Prognostic and Predictive Factors in Breast Carcinoma

18

Simona Stolnicu

Regarding breast cancer, there are well-known clinical, pathological, and molecular prognostic and predictive factors documented by several studies especially within the last decades. From a pathological point of view, these factors need to be evaluated while examining a breast carcinoma, and it is the pathologist's important role to perform this in each case and to include this information in the final pathology report. Based on these factors, patients with breast cancer are divided into those with good prognosis and those with bad prognosis. Also, based on these factors, management is established in every case and the response to the treatment is estimated. Since the evaluation of these parameters is so important in breast pathology, it is necessary for the pathologist dealing with breast carcinoma cases to have experience in this field. Also, especially regarding the evaluation of the markers performed to classify a tumor from a molecular point of view, every laboratory performing these tests is responsible for providing accurate and reproducible results.

Prognostic factors are those parameters that provide information on tumor progression and outcome independent of systemic therapy, while predictive factors indicate the sensitivity or resistance to a particular type of therapy. Related to breast cancer, there are well-known clinical, pathological, and molecular prognostic and predictive factors documented by several studies especially within recent decades. From a pathological point of view, these factors need to be evaluated while examining a breast carcinoma, and it is the pathologist's important role to perform this in each case and to include this information in the final pathology report. Usually, the value of any of these prognostic and predictive factors is established after multivariate statistical tests.

S. Stolnicu, MD, PhD
Department of Pathology, University of Medicine and Pharmacy, Tîrgu Mureş, Romania

18.1 Clinical Prognostic Factors

18.1.1 Age

Age is a controversial clinical prognostic factor, as some studies have shown that in younger patients with breast cancer, prognosis is more limited, while other studies have shown that prognosis is more favorable, and still others that there is no correlation between age and prognosis.

18.1.2 Pregnancy

Breast cancer associated with pregnancy is clinically defined as a carcinoma diagnosed during pregnancy or in the first year postpartum, and literature insists on separating these two groups of patients. The incidence of breast cancer associated with pregnancy is 0.2–3.8% and about 15% in women under the age of 40 [1]. Traditionally, it has been thought that pregnancy is a factor that aggravates the prognosis of breast cancer. However, studies failed to demonstrate that pregnancy is an independent prognostic factor in breast cancer. Bad prognosis is, rather, related to the fact that pregnancy usually occurs in younger patients and breast tumors are more difficult to detect during pregnancy or breastfeeding, owing to the breast parenchyma edema (especially if the tumor is small in size), and consequently there is a delay in the diagnosis [2, 3]. A large proportion of patients diagnosed with breast carcinoma during pregnancy have already developed axillary metastases at the time of diagnosis and are at higher stage. Also, according to more recent papers, in a small group of susceptible patients, pregnancy can lead to the development of an aggressive form of breast cancer [4]. The management of such cases is greatly dependent on the patient's choice, together with a multidisciplinary team approach.

S. Stolnicu, I. Alvarado-Cabrero (eds.), *Practical Atlas of Breast Pathology*, https://doi.org/10.1007/978-3-319-93257-6_18

18.1.3 Bilaterality

Patients with breast cancer have an increased risk of developing such a tumor in the contralateral breast. The risk of developing a contralateral metachronous breast cancer is approximately 1% in the year after mastectomy, while the risk of bilateral synchronous breast cancer is 0.2–2% [5–9]. Synchronous breast cancer is an identified carcinoma within the first two months of primary tumor detection, whereas metachronous breast cancer is a mammary cancer detected more than 2 months after primary tumor diagnosis. The second tumor may be of *in situ* or infiltrating type. By introducing bilateral mammography and screening, the number of patients found to have synchronous breast cancer increased. Also, the frequency of contralateral carcinoma varies among studies because of the patient selection and diagnostic and grossing method. Parameters associated with primary breast cancer that can predict the risk of developing breast cancer in the contralateral breast are: age, tumor size, location, clinical stage, microscopic type and grade (lobular and infiltrating tubular carcinomas and grade 3 carcinomas in general are more commonly associated with bilateral tumors), multicentricity, family history of breast cancer, and association with Peutz-Jeghers syndrome. Some studies have shown that bilateral breast cancer is associated with a more limited prognosis, while other studies have shown that the presence of bilateral breast tumors does not change prognosis [10]. Some of the patients who develop bilateral breast cancer probably have a genetic predisposition.

18.1.4 Multicentricity

In routine practice, most breast carcinomas are diagnosed as unifocal, while a variable proportion is represented by multiple tumors (Fig. 18.1). Data available in the literature regarding the incidence, definition, morphological and molecular profile, treatment, and prognosis of multiple carcinomas are currently contradictory. The incidence of multiple breast carcinomas varies between 6.1% and 77%, due to differences in definition, inclusion/selection criteria, preoperative diagnostic methods (the incidence is 15% when detected with mammographic examination and 35% when detected with MRI and ultrasound), and differences in sampling methods and their correlation with preoperative radiological examinations used in different oncologic hospitals [11, 12]. More recent studies that histopathologically analyzed consecutive cases using the "wide section" method have revealed the presence of multiple foci in most breast carcinoma patients [13]. Traditionally, multiple carcinomas have been classified in two categories: multifocal and multicentric. These definitions were not applied in a uniform manner and these terms are sometimes used together, which can lead to confusion. Also, the distinction between multifocal and multicentric carcinomas was made using several criteria: topographic, histological pattern, and tumor origin. A delimitation between multifocal and multicentric carcinomas was also attempted by using an arbitrary distance between tumor foci. Other authors [14–17] used both terms together, without making a distinction between the two entities by avoiding "quantitative" delimitations. They considered breast carcinomas to be multiple when multiple invasive foci separated by benign breast tissue are seen, regardless of the distance between foci; topographic criteria and distance between tumor foci are considered by these authors to be parameters of debatable biological significance [13]. This definition suggests that, according to more recent studies, the morphology and molecular profile of multiple tumor foci are more important parameters to determine the prognosis than are the location and the distance between multiple foci within the breast.

The latest editions of AJCC and TNM systems define ipsilateral synchronous multiple breast carcinomas as the presence of at least two invasive tumor foci located within the same breast, macroscopically distinct, and assessable using clinical and pathological methods [18, 19]. The multiple foci should only be assessed in terms of their number, which should be reported between parentheses in the final pathology report. However, reporting the histological type, grade,

and molecular profile of each tumor focus is imperative, since multiple studies have demonstrated that there is a morphological and molecular heterogeneity among multiple tumor foci, and this should have an impact on management and prognosis [20, 21]. Although multicentricity does not constitute an independent prognostic factor in multivariate analysis, multiple breast carcinomas have a worse prognosis than unifocal ones, and this should be taken into consideration by members of the multidisciplinary tumor board when establishing the treatment [22].

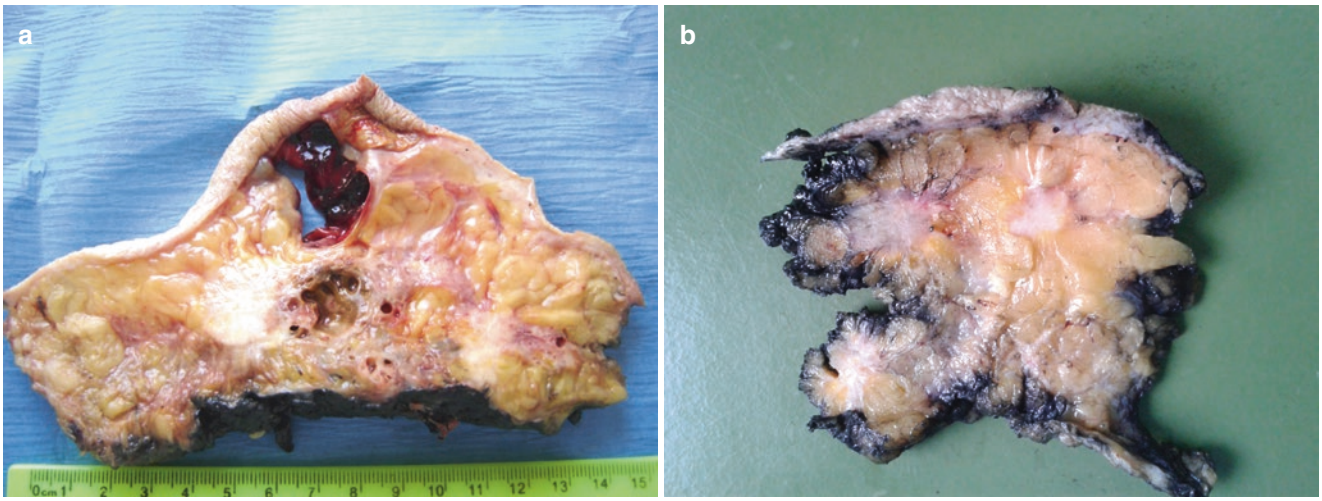

Fig. 18.1 Multiple breast carcinoma: (**a**) Mastectomy specimen with multiple grossly identifiable tumors with infiltrative margins, some of which are of cystic appearance while others are of solid type; (**b**) Quadranectomy specimen with multiple infiltrating breast carcinomas—the number of the tumor foci together with the distance of each focus from the surgical margins must be provided by the pathologist while grossing the specimen

18.1.5 Stage

Stage is an important prognostic factor, but it also serves to determine the type of treatment, and allows for the comparison of outcome results across institutions and national or international clinical trials. This is one of the numerous reasons why it is advisable that all the medical centers involved in diagnosis and treatment of breast cancer should use the same staging system. In 1954, the International Union Against Cancer (UICC) proposed the TNM system, a staging system for breast cancer based on the assessment of the primary tumor (T), regional lymph nodes (N), and distant metastases (M). Within this staging system, regional lymph nodes—axillary, transpectoral, and internal mammary—are taken into account. Therefore, metastases in these lymph node groups (as well as metastases to the intramammary lymph nodes) are considered metastases in N category, while all other metastases are considered distant metastases included in the M category. The TNM system consists of four stages (named I, II, III, IV, in ascending order of severity), and each stage comprises a group of tumors with a similar prognosis. The TNM stage may be clinical (cTNM), based on physical examination and a combination of radiological examinations, or pathological (pTNM), requiring the examination of the primary tumor tissue and regional lymph nodes. Of interest, the clinical and pathological TNM stage do not always correlate. After the UICC proposal in 1954, The American Joint Committee for Cancer Staging and End Results Reporting (AJCC) soon adopted a modified version of the TNM system. The TNM has undergone a number of changes over time, the latest of which was adopted in 2010 and provides more directions related to the specific methods of clinical and pathological tumor size measurement, clarifications of the post-treatment yT and yN classification that are determined after surgical procedure, clarification of the classification of isolated tumor cells and micrometastases in lymph nodes, and definitions of a new category of tumor cells microscopically detectable in bone marrow or circulating blood or found incidentally in other tissues with a size of less than 0.2 mm and without associated symptoms [18]. There are other staging systems for breast carcinoma, but they are not used as often internationally.

18.2 Pathological Prognostic Factors

18.2.1 Tumor Size

Tumor size is an important prognostic factor in that the smaller the size of the tumor, the better the tumor prognosis [23]. The bigger the tumor, the more likely it is to associate with axillary metastases [6]. The way of reporting the size of the tumor is very important, and it varies among pathologists and medical institutions. First of all, the tumor size is reported by the clinicians (through palpation during physical examination) and by the radiologists (using different methods such as ultrasound examination). Of note, all these methods provide information about the tumor; however, the pathological method is best method with which to measure the size. The clinical and pathological size may vary in up to 54% of cases, and a good correlation is needed in all cases.

