needle aspiration cytologies for the preoperative diagnosis of primary tumor or of distant metastases. The foremost means of communication with treating physicians, surgeons, radiologists and radiotherapists (and ultimately the patients) is represented by the pathology report.

Pathology reports may look different in appearance, at the discretion of each specific pathologist, taking into account clinician preferences, institutional policies, and individual practice, but it is mandatory they provide all clinically relevant information. An accurate and detailed pathology report of breast cancer, in addition to the histopathological diagnosis, must include all the prognostic parameters derived from the morphological examination and the immunohistochemical and molecular assessments and the predictive parameters useful for evaluating the efficacy of local and systemic treatments. In this regard, several guidelines for pathological reporting have been issued in the past years, and the most widely used are those of the Association of Directors of Anatomic and Surgical Pathology (ADASP), the College of American Pathologist (CAP), and the Royal College of Pathologists (RCP). These recommendations are drafted as a sort of checklist, a framework to assist pathologist in the completion of an exhaustive pathology report, encouraging healthcare professionals to use common terminology and definitions for breast diseases, and to harmonize the way of classifying breast cancer. In the following sections we will discuss general principles of specimen handling and sampling, as well as all principal parameters to be included in the pathology report, focusing on prognostic and predictive markers.

16.2 Pathology Request Form

An efficient multidisciplinary approach to patient care implies a precise exchange of information among different health-care professionals. Therefore, after the diagnostic procedures or the surgical intervention, any individual specimen submitted to the pathology laboratory should be accompanied by a comprehensive request form, providing the pathologists with all clinically relevant information, inclusive of:

- Patient personal data and demographic information, including name, surname, date of birth, sex, and ethnicity.
- Type of specimen, such as fine needle aspiration cytology; core biopsies; vacuum-assisted biopsy (VAB); lumpectomy and mastectomy, with or without locoregional lymph nodes; and number of specimen containers submitted, identifying each separately.
- Date and time of surgery.
- Clinical history and previous findings, including breast laterality, number and size of lesions, location within the breast, imaging data (mammography, ultrasound, MRI),

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history of previous malignancies, neoadjuvant therapy, including comments on clinical or radiological response. Drawings can be very helpful.
- Previous biopsy or cytology results for each lesion, with relevant details and laboratory of origin, whenever available.
- Method of localization used.
- In resected specimens, drawings or description indicating the position of the orientating clips/sutures. Surgeon should orientate all breast cancer resection specimens. Each breast unit should establish a code of orientation, using either different lengths or number of sutures and/or metal clips or ink. The code should be anatomically relevant and assist in accurate evaluation of the specimen and its margins.
- Whether any relevant marker (most frequently microcalcifications) was identified on imaging of the specimen, if performed.
- For axillary specimens: whether a sentinel lymph node (specifying if an intraoperative assessment is requested or a routine analysis on formalin-fixed, paraffin-embedded sections), a lymph node sampling or a completion axillary dissection (indicating levels dissected) is submitted for pathological examination.

16.3 Specimen Handling and Sampling

Surgical specimens must be handled to ensure good preservation of all the morphological and biological characteristics of the tumor cells. Inadequate fixation may cause extensive morphological artifacts, loss of tissue antigenicity, and degradation of nucleic acids (especially mRNA), making specimen not suitable for a reliable assessment of prognostic and predictive parameters (i.e., hormone receptors, HER2, Ki-67 labeling index, and molecular analysis).

After surgical removal, specimen should be sent immediately to the pathology lab. According to local policies, pathologists may dissect the specimens either on fresh state or after formalin fixation, but in any case a prompt and accurate fixation must be ensured, thus preventing tissue autolysis. In case of delayed sampling, the specimens should be immediately placed in an adequate volume of fixative, at least ten times that of the specimen, and cut through the tumor from the fascial plane toward the surface of the sample, to ensure adequate penetration of fixative into the tumor tissue, especially in large and fatty mammary glands. Refrigeration and vacuum packing may also be helpful in delaying autolysis. The American Society of Clinical Oncology/College of American Pathology (ASCO/CAP) guidelines advocate promptly placing the breast specimens into fixative within 1 h after surgical removal to minimize cold ischemia time and maintaining the samples in 10% neutral buffered formalin (NBF) for 6 h to 72 h, for ensuring best preservation of tissue antigenicity for assessment of HER2 and hormone receptor status [1, 2].

In samples of non-palpable lesions, intraoperative radiography of the specimen or macroscopic examination by a pathologist is particularly useful to confirm the success of the excision procedure. This is also highly recommended for wide local resections (quadrantectomies or lumpectomies), to allow confirmation of the presence of the abnormality and of its location in the specimen, thus facilitating immediate re-excision if the lesion is close to or involving a margin.

Once received in the laboratory, pathologists should examine the specimen, recording the type of excision, its dimensions along the three spatial axes, the presence or absence of skin, and/or nipple and axillary tissue. Relevant surgical margins or the entire specimen surface should be inked so that the margins of excision can be easily determined histologically. Therapeutic surgical procedures, as quadrantectomy or mastectomy, according to Surgical Guidelines for the management of breast cancer, usually require tissue removal from the subcutis to the pectoral fascia, which are considered anatomical planes rather than surgical margins. However, in case of central excision, breast tissue remains at the superficial (close to the nipple-areola complex) surface, requiring careful margin assessment. Therefore, it is important for the pathologist to be aware of the type of excision, in order to manage surgical margins properly.