The clinical and radiological measurements are usually reported in centimeters. The clinical method, utilizing palpation, also takes into account the fat tissue and the skin and using this method, the size may be overestimated. Some pathologists report the macroscopic size (during the grossing of the surgical specimen), others report the microscopic size, the one obtained by measuring the tumor tissue on the glass slide. In terms of macroscopic reporting, the tumor is measured in two dimensions, which is estimated in millimeters (this is done on the breast tissue sections during the grossing process, in the area where the pathologist considers the tumor to have the largest dimensions) (Fig. 18.2). After this assessment, the mammary gland sections are joined, and the third dimension is measured, also given in millimeters. If this dimension is greater than the first two (which is possible because breast carcinomas are not always round and symmetrical), this is the dimension that is reported, along with the next dimension (in descending order). Concerning the microscopic dimension, some pathologists only report the size of the invasive component, but others report the size of both the invasive and the *in situ* component, provided that the latter is situated at a distance of more than 1 mm away from the invasive tumor edge (Fig. 18.3). It has been shown, however, that for the prognosis of the tumor, staging, and management, the size of the invasive component is important; therefore, when there are discrepancies between the macroscopic and microscopic reported size, with regard to the invasive component, the final reporting must include the microscopically detected size. This parameter is considered when staging a malignant breast cancer according to pTNM. Tumor diameter reporting does not take into account vascular invasion foci. This method of measurement applies to unifocal tumors. The international guidelines recommend the use of the maximum diameter of the largest tumor focus in multiple carcinomas, rather than the sum of all diameters when reporting

the tumor in the final pathology report [18, 24]. However, the diameter of each tumor focus should be included in the pathology report, since it gives the oncologist an idea about the total volume of the tumor that should be treated; on the other hand, when staging, the largest diameter of the tumor foci should be used [22]. Also, according to the abovementioned international guidelines, these criteria do not apply to tumors with a single macroscopic focus associated with several separate, only microscopically detected, foci, which are called "satellite tumors." If both a macroscopic and a microscopic examination reveals a tumor spread over a variable area in the size of the "spider web" but with no distinct tumor mass, the tumor is called diffuse, and the size of the entire lesion is measured. Sometimes, especially in invasive lobular carcinoma, diffuse appearance involves the tumor only partially. In this case, if the diffuse aspect concerns less than 50% of the lesion, it is called the mixed tumor type. If a tumor was previously biopsied and preoperatively treated oncologically, the size of the tumor can no longer be determined while grossing or during microscopic examination. In these cases, the tumor diameter established on ultrasound or mammogram is considered in the final staging. As for *in situ* carcinomas, intraductal carcinomas are measured on the microscopic section (and if they form a palpable tumor, they are measured at grossing). The diameter of *in situ* lobular carcinoma is not measured.

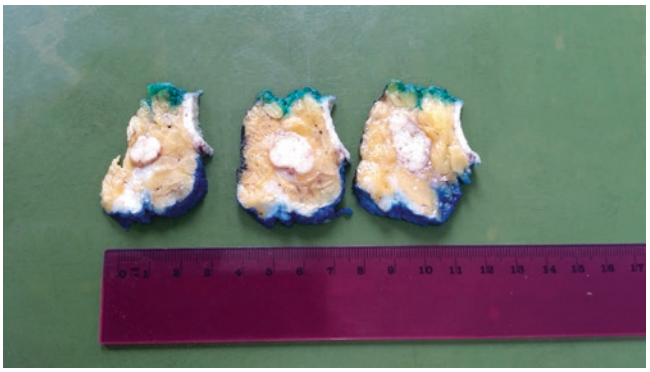

Fig. 18.2 The tumor size is measured during the grossing process and it is estimated in millimeters

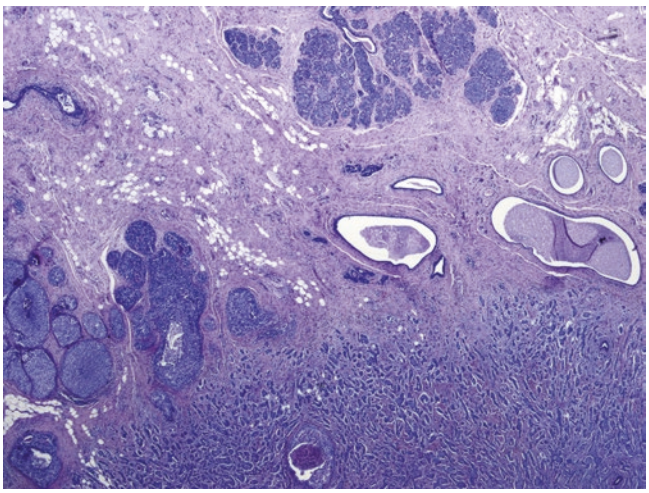

Fig. 18.3 Tumor size: both invasive and *in situ* components can be reported while measuring the size of the tumor, provided that the latter is situated at a distance of more than 1 mm away from the invasive tumor edge, as in this picture

18.2.2 Microscopic Type

Microscopic type is assessed by microscopically examining all the available sections from a tumor and applying the WHO classification [24]. However, establishing the microscopic type is subjective: There is a lack of agreement on diagnostic criteria or lack of good diagnostic criteria, and some pathologists do not recognize the mixed category. For a good assessment of the microscopic type, at least one tissue fragment per 1 cm of tumor is needed to be included in paraffin blocks and then examined under the microscope. Establishing the correct microscopic type is important because in some microscopic types of breast cancer, favorable prognosis has been demonstrated, such as in the following: invasive carcinoma of tubular type, cribriform type, mucinous hypocellular type, and adenoid-cystic type [23, 24]. As for medullary carcinoma, its prognosis is very controversial. Some authors claim that prognosis is better than no special type (NST) infiltrating carcinoma, others claim it is more reserved [24, 25]. In contrast, some microscopic types have an unfavorable prognosis, such as "signet ring" cell carcinoma, inflammatory carcinoma, and metaplastic carcinoma (some but not all of the subtypes of the latter category). Recent guidelines provide information about diverse management of different microscopic types of breast carcinomas (also, in correlation with other prognostic factors). It is advisable that the microscopic type of a breast tumor should be established using the WHO latest edition (2012) [24].

18.2.3 Microscopic Grade

Regardless of the microscopic type, invasive breast cancers are graded, and the microscopic grade is an important prognostic and predictive factor. According to the WHO 2012 criteria, the microscopic grade should be applied to all invasive carcinomas, as it provides important information about tumor prognosis [24, 26]. High-grade invasive carcinomas (grade 3) more frequently exhibit distant metastases and poor prognosis. This also applies to small tumors and even to those without axillary lymph node metastases. Also, the histological grade can provide information on the response to oncological treatment. In this respect, studies have shown that high-grade breast carcinomas respond to chemotherapy treatment better, and most cases with complete pathologic response to neoadjuvant chemotherapy are grade 3 tumors. Also, tumors of different microscopic grades show distinct molecular profiles, and there are studies suggesting that grade 1 and grade 3 tumors are two different diseases with different molecular origins, pathogenesis, and behavior [27, 28]. Currently, histological grade also remains an independent prognostic factor for ER-positive tumors. The grading system established by Patey and Scarff—later modified by Bloom and Richardson and more recently by Elston and Ellis in 1991—is used for grading invasive breast cancers [29]. This microscopic grade is established on microscopic examination by the pathologist and is obtained by adding three numbers representing the estimation of three different parameters: tubular formation (as an expression of glandular differentiation), nuclear pleomorphism, and mitotic activity. Each parameter is scored between 1 and 3 points as follows:

1. Formation of tubules:
   1 point: tubule formation in over 75% of the tumor.
   2 points: tubule formation in 10–75% of the tumor.
   3 points: tubule formation in less than 10% of the tumor.
2. Nuclear pleomorphism:
   1 point: nuclei with minimal variation in size and shape.
   2 points: nuclei with moderate variation in size and shape.
   3 points: nuclei with marked variation in size and shape.
3. Number of mitoses:
   1–3 points depending on the diameter of the microscopic field.

The three numbers are added up and a score is obtained to measure the microscopic grade as follows:

- Grade 1: 3–5 points (well-differentiated).
- Grade 2: 6–7 points (moderately differentiated).
- Grade 3: 8–9 points (poorly differentiated) (Figs. 18.4, 18.5, and 18.6).

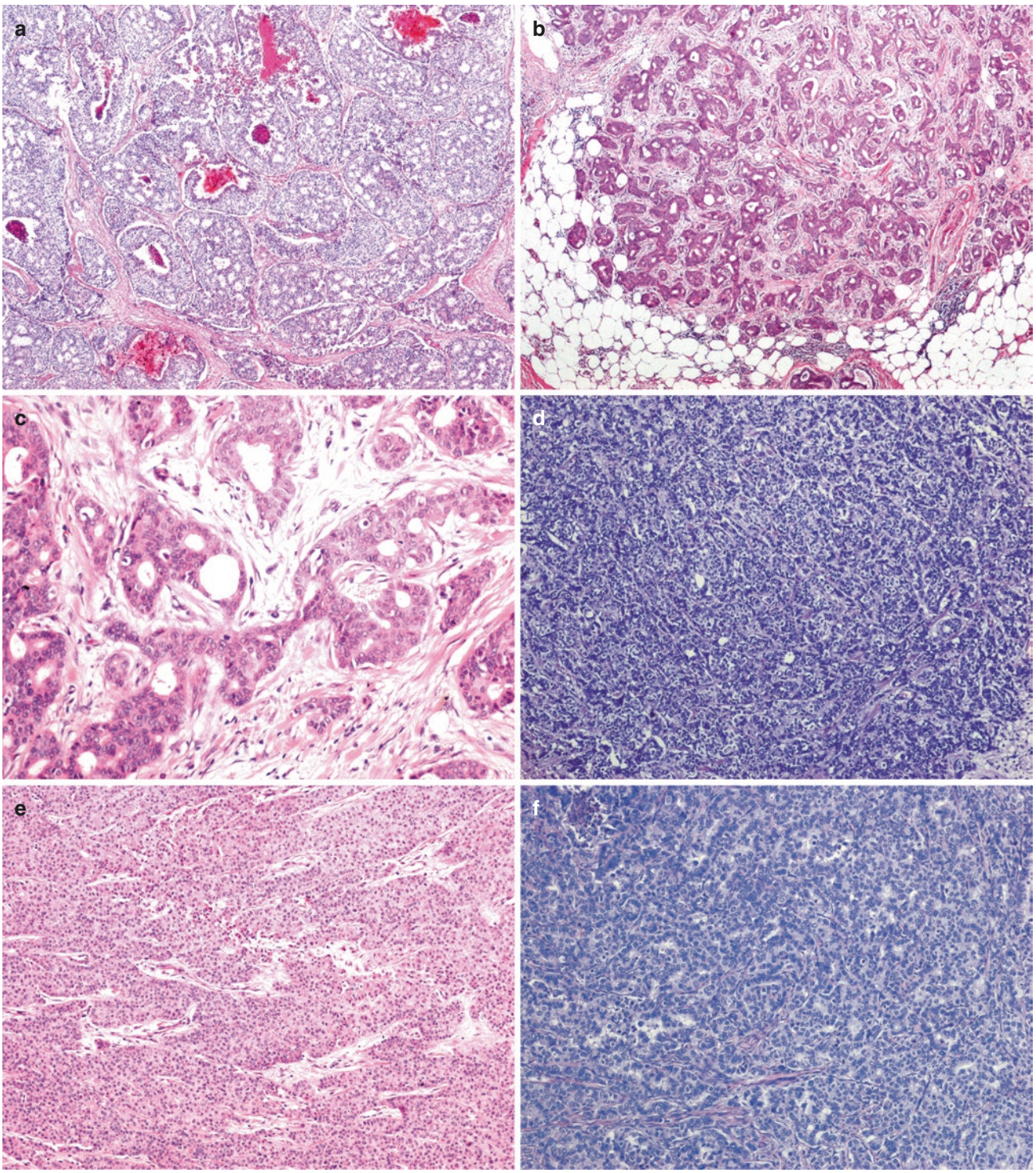

Fig. 18.4 Microscopic grade: (**a**) Tubule formation in over 75% of the tumor; (**b**) another example in which the tubule formation is identified in over 75% of the tumor—1 point; (**c**) same case as (**b**), but at high-power examination with open round lumina surrounded by polarized atypical cells with basal nuclei and apical cytoplasm around the spaces—1 point; (**d**) tubule formation in less than 10% of the tumor (3 points); (**e**) another case with tubules in less than 10% of the tumor (3 points); F, high power examination reveals presence of small round spaces but lacking an open round lumina surrounded by polarized atypical cells (pseudolumina)—3 points

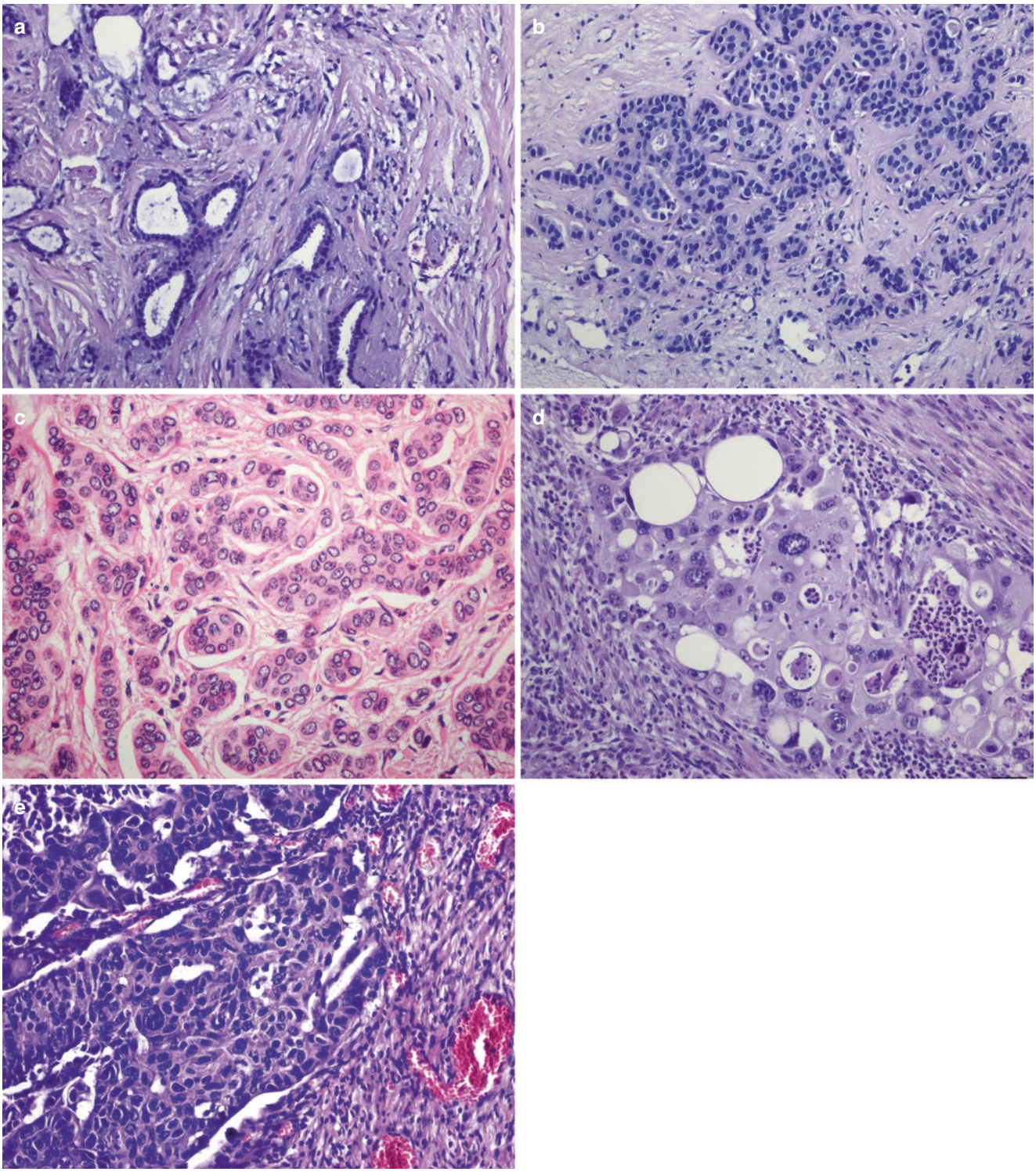

Fig. 18.5 Microscopic grade: (**a**) Nuclei with minimal variation in size and shape—1 point; (**b**, **c**) Nuclei with moderate variation in size and shape—2 points; (**d**, **e**) Nuclei with marked variation in size and shape—3 points

For reporting invasive breast carcinomas, it is advisable to use the term *grade* and specify which grade (1, 2, or 3), instead of *well*, *moderately*, and *poorly differentiated*. Grading is not done on frozen sections or on incorrectly fixed (especially delayed in fixation), cut, or stained sections. Grading can be done on biopsies, but due to the limited quantity of tissue, the ability to accurately identify the number of mitoses is also limited. This may lead to underestimation of the grade on such specimens, and it is advisable to repeat the grading on the surgical specimen. If for some reason the

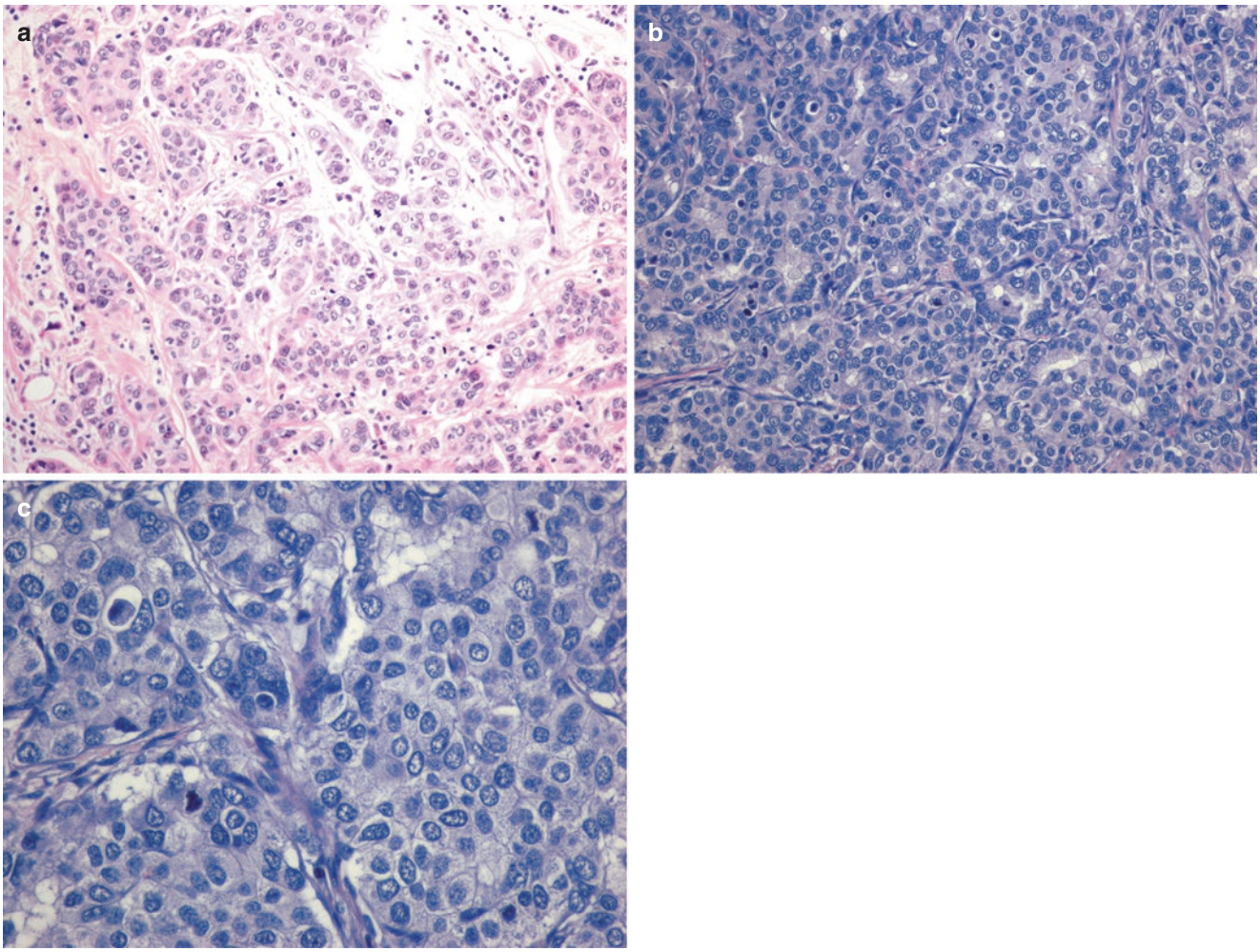

Fig. 18.6 Microscopic grade: number of mitosis will get, depending on the diameter of the microscopic 1 point (**a**) or 3 points (**b** and **c**)

section is not appropriate, it is advisable not to grade the tumor. A good relationship with the surgery department that provides the specimens, as well as training of the staff technicians in the pathology laboratory, are required to properly handle and fix the breast tissue. It is advisable that microscopic grading be done by two pathologists experienced in this field, and in case of inconsistency, grading should be established by consensus. With time and more experience, even one specialized pathologist can grade a breast tumor; however, it is recommended that the pathologist periodically re-check a sample of cases without knowledge of the previous result. Recurrences or tumors treated preoperatively are not graded. If there are multiple tumors present, they must be graded and reported separately. With respect to the formation of tubular structures, only those that have an open and obvious lumen in the center (and surrounded by polarized tumor cells) are considered, and their proportion is appreciated. By tubular formation, one must also take into account the acini, glands, and papillae. Tubular formation is appreciated by examining the entire tumor, including the most undifferentiated areas, on at least three sections from each tumor (it is advisable to examine 4–6 sections, depending on the size of the tumor). Areas of tumor necrosis are avoided. Tubular formation is appreciated on low-power examination. Nuclear pleomorphism is more subjectively appreciated and is done by comparing the appearance of tumor cell nuclei between them and in comparison with normal epithelial cell nuclei in the normal breast tissue adjacent to the tumor. If the normal breast is missing from the slide, then comparison with normal lymphocytes is useful. If one sees only focal pleomorphic nuclei, this should not automatically result in a score 3 for pleomorphism. The pleomorphic nuclei should be found in at least one quarter of the tumor before a score of 3 is allocated for this parameter [29]. Evaluating mitotic figures is even more difficult. Only atypical mitotic figures are considered, and they should not be confused with hyperchromatic nuclei, apoptotic bodies, pycnotic nuclei, or lymphocytes. The total number of mitoses per 10 high-power microscopic fields is calculated. Calculation is done at the periphery of the tumor (the area with the most common

mitoses), as well as in the most undifferentiated areas of the tumor, with attempts to assess this parameter also in the central areas within the same tumor (which is less mitotically active). Areas of necrosis are avoided. Of interest, breast carcinomas are heterogeneous, and some degree of variation may occur from one part of the tumor to another concerning the microscopic grade in general, but especially concerning the number of mitoses. To determine the number of mitoses, regardless of the type of the microscope used, it is necessary to determine the field diameter in advance. To obtain this diameter, the following formula is applied:

$$\text{Field diameter} = \text{number of the field} / \text{objective magnification} \times \text{intermediate magnification.}$$

The number of the field is set for each microscope by the manufacturing company. On each lens, regardless of the type of microscope, both lens and intermediate zooming are indicated. Once the field diameter is determined, the number of mitoses/ten microscopic high-power fields is calculated and then the score is evaluated using the chart provided in the NHS Breast Screening Program (1997) published by the ENHSBSP [23].

This method of appreciating the microscopic grade is applied for each microscopic type of infiltrating breast carcinoma. In mixed type of infiltrating carcinoma (in which two or more microscopic types are identified), each component is graded and reported separately.

Variants of infiltrating breast carcinoma (including the lobular infiltrating carcinoma) are graded according to the same method [29].

Some microscopic subtypes are always grade 1 (like tubular carcinoma) while others are always grade 3 (like medullary carcinoma). Metaplastic carcinoma raises most problems with grading. Squamous carcinoma is graded according to nuclear pleomorphism and cytoplasmic differentiation. In the case of this tumor, the grading system of infiltrating ductal carcinoma is not applied. Adenosquamous carcinoma can be low-grade or high-grade. Regarding adenoid-cystic carcinoma, the same grading system was proposed as in the case of a similar tumor with localization in the salivary gland. The grading system comprises three grades: grade 1 is characterized by the presence of glandular and cystic areas without a solid component; grade 2 contains solids that make up less than 30% of the invasive component; in grade 3, the solid component represents more than 30% of the tumor [30]. Another grading system for mammary adenoid-cystic carcinoma was proposed by Rosen in 1989 [31]. This grading has two categories: low-grade malignancies are characterized by a predominantly glandular or tubular appearance and the solid component is minimal or absent; high-grade malignant tumors are characterized by a predominantly solid appearance.

The microscopic grade set by Ellis and Elston is not perfect, and intraobserver disagreement has been reported by some studies. The subjective criteria are the main reason for reproducibility problems. However, subjectivity can be diminished and reproducibility can be improved with increased experience of the breast pathologist. Grading systems comprising only two grades may be developed in the future.