Afterward, pathologist should slice the specimen at intervals of approximately 3–5 mm, preferably along sagittal planes, enabling easy X-ray mapping of the specimens in case of non-palpable lesions with calcifications or tissue distortion, in order to ensure high-confidence localization. The sampling technique, however, may vary according to the type and size of the samples and also according to local protocols or pathologists' preferences; therefore, some degree of flexibility is allowed. The number of blocks of invasive tumors to be prepared for microscopic examination can vary with tumor size, but it is usually of at least three blocks per tumor nodule. The peritumoral tissue should also be submitted for histology to identify associated DCIS, peritumoral lymphovascular invasion, and to allow surrounding normal breast tissue to be used as an internal immunohistochemical control for the assessment of hormone receptor and HER2 status. It may be possible to sample the lesion and its adjacent radial margin in one block in case of very small lesions, but in the vast majority of the cases, resection margins must be examined in several blocks. Particular attention must be paid to the areolar margin, due to high gland density and possible tumor extension

in lactiferous ducts, particularly for DCIS. In mastectomy specimens, sagittal sections of the nipple should be taken to exclude Paget's disease, while a coronal section of nipple and retro-areolar tissue is recommended to assess possible nipple duct involvement by DCIS.

For DCIS specimens, the number of blocks sampled is variable according to the size of the specimen and of the lesion. For small specimens, especially when radiologic assessment is unavailable, sampling of the entire tissue is recommended. For larger specimens, the pathologist should sample representative blocks (at least one block for each centimeter of the lesion) from the entire involved area, to scrutinize the sample for any possible area of invasive carcinoma, and including the site of any previous core biopsy.

Ultimately, details of the macroscopic features of the specimen must be recorded, especially tumor size and distances to all margins. In the presence of multiple tumors, the distance between tumors themselves and between each tumor and resection margins should be recorded. It is recommended to sample the tissue between tumor nodules, to ascertain if the neoplastic foci are truly separated (multifocal or multicentric tumors) or instead interconnected. The axillary tail of the specimen should be inspected for the presence of intramammary or low axillary lymph nodes.

Neoadjuvant systemic therapy is frequently administered to patients with large, locally advanced, or inflammatory breast cancers, with the aim to reduce the tumor size allowing breast-conserving surgery and tumor downstaging. It also provides the opportunity to assess response to treatment after a reasonably short time of exposure to the treatment (see related chapter). However, significant difficulties and variability exists in methods for pathologic assessment of response to neoadjuvant therapy. Recently guidelines issued by the Breast International Group-North American Breast Cancer Group (BIG/NABCG) [3, 4] recommend practical methods for a standardized pathologic assessment of the breast specimen following neoadjuvant therapy. Briefly, it is mandatory to identify macroscopically the tumor bed before any sampling and to record the two axes of the largest cross section of the entire area involved. Obvious remaining tumor should also be measured. It is strongly recommended that an image of the sliced specimen be taken (photograph or drawing) and then used to create a map of the carefully oriented tissue blocks collected. This will allow pathologists to obtain an accurate and comprehensive histological image of residual tumor, ultimately assuring a precise assessment of residual disease and staging. Extent of sampling should be determined by the pretreatment tumor size; an entire cross section of the tumor bed taken for each cm of the pretreatment tumor size (for a total number of approximately 15 blocks in most cases) should be sufficient to reliably document the pathological response.

16.4 The Pathology Report: A Synopsis

The following are the main parameters that should be carefully evaluated and clearly reported in the pathology report. Their assessment methods and clinical relevance will be briefly discussed.

16.5 Tumor Type

Breast cancer is considered a heterogeneous disease, made up of several different subtypes with variable morphological and biological features, different prognosis and response to systemic therapy. WHO histopathologic classification is based on characteristics seen upon light microscopy of biopsy specimens [5]. Two most common histopathological types collectively represent approximately 70–80% of breast cancers, namely, invasive ductal carcinoma, no special type (IDC NST) or invasive lobular carcinoma (ILC).

Among less common tumor histotypes, some "special" tumor types are per se associated with intrinsically peculiar prognostic profile. Tubular and cribriform carcinomas, for example, are characterized by an almost indolent clinical course with an extremely good overall survival [6], and the adenoid cystic carcinomas carry a very favorable prognosis in the vast majority of the cases [7]. On the contrary, metaplastic carcinomas are associated with significant worse clinical outcome then the IDC NST [8].

The fact remains, however, that for the vast majority of breast cancer (IDC NST and ILC), morphological classification is unable to meaningfully reflect the vast heterogeneity in terms of biological features, prognosis and response to systemic therapy, failing to assist the oncologist in planning adequate systemic treatment. It is also arguable if IDC and ILC do actually reflect clinical differences, and whether ILC per se constitutes a prognostically favorable group [9, 10].

16.6 Tumor Size

An accurate measurement of tumor size is mandatory, as it represents the first parameter of breast cancer staging. TNM classification still represents one of the most powerful prognosticators in breast cancer [11], being statistically correlated with risk of recurrence, metastatization, and overall survival. Identification of the tumor edge is also a prerequisite for a reliable assessment of resection margin status.