18.2.4 Vascular Invasion

Vascular invasion is an important prognostic factor. The presence of tumor emboli in vessels is associated with increased risk of metastasis in axillary lymph nodes, with increased frequency of local recurrences and with poor prognosis [32]. Also, the presence of both vascular invasion and lymph node metastases is associated with a worse prognosis than either alone. Of interest, it is important to identify which patients with breast cancer with free regional lymph nodes present vascular invasion, since this group of patients is at higher risk to develop distant metastases. No distinction should be made between the types of vessels (lymphatic or blood), as there is no prognostic significance. Most of the time, however, it cannot be established precisely whether the vessel is of the lymph or blood type, even if immunohistochemical examinations for Factor VIII, CD 31, CD 34, or Ulex europaeus are performed. As a rule, the presence of tumor emboli should be sought around and outside the tumor (at least 1 mm outside) (Figs. 18.7 and 18.8). It should be differentiated from tumor cell nests that are located within empty spaces inside the tumor and represent artefacts produced by stroma retrieval during technical processing (Fig. 18.9). Also, when the tumor cells fill the vascular spaces, it must be differentiated by nests of ductal carcinoma *in situ* (DCIS), which are surrounded by an outer layer of myoepithelial cells (which can be demonstrated with the use of myoepithelial markers). Care must be taken not to over-diagnose foci of micropapillary-infiltrating carcinomas as tumor emboli. To highlight the tumor emboli in the vascular space, it is necessary to visualize a layer of endothelial cells around the space. The presence of erythrocytes or thrombi inside the vascular lumen is helpful. Also, the adjacent presence of a venous or arterial lumen is useful. A useful criterion is the shape of the tumor emboli, which in general is not identical to the shape of the space around it. Inflammatory carcinoma demonstrates a particular aspect of this. In this case, the presence of tumor emboli in dermal lymphatic vessels establishes the diagnosis and, as a consequence, the breast skin becomes red, edematous, and warm. Of note, the presence of vascular invasion alone, not associated with residual tumor tissue after neoadjuvant chemotherapy, is resistant to treatment and is associated with a poor prognosis.

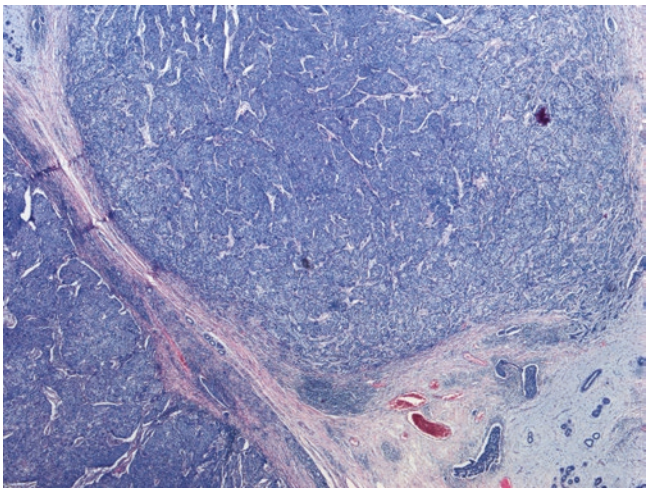

Fig. 18.8 Tumor emboli identified outside the infiltrative tumor margins

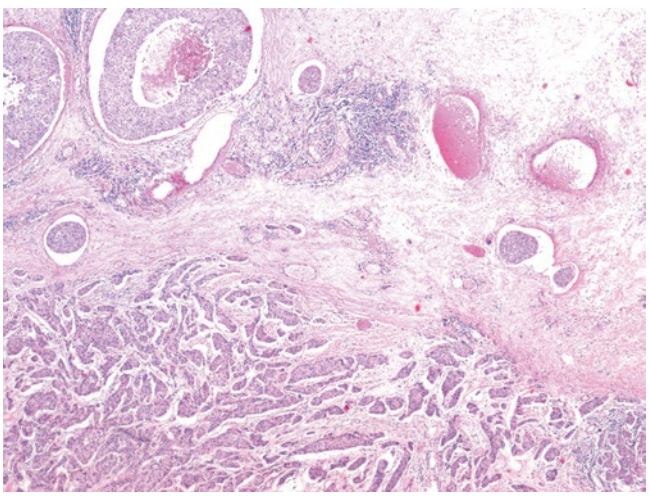

Fig. 18.7 The presence of tumor emboli should be sought around and outside the tumor (at least 1 mm outside)

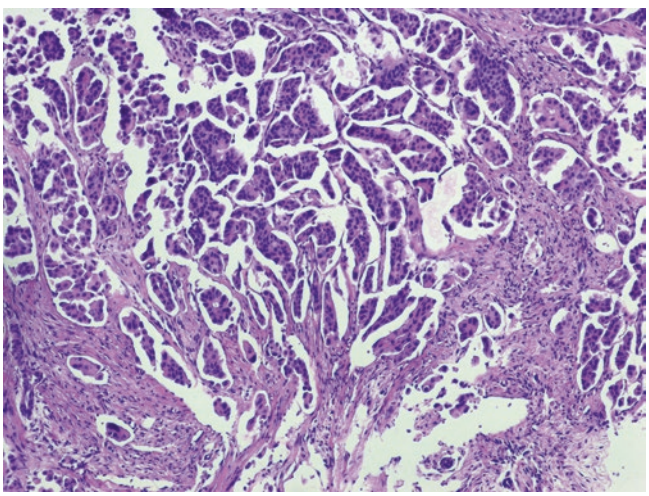

Fig. 18.9 Tumor emboli should be differentiated from tumor cell nests that are located within empty spaces inside the tumor and represent artefacts produced by stroma retrieval during technical processing

18.2.5 Tumor Necrosis

Tumor necrosis is associated with poor prognosis, lack of response to treatment, and early recurrence of the tumor process [33]. The presence of necrosis correlates with tumor size and microscopic grade. In contrast to some invasive mammary carcinomas in which necrosis is focally present, there is a category of such lesions in which necrosis is extensive, located centrally with a geographic-like shape and surrounded by a marginal tissue tumor at the periphery. This latter category is usually basal-like from a molecular point of view and triple negative, and it has a more aggressive prognosis, being frequently associated with pulmonary and cerebral metastases (Fig. 18.10) [34].

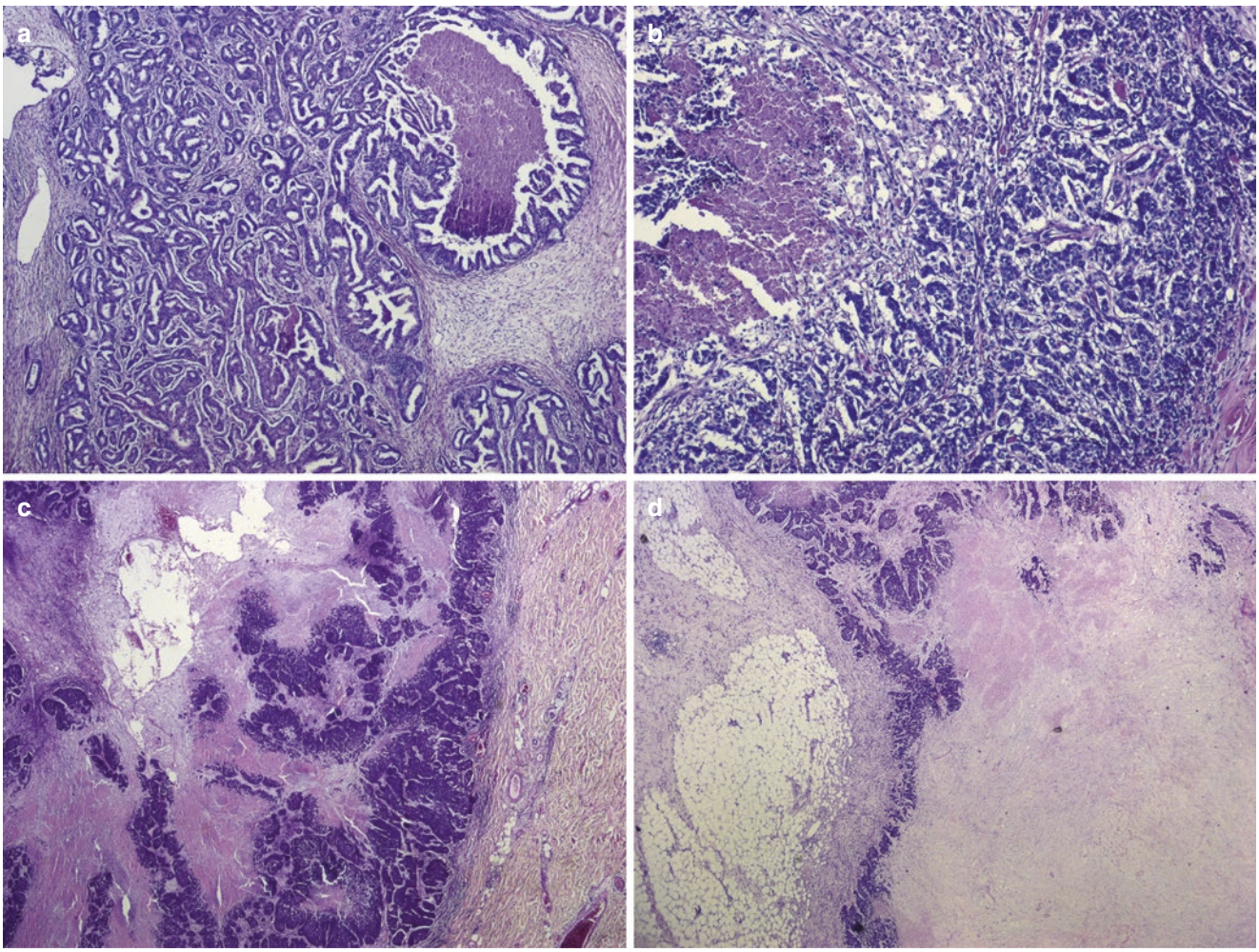

Fig. 18.10 Tumor necrosis: (**a**) Grade 1 invasive mammary carcinomas of NST type in which necrosis is focally present; (**b**) Larger area of necrosis in grade 2 invasive breast carcinoma of NST type; (**c**) Grade 3 infiltrating breast carcinoma of NST type with extensive necrosis; (**d**) Extensive necrosis located centrally with a geographic-like shape and surrounded by a marginal tissue tumor at the periphery in a basal-like triple negative grade 3 infiltrating breast carcinoma

18.2.6 Tumor-Infiltrating Lymphocytes

Several recent papers have evaluated the prognostic and predictive importance of the tumor-infiltrating lymphocytes in breast cancer. In 2013, a consensus meeting sought to provide recommendations for the evaluation of tumor-infiltrating lymphocytes in breast cancer, especially for the clinical routine practice, with a special focus on what area to examine in order to report tumor-infiltrating lymphocytes and how to score the presence of tumor-infiltrating lymphocytes [35]. The presence of tumor-infiltrating lymphocytes in the stroma is most often found in triple-negative, HER2-positive, and poorly differentiated breast carcinomas; also, its presence (and number of tumor-infiltrating lymphocytes) is associated with pathological complete response to neoadjuvant therapy, better disease-free survival, and better overall survival, being an independent prognostic factor [35–37]. The original method to evaluate this parameter was described by Denkert in 2010 [38]. The tumor-infiltrating lymphocytes should be reported for the stromal compartment as a percentage of the area of stromal tissue occupied by these cells over total intratumoral stromal area (as an average, not focusing on hot spots) (Fig. 18.11). As a consequence, tumor-infiltrating lymphocytes should only be evaluated within the borders of the invasive tumor component. The lymphocytes should be evaluated using a 20× or 40× objective on a Hematoxylin-eosin stained slide; immunohistochemical markers such as CD45, CD8, and CD3 can also be used (although there is no added value from these markers according to some studies) [35]. The international recommendations advise counting the stromal compartment (rather than the intratumor lymphocytes, that is, those cells in direct contact with nests of tumor cells) [35]. Also, it is advisable to avoid areas of necrosis, fibrosis (including biopsy-site) and crushing artifacts. Only mononuclear cells (lymphocytes and plasma cells) should be counted, while polymorphonuclear leukocytes, dendritic cells, and macrophages must be excluded. The counting should be done, if possible, on one full tumor section; core biopsies can be used only in the pre-treatment neoadjuvant setting. At this time there is no international clinical relevant tumor-infiltrating lymphocyte threshold (which vary between 50% and 60% stromal lymphocytes) [35].

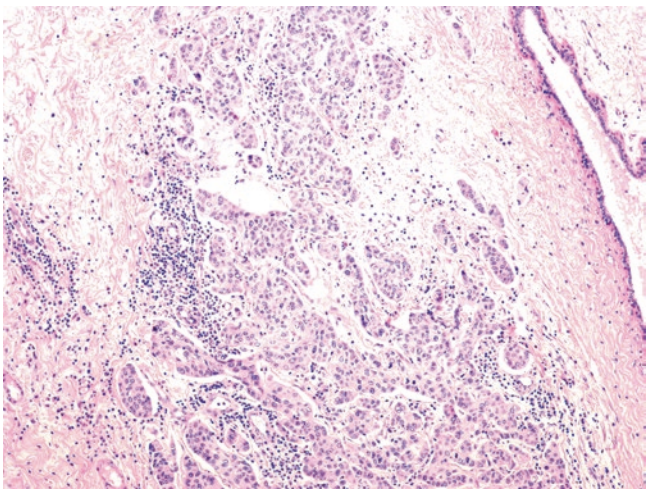

Fig. 18.11 The tumor-infiltrating lymphocytes should be reported for the stromal compartment as a percentage of the area of stromal tissue occupied by these cells over total intratumoral stromal area

18.2.7 An Extensive *In Situ* Component

The presence of an extensive *in situ* component is associated with a higher rate of local recurrence, especially in patients treated with conservative surgery associated with radiotherapy (the gold standard for patients with breast cancers) [39]. Those patients presenting an extensive DCIS component are more likely to have extensive DCIS in the remaining breast tissue after quadranectomy, and are the ones more likely to develop local recurrences, a fact that has been confirmed by numerous studies [40]. The presence of the extensive *in situ* component must be included in the final report, as well as the distance to the surgical margins (Fig. 18.12). Regarding the surgical margins, for pure DCIS, margins of at least 2 mm are associated with a reduced risk of local recurrence if the surgery is followed by radiotherapy [41], while for patients treated only with surgery, the optimal margin width is unknown, but should be at least 2 mm.

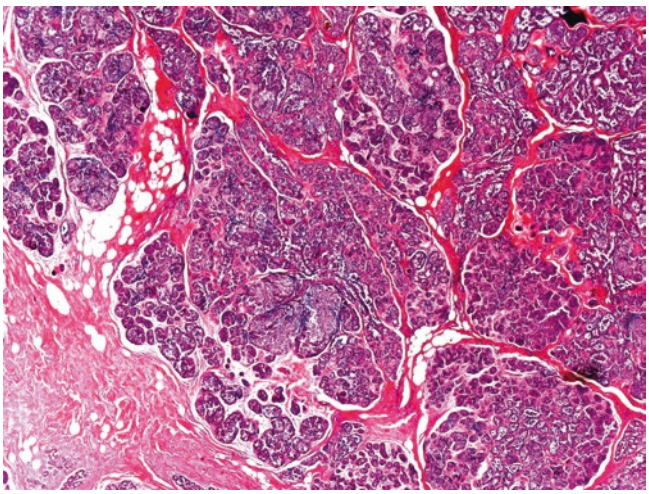

Fig. 18.12 Extensive lobular *in situ* carcinoma: the presence of this component should be reported in the final histopathological report

18.2.8 Status of Surgical Margins

Especially after conservative surgical treatment became the gold standard in breast cancer, evaluation of the surgical margins was considered a very important parameter, and the aspect of these margins is a prognostic factor when it is positively related to development of local recurrences [42]. The surgical margins are evaluated by the pathologist while grossing the surgical specimen, and on microscopic examination, but also by cytological examination, inking the specimen or separate examination of the cavity post-quadranectomy (Figs. 18.13 and 18.14). Defining a positive or negative surgical margin is very difficult, and the definition varies among pathologists, clinicians, and medical institutions. However, by establishing a good and evidence-based definition, one might avoid unnecessary surgery, morbidity, and high costs, and one can improve the cosmetic aspect of the remaining breast. Most data originate in retrospective studies and cutoffs of 1, 2, 5, and 10 mm have been used. According to the latest international guidelines, only the presence of ink

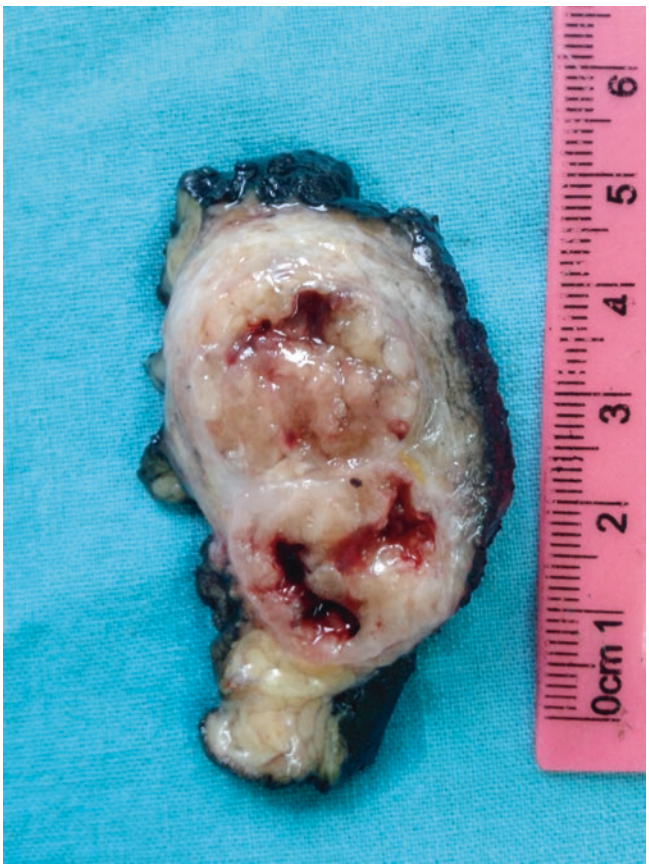

Fig. 18.13 Status of the surgical margins: the surgical margins are evaluated by the pathologist while grossing the surgical specimen; in this case, an infiltrating breast carcinoma of large size is infiltrating the surgical margin, marked with black ink

to the tumor cells is considered a positive margin, the goal not being to ensure that there is no residual tumor left after surgery, but to identify those patients more likely to have a large residual tumor burden and who would require further surgery (Figs. 18.15 and 18.16) [41, 43]. Requirements for optimal margin evaluation include: orientation of the surgical specimen, description of the gross and microscopic margin status, and reporting of the distance and orientation of any type of tumor (*in situ* or invasive) in relation to the closest margin [41].

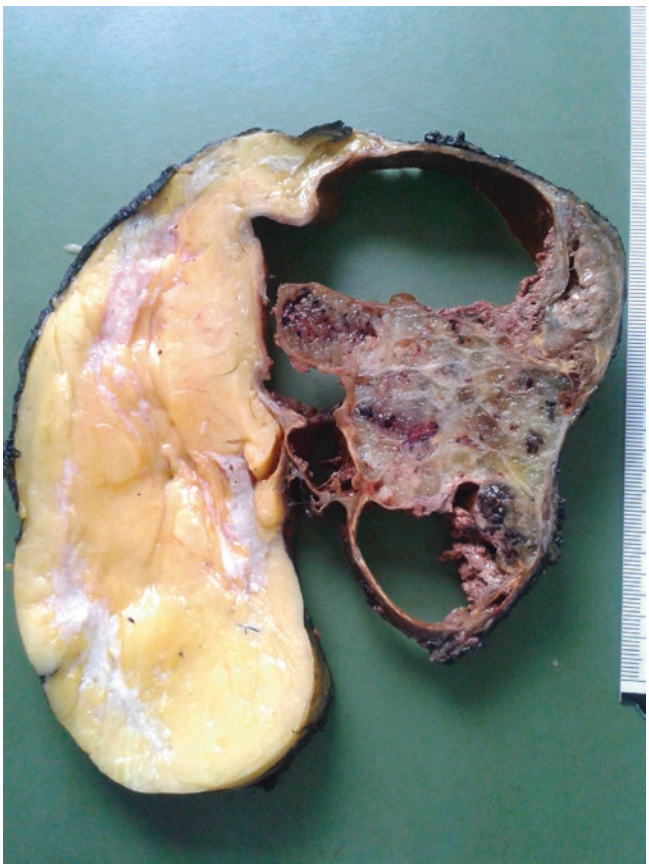

Fig. 18.14 Status of surgical margins: at the macroscopic examination, the malignant tumor is infiltrating the surgical margin (inked with black color)

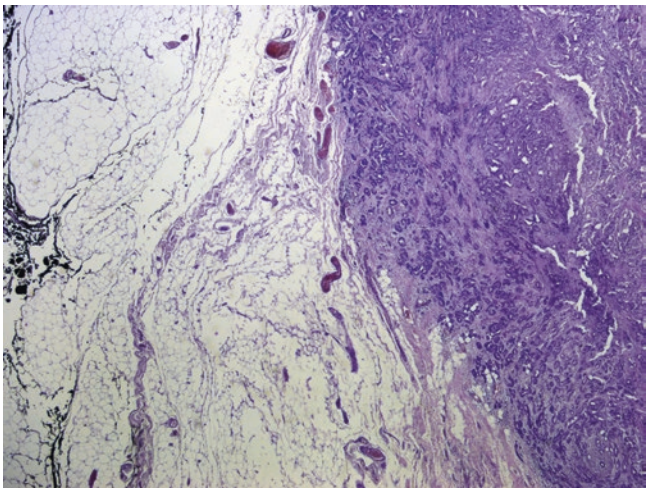

Fig. 18.15 Grade 1 infiltrating breast carcinoma: at the microscopic examination, the pathologist can appreciate that the tumor is located at distance from the surgical margin; however, the distance from the closest margin must be reported

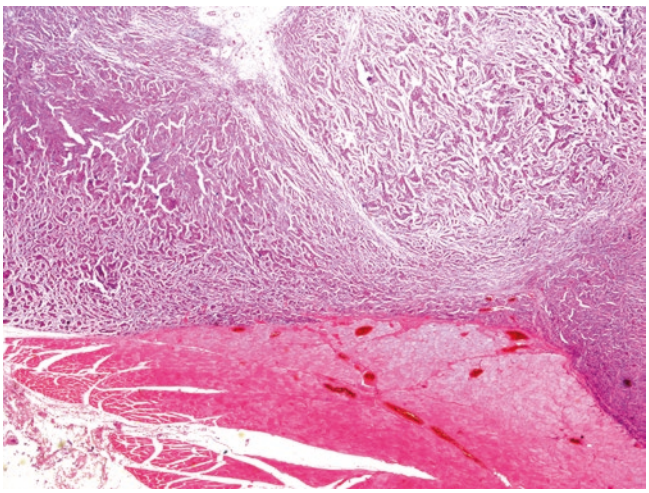

Fig. 18.16 Infiltrating breast carcinoma: the tumor is infiltrating the pectoral muscle, but is at a distance from the surgical margin

18.2.9 Lymph Node Metastases

Lymph node metastases are the most important prognostic factor for patients with breast carcinoma and can be assessed only via histopathological examination [24, 44, 45]. Patient survival depends on the presence or absence of lymph node metastases, the number of lymph nodes affected, the group of affected lymph nodes, the extent of metastasis, and the presence of tumor cells in peri-lymph node vessels and peri-lymph node fat tissue [46]. It is important to detect and sample as many axillary lymph nodes as possible. The number of normal lymph nodes present in one axilla varies from one patient to another. Also, the size of metastasis is important, but studies have shown that although small metastases (micrometastases) do have statistical significance, their impact on prognosis is less than 3% at 5 and 10 years when compared with node-negative patients, and they are not a discriminatory variable for predicting either recurrence or survival [47–50]. In micrometastasis, the metastasis is larger than 0.2 mm in diameter, but smaller than 2 mm in the largest dimension, and in macrometastasis, the metastasis is larger than 2 mm in diameter (Figs. 18.17, 18.18, 18.19, 18.20, and 18.21). There is also a third category of patients, in which only isolated tumor cells or small groups of tumor cells are present, called isolated tumor cells, with a diameter of less than 0.2 mm in the largest diameter (Fig. 18.22). According to the latest pTNM classification, these isolated tumor cells have no metastatic capacity and should therefore be classified as pN0 [24]. Macrometastases are easily detected even at low-power examination, while micrometastases and isolated tumor cells require more careful examination (especially examination of the subcapsular area), and in some situations also the use of ancillary stains (Figs. 18.23, 18.24, 18.25, and 18.26). All parameters to be specified in the status of axillary lymph nodes must be reported within the pTNM classification. If multiple foci of metastases are detected in an axillary lymph node, the size of the largest confluent focus is recorded (same as for multiple primary tumors) [18, 19]. When only clusters of tumor cells can be identified within afferent lymphatics of the node but not within the node parenchyma, the tumor should be classified according to the AJCC rules for metastases present, and this should not be confused with extranodal extension (extranodal extension should be reported separately). There are cases in which clinically or macroscopically one or more axillary lymph nodes have a size greater than 2 cm, but the microscopic examination does not reveal the presence of a metastasis. In these cases, only the changes observed during the microscopic examination are described, and they are classified in the pN0 category. Also, the presence of extracapsular extension (and its size) as well as the presence of tumor cells in the peri-lymph nodular vessels, must be recorded according to the pN classification (Fig. 18.27). If metastasis occurs in internal mammary lymph nodes, the survival of the patient is even shorter. The presence of metastases in internal mammary lymph nodes is particularly associated with malignant tumors located in internal quadrants. Usually, patients with metastases in internal mammary lymph nodes also have axillary metastases.

Isolated tumor cells or foci of micrometastases can be recognized with ancillary studies like Cytokeratin stain; however, there are also other cells positive for cytokeratin, such as interstitial reticulum cells (usually positive for pan-Cytokeratin CAM 5.2 but not for pan-Cytokeratin AE1/AE3). Pan-Cytokeratin AE1/AE3 should be better used to identify isolated tumor cells.