The maximum dimension of invasive tumors should be measured macroscopically, paying attention to irregularly shaped or multi-lobulated lesions. When tumor measurement is not feasible, then the tumor size identified by imaging, based on ultrasound, mammography, or MRI, should be used

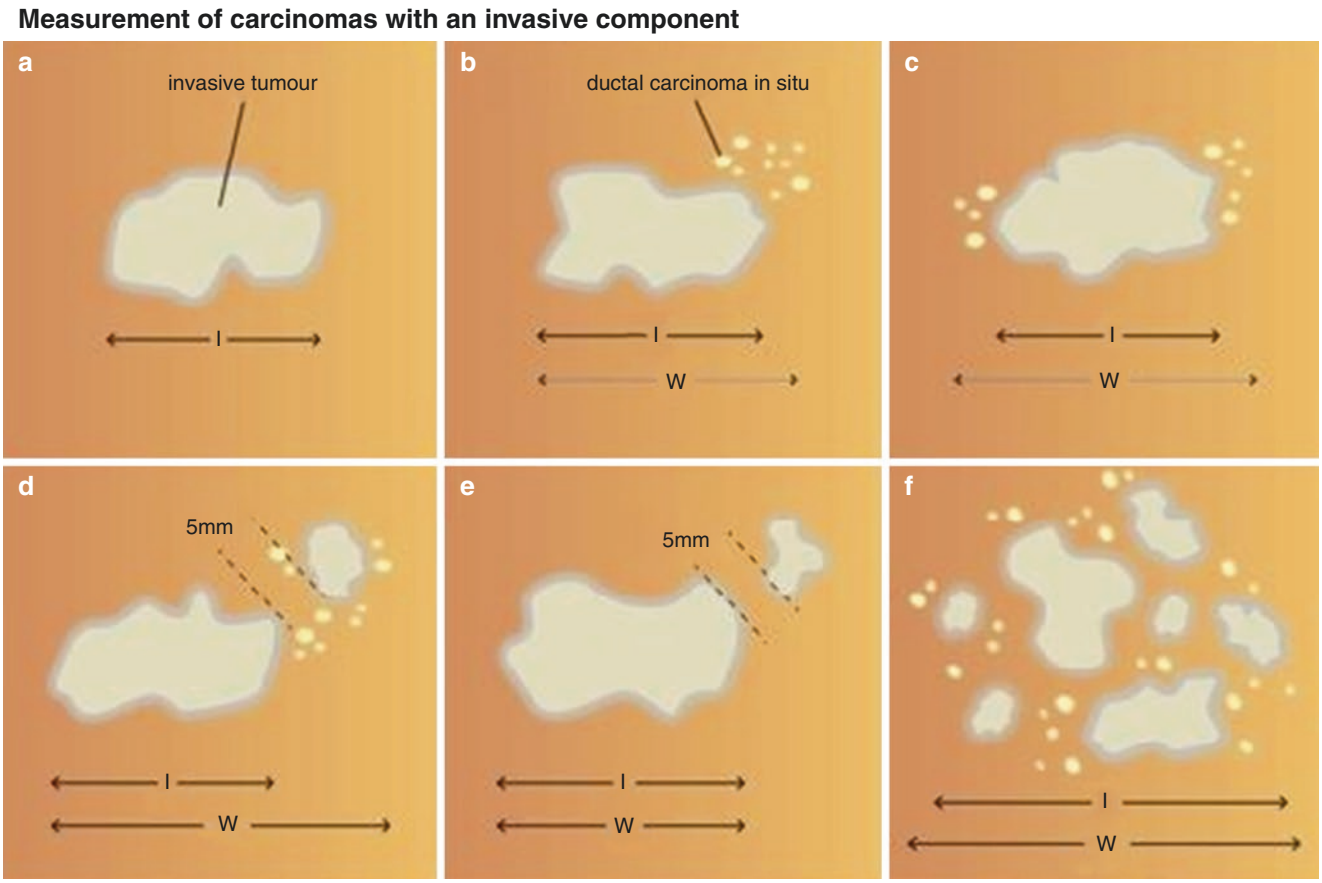

Fig. 16.1 Illustrations of how to measure invasive and whole tumor sizes in various scenarios [12]

as the best available record of true tumor size, replacing pathological size assessment. In case of discrepancy between the macroscopic and the microscopic size occurs, then the latter should be recorded, provided that the plane of the maximum dimension of the lesion has been included in the slide. The assessment of the whole tumor size including in situ carcinoma should be recorded, reporting also relative percentages of invasive tumor and DCIS. In tumors composed predominantly of DCIS but with multiple foci of (micro)invasion, measurement of the invasive tumor should correspond to maximum axis of the area occupied by invasive foci, as shown in Fig. 16.1, along with other frequent scenarios.

On rare occasions, pathologists may find it challenging to determine whether two adjacent tumor foci represent satellite foci or one lesion mimicking this process due to plane of sectioning. In this regard, the presence of intervening normal tissue and increasing distance between foci are features suggesting that these are more likely to be multiple foci than a single process. A distance of 5 mm or greater is often used to define separate foci. In case of clear-cut distinct multiple tumor masses, pathologists should record if the neoplastic foci are in the same breast quadrants or at a distance of less than 5 cm (multifocal tumors) or in different quadrants or at a greater distance (multicentric tumors).

16.7 Histological Grade

Invasive carcinomas are routinely graded, and grade is now widely recognized as a powerful prognostic factor, significantly associated with clinical outcome [13–15]. Assessment of histological grade has become more objective with modifications of the Patey and Scarff [16] method first by Bloom and Richardson [17] and more recently by Elston and Ellis [18]. Histological grading involves the assessment of three components of tumor morphology: tubule/acinar formation, nuclear atypia/pleomorphism, and number of mitoses. Each parameter is scored from 1 to 3, and the sum gives the overall histological grade as follows: Grade 1 (well differentiated) = scores of 3 to 5, Grade 2 (moderately differentiated) = scores of 6 to 7,

and Grade 3 (poorly differentiated) = scores of 8 or 9. Below are briefly discussed criteria involved in tumor grading:

- *Tubule/acinar formation*: all tumor area should be scanned, assessing semiquantitatively the proportion occupied by tubule formation. This assessment is generally carried out during the initial low-power scan of the tumor sections. Tumors showing >75%, 10–75%, or <10% of tubule formation are scored 1, 2, or 3, respectively.
- *Nuclear atypia/pleomorphism*: assessed comparing tumor nuclear size and shape with normal luminal cells. This is the parameter mostly affected by interobserver variability; breast specialist pathologists seem to report higher grades than nonspecialists [19].
- *Mitoses*: accurate mitotic count requires optimally fixed and processed specimens. Mitoses should be counted in ten high-power fields (40× objective). The mitotic score is dependent
- on the high-power field diameter; in this regard tables of conversion with different scoring tiers according to the actual field diameter of the microscope are available. At least ten fields should be counted at the periphery of the tumor, where it has been demonstrated that proliferative activity is greatest [20, 21]. If there is variation in the number of mitoses in different areas of the tumor, the area with the highest mitotic count should be taken into account. If the mitotic score falls very close to a score cut point, additional ten high-power fields should be evaluated, assigning the highest score.

In core biopsies, notwithstanding paucity common low cellularity of the samples, assessment of grade is recommended, especially if the patient is a candidate to neoadjuvant treatment. There is about 70% agreement of grade on core biopsy with the corresponding surgical specimen [22, 23]. If both core biopsy and surgical specimen are available, grading should be scored on the latter. Assessment of grade in the surgical specimens after neoadjuvant systemic therapy may be unreliable, due to the effect of the cytotoxic drugs on the morphology and the mitotic index of the tumor cells.

16.8 Peritumoral Lymphovascular Invasion

Lymphovascular invasion (LVI) mirrors the ability of cancer cells to invade lymphatics and blood vessels, and it is correlated to a higher likelihood of nodal or distant metastases. LVI in a peritumoral location is unanimously regarded as an important prognostic factor in patients with lymph node-negative invasive breast cancer, providing independent information about both local recurrence and survival [24–26]. It is therefore important to record in the pathology report whether or not it is present. Given the difficulties in the morphological distinction between lymphatics and blood vessels, findings should be categorized as "lymphovascular spaces" rather than as specific channels. This is supported by evidence identifying that most tumor emboli are present in lymphatic channels.

At the microscopic level, stromal retraction artifact around neoplastic cell nests can mimic vascular invasion; therefore, a clear rim of endothelium should be identified. Other clues in recognizing lymphovascular invasion is the presence of nearby vascular channels or the location of tumor cells within spaces with erythrocytes and/or thrombi

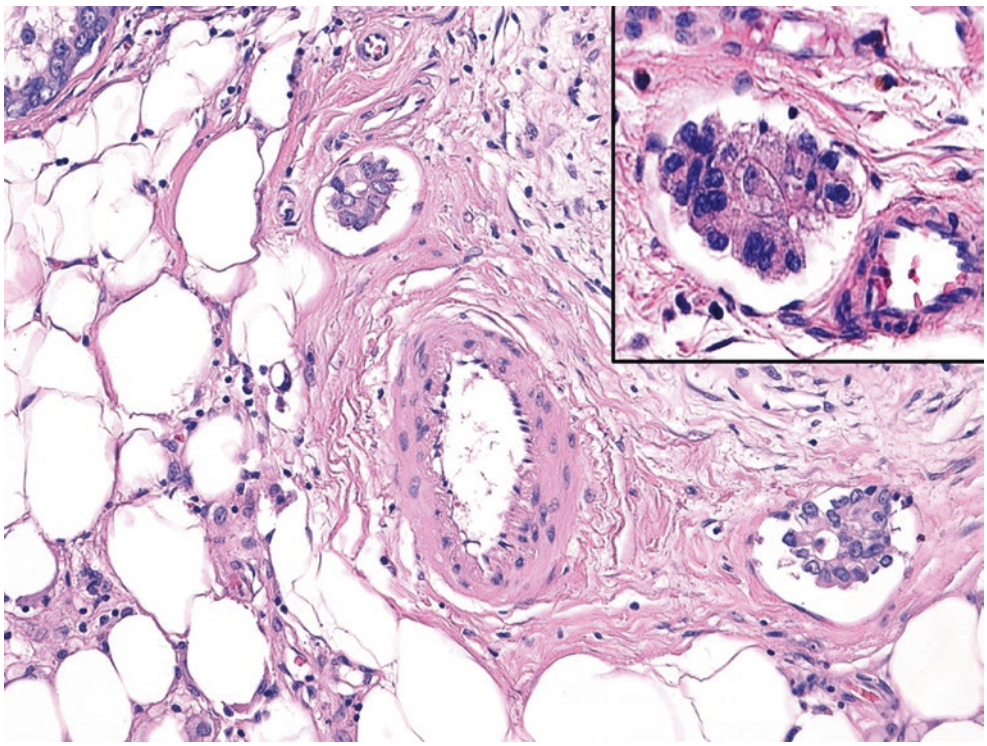

Fig. 16.2 An example of lymphovascular invasion

(Fig. 16.2). In difficult cases immunohistochemistry can be of help to identify the endothelial lining (CD34, CD31, and D2-40 antibodies) [27].