Axillary lymph nodes are, in most cases (75%), filtration stations of tumor cells from a breast cancer. Research has shown that lymphatic drainage of the breast is done sequentially and, initially, a lymph node located in the lymph drainage path (called sentinel lymph node) is affected. Multiple studies have shown that sentinel lymph node biopsy, in addition to being minimally invasive, is a reliable method (accuracy close to 100%) for determining the status of regional lymph nodes in patients with breast cancer. The absence of metastases in the sentinel lymph node predicts the absence of metastasis in other lymph nodes in a proportion of 98%. In these cases, it is possible to apply a conservative surgical therapy [51].

There is no consensus on the technical processing and examination protocol of the sentinel lymph node in terms of pathology. Pathologists who have dealt with the detection of metastases in sentinel lymph nodes have developed different protocols for microscopic examination. The identification of the sentinel lymph node is made intraoperatively, and its microscopic examination is done on frozen sections, the sections being stained with hematoxylin-eosin. Subsequently, sentinel lymph node fragments cut at 2 mm apart from one another are embedded in paraffin blocks. The protocol of sectioning, examining, staging, and reporting the sentinel lymph node in breast pathology is presented in Chapter 23.

Lymph node metastases of breast cancer should not be confused with other pathological processes that affect a lymph node.

Of the reactive processes that may involve an axillary lymph node, one can encounter hyperplasia of lymphoid follicles and sinus histiocytosis (the presence of both lesions is associated by some reports with a better immune response to the presence of the breast carcinoma and a better survival) (Figs. 18.28 and 18.29). Also, an axillary lymph node can present fat tissue metaplasia, when normal lymphoid tissue is replaced by adipose tissue (this situation occurs more frequently in obese patients) (Fig. 18.30). The characteristic changes of toxoplasmosis type granuloma may also be encountered. Also, a sarcoid-like granulomatous lesion may sometimes occur, especially during chemotherapy or tumor necrosis factor blocker therapy, and sarcoidosis and tuberculosis must be ruled out [52] (Fig. 18.31). Very rarely, true

sarcoidosis or tuberculosis affect the axillary lymph nodes (Fig. 18.32). Benign-appearing groups of nevus cells with a capsular location can sometimes occur in a lymph node. This is always an incidental finding (Fig. 18.33). The cells are oval, with pale cytoplasm, indistinct borders, sometimes contain melanin pigment within the cytoplasm, and present bland nuclei. Absence of mucin production, presence of melanin pigment, location within the capsule, and the immunohistochemical profile help to differentiate this lesion from metastatic process. Staining for HMB-45 and S-100 protein allows for identifying the melanocytic origin of these cells together with Cytokeratin, since some tumor cells may be positive for S-100 protein (Fig. 18.34). Of note, nevus cells can occur within the same node with metastases. Also, a lymph node may have benign glandular inclusions (also called endosalpingiosis), which are formed by epithelial cells without atypia, line the cystic spaces, and have varying

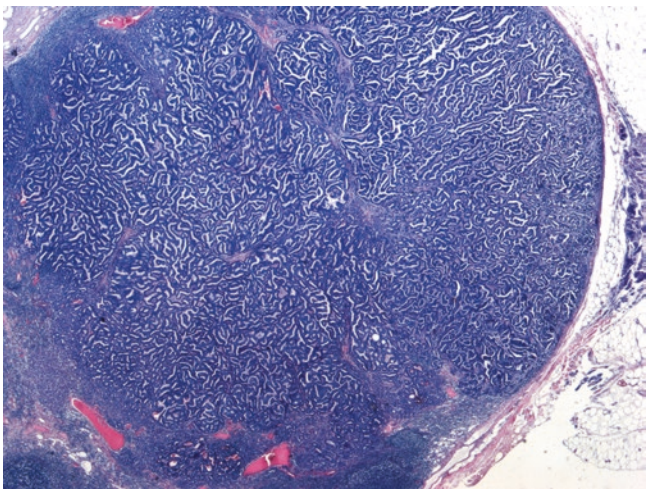

Fig. 18.17 Axillary lymph node macrometastasis from a grade 1 infiltrating breast carcinoma

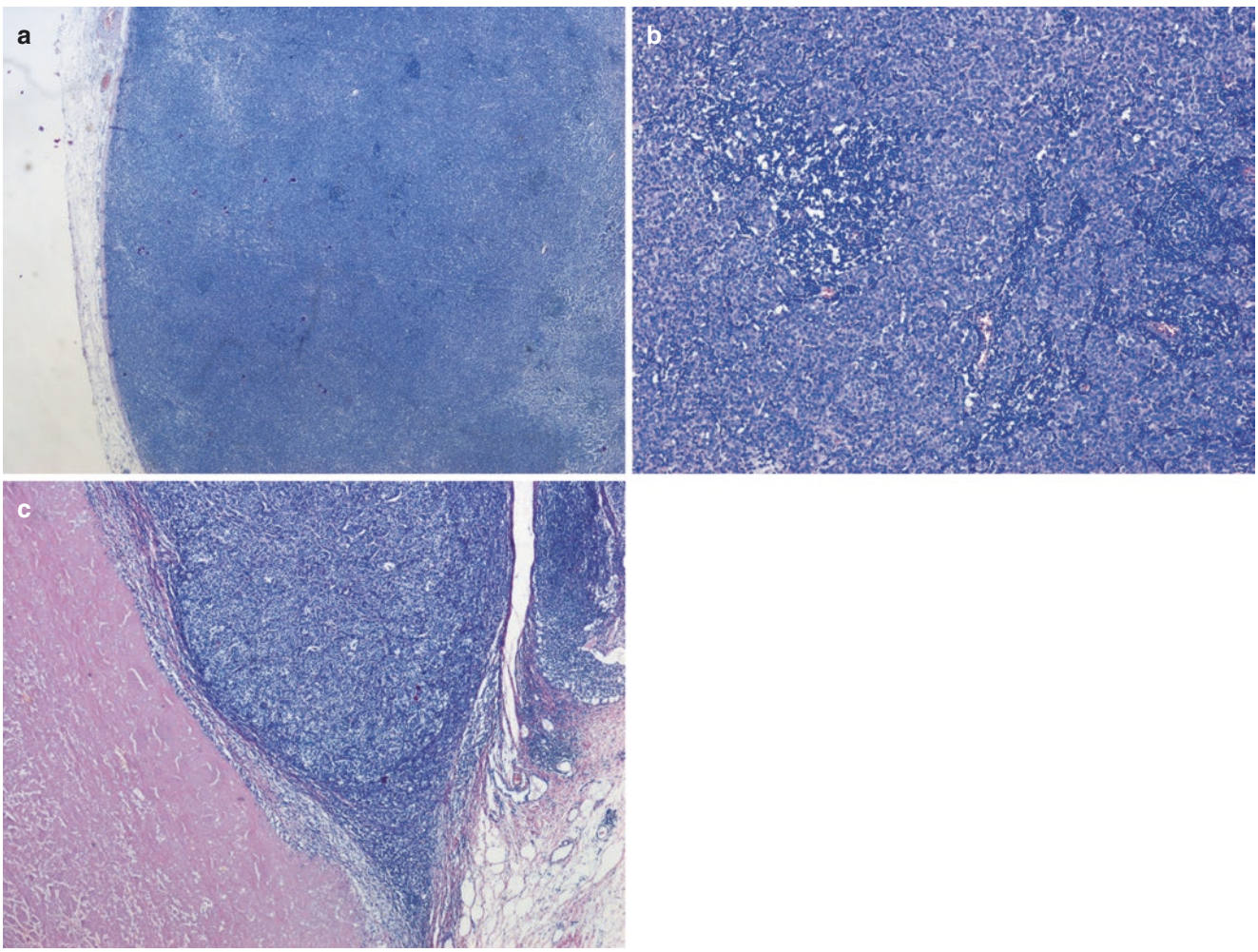

Fig. 18.18 Axillary lymph node macrometastasis from a grade 3 infiltrating breast carcinoma: (**a**) At low power, the lymph node architecture is replaced by tumor cells; (**b**) Higher power reveals that the tumor cells are of high grade and not forming tubular structures; (**c**) other areas contain also tumor necrosis

sizes (comparison with primary breast tumor morphology is of great help, as well as the positivity for WT-1, CA125, and estrogen receptors, and negativity for GCDFP-15 and GATA 3) (Fig. 18.35). This must be known by practicing pathologists and should not be confused with metastatic breast carcinoma, especially when the endosalpingiosis is of florid type [53]. Of interest, benign glandular inclusions represented by breast acini and ducts can also occur within axillary lymph nodes. This is important to know, since benign or malignant lesions similar to the ones in the breast can develop out of these inclusions. These acini and ducts are lined by epithelial, myoepithelial cells and have a basal membrane at the periphery. Silicone lymphadenopathy can occur, especially in the presence of silicone breast implants (empty or clear round spaces associated with inflammatory infiltrate, vacuolated histocytes, and multinucleate cells are present) (Fig. 18.36). Megakaryocytes can also be found in axillary lymph nodes, especially following neoadjuvant chemotherapy for local advanced breast cancer, being a potential source of false-positive diagnosis of axillary metastases from breast cancer [54, 55]. Ancillary immunohistochemical studies, however, can rule out metastases. Lymph node metastases of breast cancer should not be confused with lymph node metastases of primary tumors from another location (especially from a thyroid carcinoma).

Displaced epithelium from benign breast tissue may occur, as might benign proliferative lesions or after the biopsy (Fig. 18.37) of breast lesions, especially after the biopsy of breast papillary lesions (of benign or malignant type) that are fragile. They are typically detected within the subcapsular sinus. Sometimes it is impossible to differentiate between benign or malignant cells (however, the presence of degenerative cells together with the presence of hemosiderin containing macrophages are in favor of displaced cells due to a biopsy). It is advisable that in patients with invasive carcinoma or DCIS, an explanatory note should be included at the end of the report mentioning that the possibility of metastasis cannot be excluded.

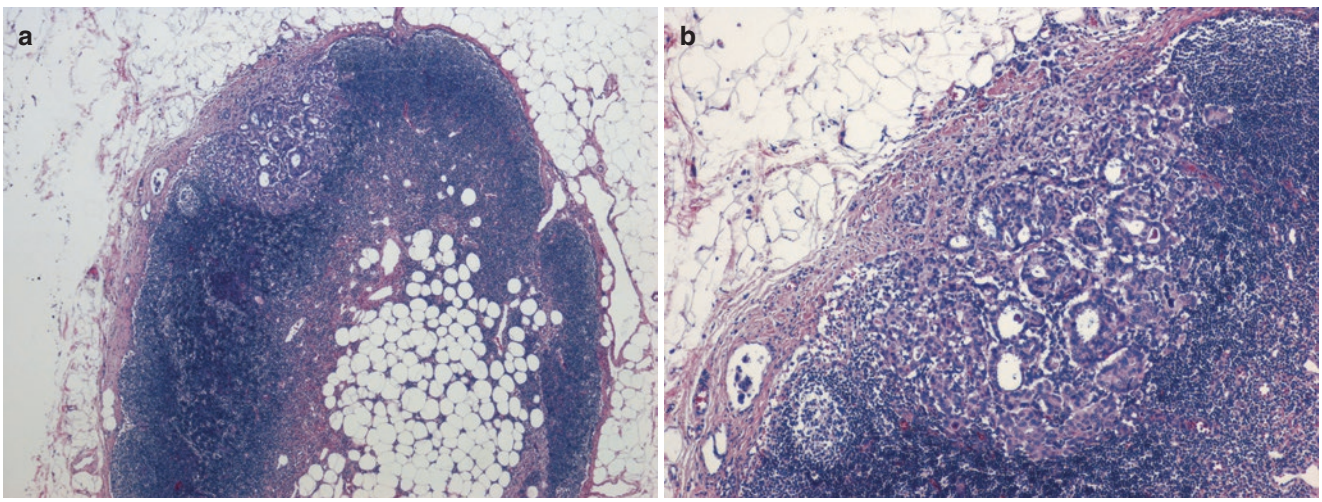

Fig. 18.19 (**a**) Micrometastasis in axillary lymph node; (**b**) High power reveals the presence of extracapsular extension and tumor emboli