16.9 Surgical Margins Status

Breast-conserving surgery (BCS) in combination with adjuvant local and systemic therapies has become the standard of care for early small tumors, thus reducing physical and psychological morbidity. Despite these advantages, BCS has a higher risk of local recurrence than mastectomy [28–33]. The strongest predictor of local recurrence is surgical margin status [34–36].

According to the number of positive margins and the remaining amount of breast tissue, positive margins are managed with re-excision or mastectomy, eventually resulting in poor cosmetic outcome and high medical cost. Since early invasive cancer and DCIS nowadays represent a significant fraction of breast surgical specimens, the pathologic assessment of surgical margins is crucial, requiring close correlation between the surgical procedure and pathological examination. In particular, pathologists should be aware of the depth of tissue excised and whether the surgeon has excised all the tissue from the subcutaneous to the pectoral fascia. All distances between invasive cancer and DCIS should be recorded or, at least, the closest ones. According to current recommendations, margins are considered free of invasive tumor when the ink does not touch the tumor. For DCIS, margins are considered free when the closest tumor nest is 2 mm away from the surgical margin.

As previously mentioned, careful orientation of the surgical specimen is mandatory, as this prevents discordance with postoperative margin orientation, which occurs in 31% of surgeries [37]. In comparison to permanent histopathologic staining, intraoperatory pathological assessment of surgical margins may result in significant decrease of re-excisions. In particular, macroscopic examination, frozen section analysis, and imprint cytology have been demonstrated to be associated with re-excision rate of 3–11%, against an average 35% rate for permanent histopathologic staining-surgical margin assessment [38, 39].

16.10 Nodal Status and Sentinel Lymph Node Biopsy

Axillary node status is the most important prognostic indicator in breast cancer. Therefore, careful assessment of nodal status is mandatory. If axillary dissection has been performed, all lymph nodes must be carefully dissected and examined histologically. Pathology report should include the total number of lymph nodes identified and the number of involved lymph nodes, specifying whether macro- or micrometastases. Of note, nodes with isolated tumor cell only (<0.2 mm) are not considered positive for metastasis [40, 41].

However, axillary dissection is not infrequently associated with side effects, such as arm lymphedema, paresthesia, pain, and motor deficit. Moreover, with the implementation of screening programs, an increasing number of patients with node-negative early breast cancer were subjected to unnecessary axillary dissection. Hence, the need for a diagnostic procedure capable of discriminating patients for whom completion axillary dissection could be avoided. Sentinel lymph node biopsy (SLNB), a technique initially devised for penile cancer and melanoma, relies on the assumption that lymphatic spread of cancer cells occurs orderly and sequentially along the lymphatic drainage; hence, there must be a lymph node supposed to be the first metastasis recipient and from which the disease can subsequently spread to the remaining lymph nodes. Phase III clinical trials by Veronesi's and Giuliano's groups convincingly demonstrated that breast cancer patients with a negative SLNB could safely avoid axillary dissection [42–44]. Since these seminal works, SLNB entered the clinical arena, and pathologists put their efforts in defining the most accurate way for SLNB analysis, leading to a surprisingly variegated panorama, with no universally accepted protocols. Some institutions adopted an intraoperative frozen section assessment, to avoid a second surgery in case of a positive SLNB, using different protocols with regard to the number of sections examined and the cutting intervals; others adopted assessment of lymph node status on permanent sections, also using immunohistochemical stains, with the goal of achieving high sensitivity. More recently, intraoperative molecular assessment, using reverse transcription-PCR assays for cytokeratin-19 (one-step nucleic acid amplification, OSNA), entered the clinical practice [45–47]. The obsession to look for even minimal sentinel lymph node involvement, however, was eventually challenged by the evidence that patients with isolated tumor cells or micrometastasis only in the sentinel lymph node could be safely spared completion axillary dissection without any adverse effect on outcome [48]. This led to questioning the need for axillary dissection also for patients with small and clinically node-negative breast cancer, but histologically positive sentinel node. The ACOSOG Z0011 trial randomized 891 patients with clinically T1–2, breast cancer, and histologically positive SLNB, to undergo axillary dissection or not. When the ACOSOG Z0011 was initially reported with a median follow-up of 6.3 years, regional recurrence after SLND alone for women with 1 or 2 positive sentinel lymph nodes was surprisingly low (0.9%), and completion axillary dissection did not significantly reduce regional recurrence or improve survival [49, 50].

Further data analysis, with nearly 10 years of median follow-up, still showed a remarkably low regional recurrence rate of 1.5% for SLND alone [51].

Following the report of these results, completion axillary dissection is no longer recommended for patients with small early breast cancer undergoing breast-conserving surgery and whole breast irradiation, even in case of metastasis to one or two sentinel lymph nodes.

16.11 Biological Features of Breast Carcinoma

Adjuvant systemic therapy of breast cancer is mainly informed by the biological characteristics of the primary tumor, including hormone receptor and HER2 status, and the assessment of the proliferation fraction [52]. It is therefore mandatory that the final pathological report includes an accurate evaluation of these parameters.