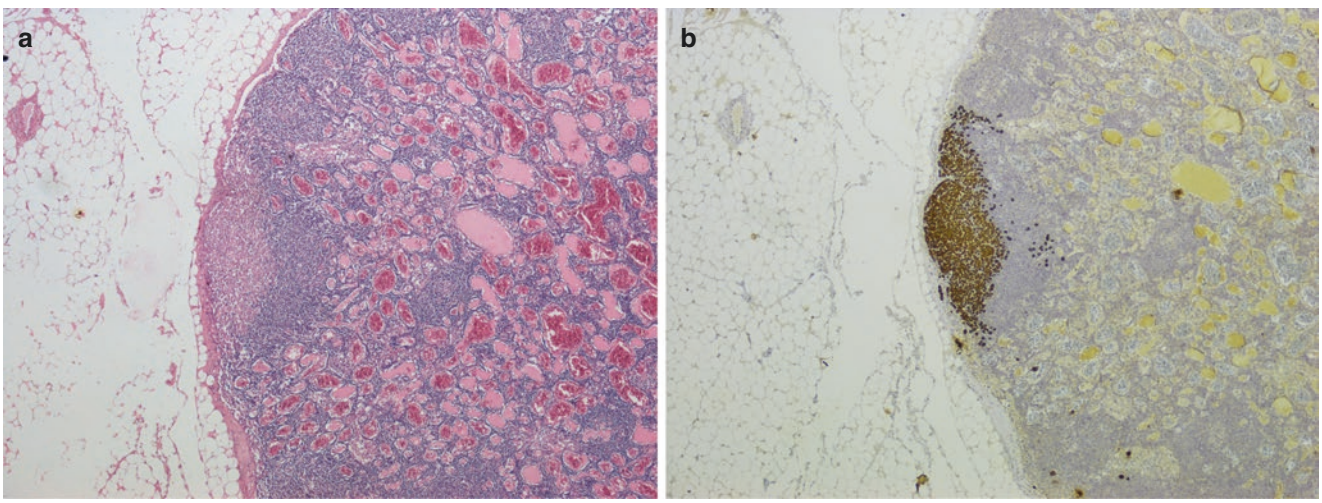

Fig. 18.20 Micrometastasis in axillary lymph node: (**a**) Especially in lobular carcinoma it is sometimes difficult to detect small size metastases on Haematoxylin-Eosin stain; (**b**) Ancillary stains can help, especially the use of Cytokeratin since the tumor cells are positive for this marker

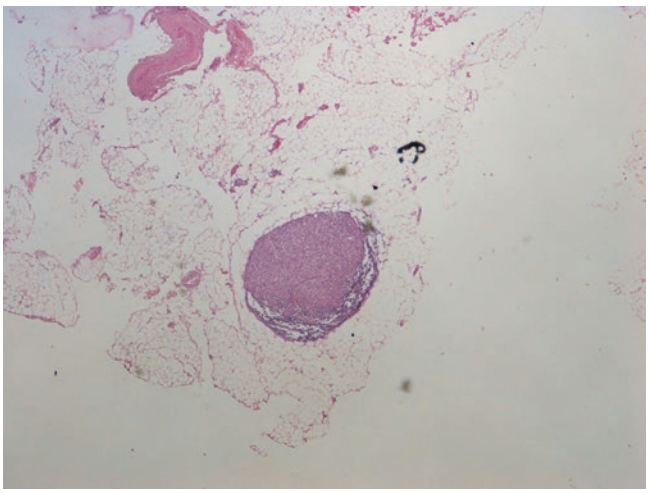

Fig. 18.21 Micrometastasis in a very small axillary lymph node demonstrating that all lymph nodes should be examined at the microscope for the detection of the metastases, despite their size

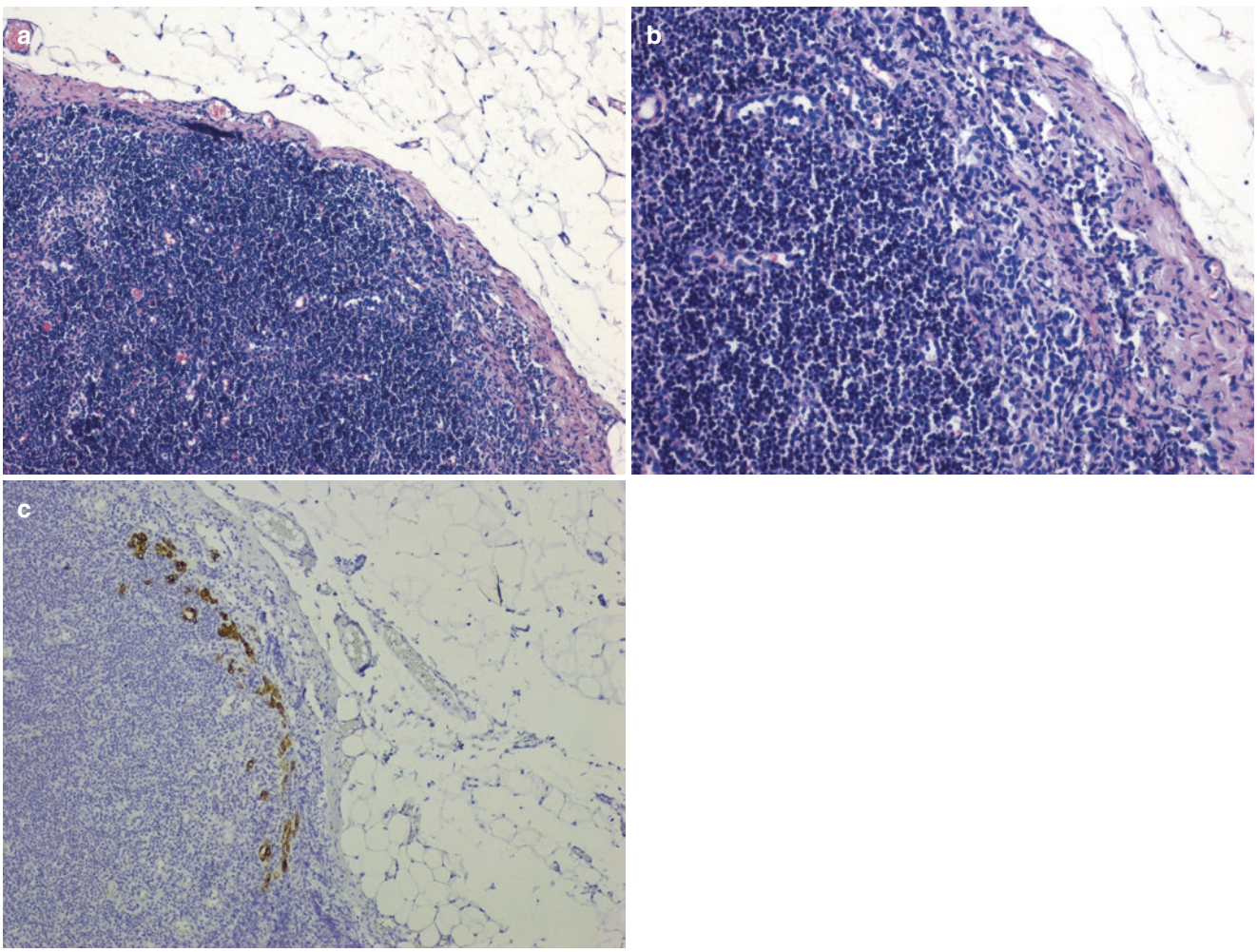

Fig. 18.22 Isolated tumor cells: (**a**) Small groups of tumor cells present in the subcapsular area; (**b**) Higher power examination reveals the presence of isolated small groups of atypical cells; (**c**) Cytokeratin stain can detect these cells better and the size of the lesion (less than 0.2 mm in the largest diameter) is better appreciated

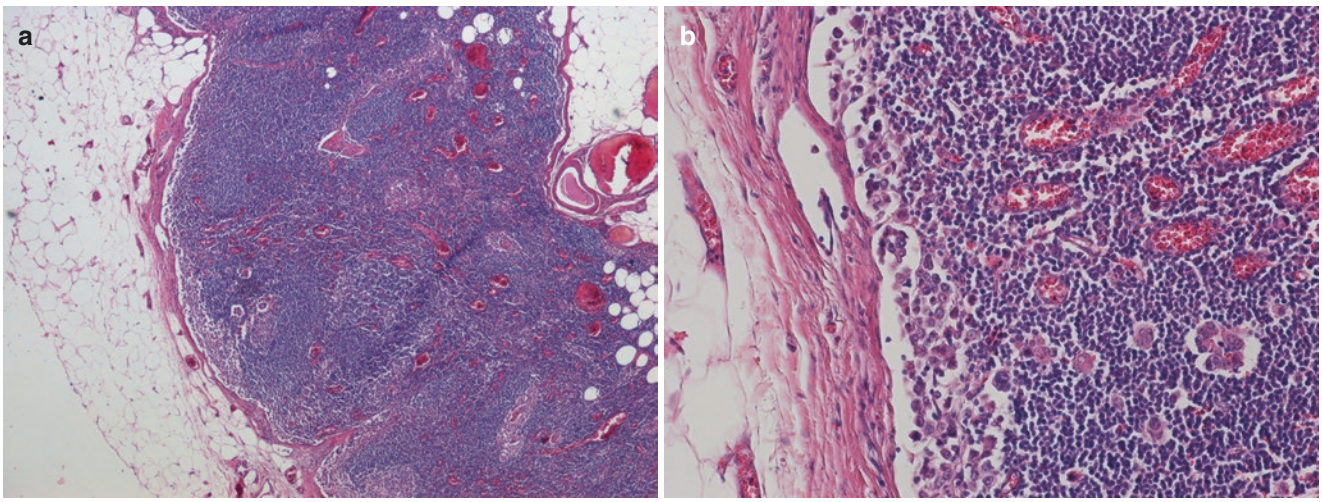

Fig. 18.23 (**a**) Small size metastases, especially when originating in lobular infiltrating breast carcinoma, are very difficult to detect while scanning at low-power examination; (**b**) Each suspected area has to be examined at high power as well

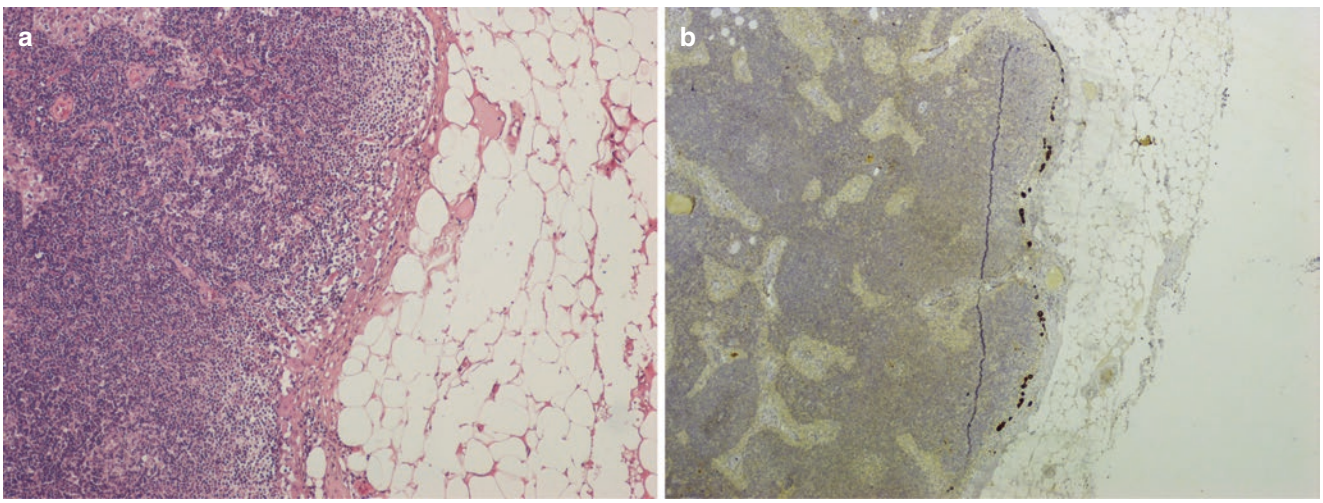

Fig. 18.24 (**a**) Isolated tumor cells requires more careful examination (especially examination of the subcapsular area) and (**b**) in some situations, also the use of ancillary stains such as Cytokeratin