As previously mentioned, a reliable assessment of these biological features requires an optimized pre-analytical phase, with proper fixation of the specimen. One of the most important steps for optimal testing is the choice of the block to be submitted for the assays: it should be taken at the invasive edge of the lesion, including normal breast parenchyma, and must be representative of the invasive component of the tumor. In bilateral breast cancer, samples from both tumors should undergo biological characterization, given the high frequency of phenotype discordances in bilateral cancer; for multifocal or multicentric disease, ideally all the different foci should be evaluated, but in the vast majority of cases they exhibit similar morphological and biological phenotype. A reasonable approach would be to assess first whether the different tumor foci show the same morphological features (i.e., tumor type and grade) or they are different. In the former case, it may be acceptable to assess hormone receptors, HER2 and proliferative index in only one nodule, whereas in the latter it is recommended to test all the foci that are morphologically different. In multifocal or multicentric disease, in case the nodule assessed for biological characteristic shows a triple negative phenotype, it is highly recommended to test further foci, seeking for clones with different biological features amenable to hormonal or targeted therapy.

16.12 Estrogen and Progesterone Receptor Status

ER plays crucial roles in breast carcinogenesis; it was first identified in the 1960s and used in breast cancer clinical management since mid-1970s. It is universally considered one of the most important biomarkers for breast cancer classification, as a primary indicator of endocrine responsiveness, thus guiding oncologist in planning patient treatment [53]. ER status has been shown to be the major determinant of breast cancer molecular subtype by gene expression profiling studies [54]. ER-positive tumors comprise up to 75% of all breast cancer patients [55] and are largely well differentiated, less aggressive, and associated with better outcome after surgery than the ER-negative ones [56, 57]. ER has been considered as the most powerful single predictive factor identified in breast cancer [58–60], given the fact that approximately 50% of patients with ER-positive disease benefit from endocrine therapy [61].

The panelists of the St. Gallen Consensus in 2009 suggested to consider positive for ER and progesterone receptor (PgR) those tumors showing at least 1% immunoreactive cells [62]. This definition has been subsequently endorsed by the ASCO/CAP guideline recommendations for ER and PgR immunohistochemical testing [63]. Reporting the actual percentage of neoplastic cells showing definite nuclear immunoreactivity has also been recommended, because the higher the number of positive cells the larger is the expected benefit from endocrine therapies. Scoring system taking into account also the staining intensity (like the H-score or the Allred score) is considered optional. The ASCO/CAP guidelines covered technical aspects of the pre-analytical and analytical steps of the immunohistochemical, interpretation, scoring, and reporting of the results, aiming to increase accuracy and reproducibility. One of the most useful recommendations to avoid false-negative results in ER testing is to evaluate systematically the immunoreactivity of the nonneoplastic breast tissue surrounding the tumor. Ductal and lobular luminal cells are invariably heterogeneous for ER and PgR immunoreactivity, whereas myoepithelial and stromal cells are invariably negative thus providing a built-in positive and negative control of the sensitivity and the specificity of the reaction (Fig. 16.3).

While in certain subsets of cases ER-positive tumors may be negative for PgR, conferring lower sensitivity to antiestrogen therapy, especially in the metastatic setting, the reversed phenotype (ER negative and PgR positive) is very rarely true. Almost all the cases with such an aberrant phenotype are due to a false-negative assay for ER or, less frequently, a false-positive assay for PgR, and the pathologists should be encouraged to repeat the test, possibly on a different block before rendering this unusual report.

16.13 HER2

Human epidermal growth factor receptor 2 (HER2)-positive [1] breast cancer (BC) accounts for 15–20% of early breast cancer, and it is characterized by an aggressive behavior and poor response to conventional chemotherapy (CHT) [64, 65]. HER2 drives tumorigenesis mostly through protein overexpression in his wild-type form and pathway hyperactivation. Cancer promotion by HER2 kinase domain activating

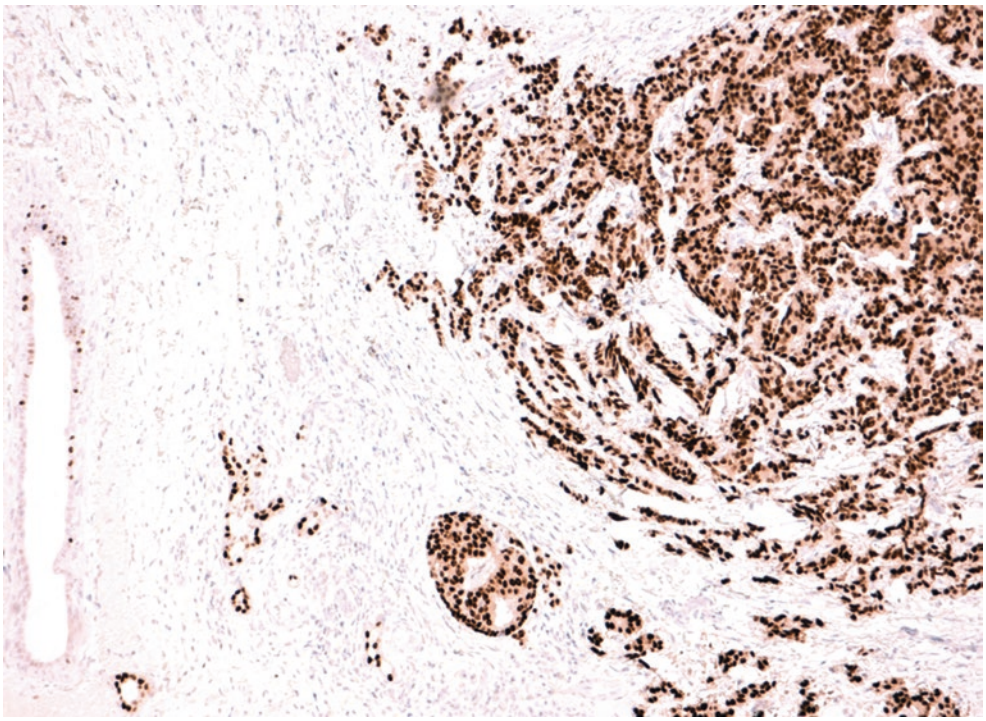

Fig. 16.3 Invasive ductal carcinoma NST showing diffuse (100%) intense positivity for ER. On the left a normal breast duct, showing heterogeneous staining

mutations has been rarely (3%) reported in the absence of protein overexpression [66–68]. The development and the clinical use of HER2-targeted therapies (antibodies and tyrosine kinase inhibitors) [69] led to a dramatic improvement of the outcome for patients with HER2-positive (HER2+) breast cancer [70–75]. HER2 pathways may be even more efficiently inhibited by combination therapies (dual blockade), as demonstrated in the metastatic and neoadjuvant setting [76–79], and currently tested within phase III randomized trials in the adjuvant setting [80]. Despite the efforts for standardizing HER2 testing, its reproducibility still represent a significant issue: central pathology review of locally assessed samples collected within prospective clinical trials reported concordance rates in the assessment of HER2 status by immunohistochemistry or in situ hybridization assays ranging from 77.5 to 96% [81–83]. In this regard, guidelines describing how to optimally perform the immunohistochemical (IHC) and in situ hybridization (ISH) assays for assessing HER2 status and evaluate and score the results have been issued and regularly updated [1]. Briefly, the 2013 ASCO/CAP guidelines define HER2-positive (score 3+) breast carcinoma as tumors containing more than 10% of cells with complete and intense circumferential membrane staining by IHC. ISH-positive breast carcinoma is defined as showing an average HER2 copy number ≥6.0 signals/cell or average HER2 copy number ≥4.0 signals/cell and a HER2 to chromosome 17 centromere (CEP17) ratio ≥2.0. Cases presenting weak to moderate circumferential membrane staining in more than 10% of tumor cells, or intense, complete and circumferential membrane staining in less than 10% of tumor cells should be classified as equivocal (score 2+) by IHC, while cases presenting HER2 to 17 centromere (CEP17) ratio <2.0 with an average HER2 copy number ≥4.0 and <6.0 signals per cell are considered equivocal by ISH. Equivocal cases require further assessment with the alternative assay or re-testing with the same assay of different tumor blocks or synchronous nodal metastases if available. Incomplete, faint, or barely perceptible membrane staining in more than 10% of tumor cells (score 1+) and no staining observed or incomplete, faint, or barely perceptible membrane staining in less than 10% of tumor cells (score 0) would confidently classify breast cancers as HER2 negative by IHC, while ISH-negative cases are characterized by a HER2 to chromosome 17 centromere (CEP17) ratio <2.0 with an average HER2 copy number <4.0 (Fig. 16.4).

16.14 Ki-67 Labeling Index

Tumor proliferation is one of the most powerful tools in breast cancer prognostication. The protein identified by the Ki-67 antibody is expressed in all proliferating cells during late G1, S, G2, and M phases of the cell cycle, peaking in the G2-M phases. In clinical practice, tumor proliferative fraction is most commonly assessed by the immunohistochemical staining of the Ki-67 antigen, using the MIB-1 monoclonal antibody [84].

The prognostic and predictive value of tumor proliferation has been extensively investigated in both the neoadjuvant and adjuvant settings [85, 86] and has been corroborated by gene expression analysis and molecular prognostic signatures, whereby the identification of intrinsic breast cancer molecular subtypes (i.e., Luminal A vs. Luminal B) or the distinction between aggressive or more indolent tumors

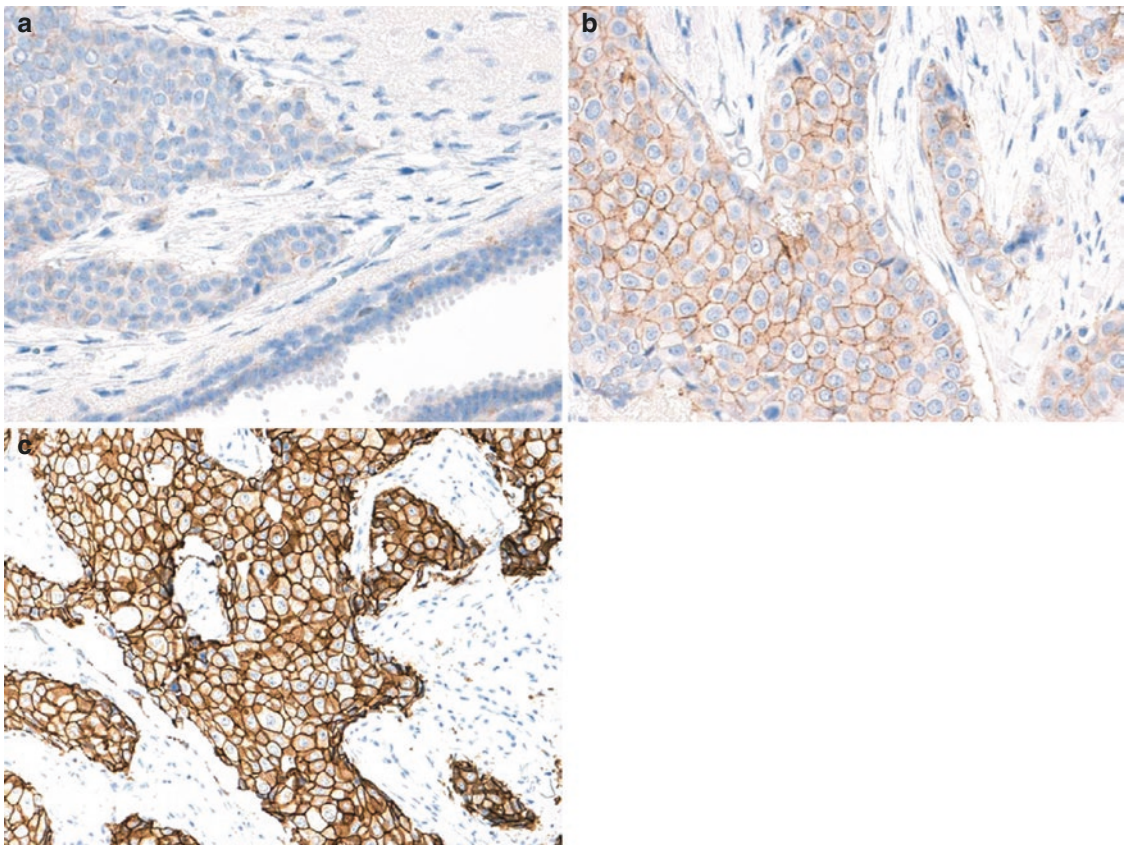

Fig. 16.4 Immunohistochemistry for HER2. Score 1+ (**a**); score 2+ (**b**); score 3+ (**c**)

relies mainly on proliferation-related genes [54, 87–92]. Therefore, accuracy of Ki-67 scoring is still a crucial issue, and huge efforts had been spent in improving consistency and in identifying the ideal cutoff value.

In 2010, the panelists of the International Ki-67 in Breast Cancer Working Group met in London and issued comprehensive recommendations aiming to achieve a harmonized, reproducible, and accurate methodology. Substantially, they suggested to assess Ki-67 labeling index on full-face sections, in at least three high-power (×40 objective) fields after an initial overview of the whole section, scoring at least 500 malignant invasive cells, preferably at the invasive edge of the tumor [93].

In their pivotal study, Cheang and colleagues [94] showed that a 14% cutoff was reliably able to discriminate tumors belonging to the Luminal A and Luminal B molecular subtypes. They found a Luminal A prevalence among ER-positive samples of approximately 60%. On the contrary, applying this same cutoff, other authors found an opposite prevalence of Luminal A and Luminal B cases. Consequently, at the 2013 St. Gallen Conference [95], the 14% cutoff for Ki-67 was challenged, and the majority of the panelists proposed to raise it to 20% for a better subclassification of luminal tumors. At the same time, Prat and colleagues [96] suggested to include high PgR expression (>20%) as an additional parameter for identifying the Luminal A subtype, along with ER positivity, HER2 negativity, and <14% Ki-67 labeling index. Maisonneuve et al. tested these new parameters in 9415 ER-positive and HER2-negative early breast cancer patients, treated between 1994 and 2006 and followed up at the European Institute of Oncology in Milan [97]. According to the 2011 St. Gallen criteria (Ki-67 cutoff of 14%), they found that 33% of the tumors would have been classified as Luminal A and 66% as Luminal B. Using the 2013 criteria (Ki-67 at 20% and adding PgR with the 20% cutoff), 43% of the tumors qualified for Luminal A and 57% for Luminal B. Interestingly, distant disease-free interval of the patients with low-proliferating tumors (Ki-67 < 14%) was not affected by PgR. Conversely, patients with tumors showing an intermediate Ki-67 labeling index (between 14 and 19%) had a significant different outcome according to PgR status, suggesting to classify as Luminal A tumors with either low (<14%) Ki-67 labeling index or with an intermediate labeling index (14–19%) and PgR of >20%. Luminal B tumors would be defined by either high Ki-67 labeling index (20% or more) or an intermediate Ki-67 and PgR _20%. Using this definition, 52% of the 9415 tumors qualified for Luminal A and 48% for Luminal B, with a significantly different clinical outcome of the patients (HR: 1.75, 95% CI: 1.42–2.11), after adjustment for clinicopathological variables including pT, pN, tumor grade, peritumoral vascular invasion, menopausal status, and systemic therapy.

16.15 Epilogue

The histopathological and biological characteristics of breast carcinoma are essential parameters to inform the choice of the systemic treatments. Hence, the pathology report of breast cancer must be complete and accurate, and include all the relevant features of the tumor. Recommendations have been issued by several national and international organizations on how to best evaluate and report these features and are continuously updated. It is the responsibility of each individual pathologist to follow all the available recommendations and guidelines strictly. The role of the pathologists in the multidisciplinary approach to breast cancer patients cannot be overemphasized, and the pathology report is their most important contribution.

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