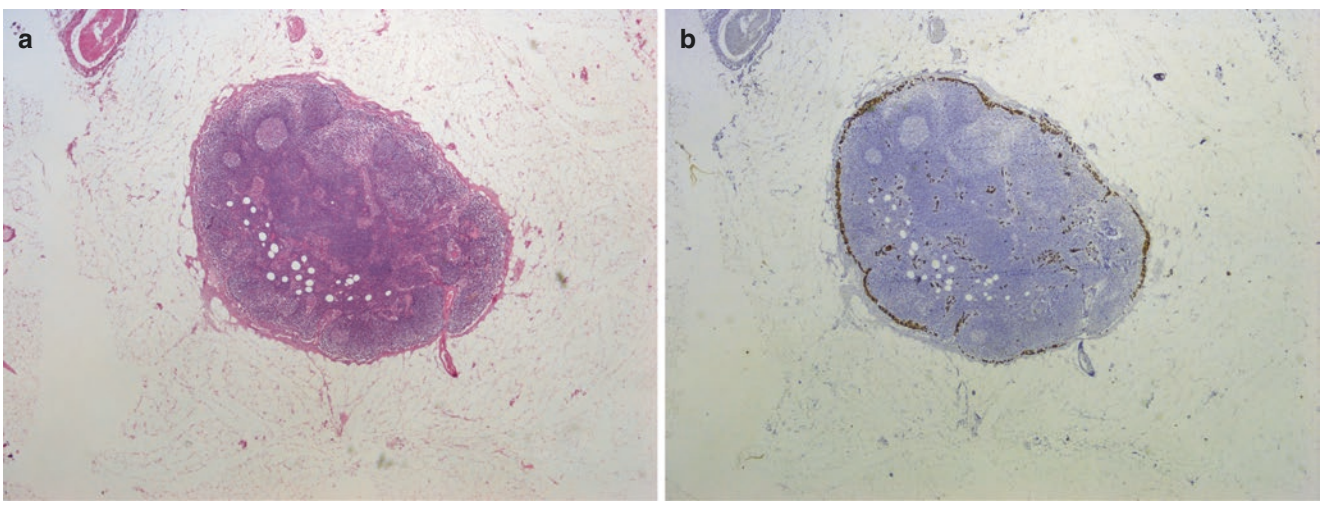

Fig. 18.25 (**a**) The presence and extent of small groups of cells within the subcapsular space (**b**) are better detected with ancillary stains such as Cytokeratin

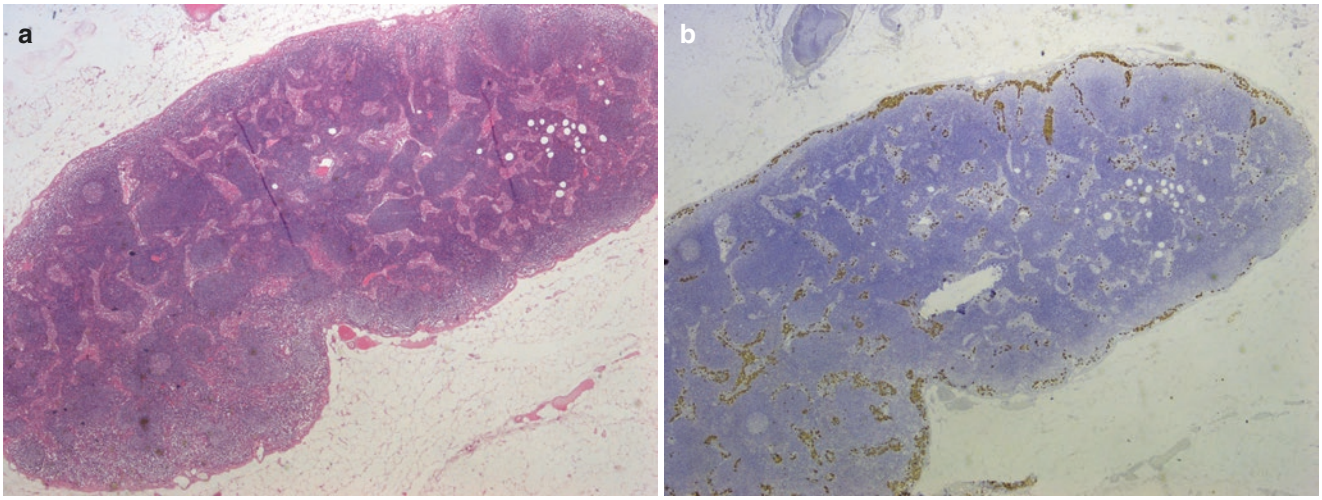

Fig. 18.26 Another case of axillary lymph node metastasis from breast carcinoma: (**a**) The presence and extent of small groups of cells within the subcapsular space are (**b**) better detected with ancillary stains such as Cytokeratin

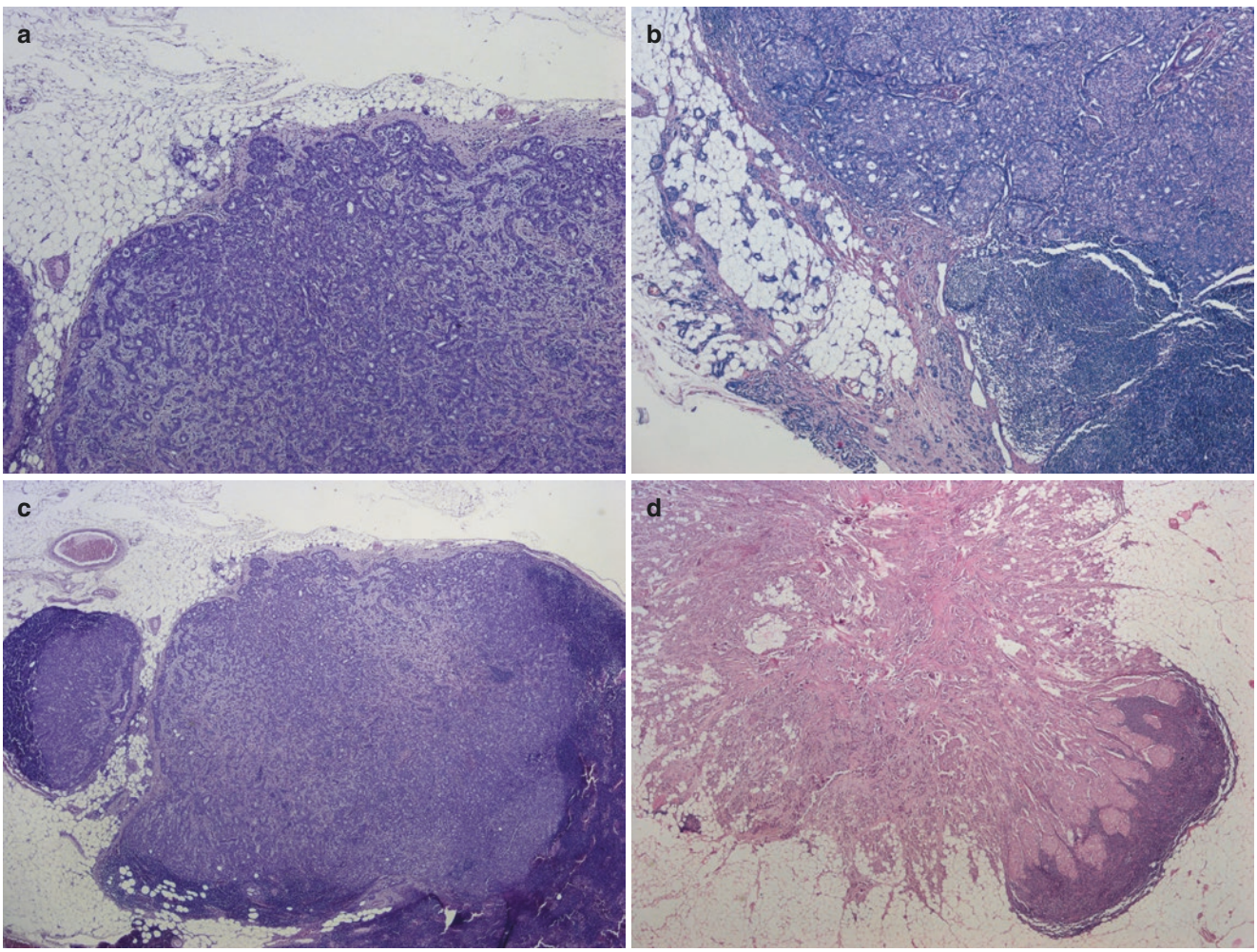

Fig. 18.27 (**a**) Extracapsular extension of lymph node metastasis from a grade 1 infiltrating breast carcinoma; (**b**) Different area with larger size; (**c**) Different case with extracapsular extension; (**d**) Massive extracapsular extension in another case—the presence and size of the extracapsular extension should be recorded in the final histopathological report

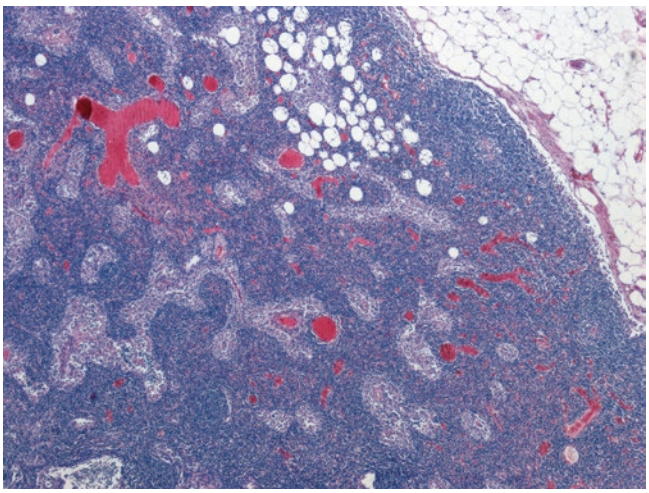

Fig. 18.28 Sinus histiocytosis involving axillary lymph node in a case of primary breast carcinoma

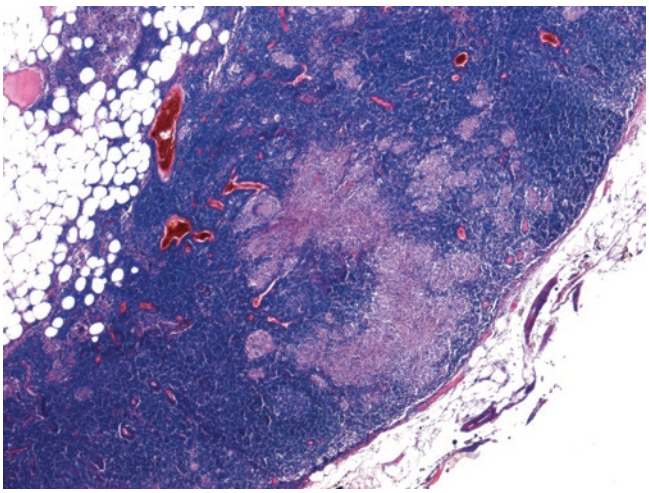

Fig. 18.31 Sarcoid-like granulomatous lesion involving axillary lymph node

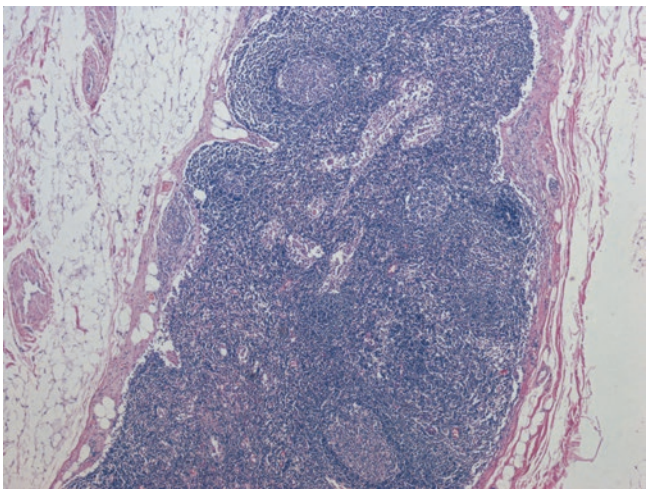

Fig. 18.29 Hyperplasia of lymphoid follicles involving axillary lymph node in a case of primary breast carcinoma

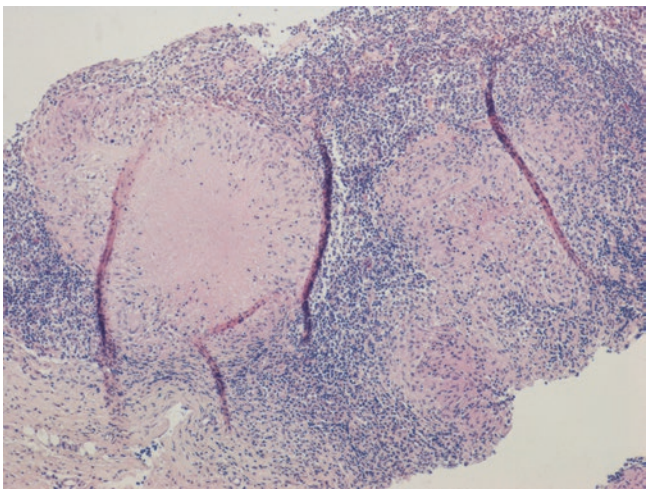

Fig. 18.32 Tuberculosis granulomatous lesions with central areas of necrosis involving axillary lymph node in a Tru-Cut biopsy

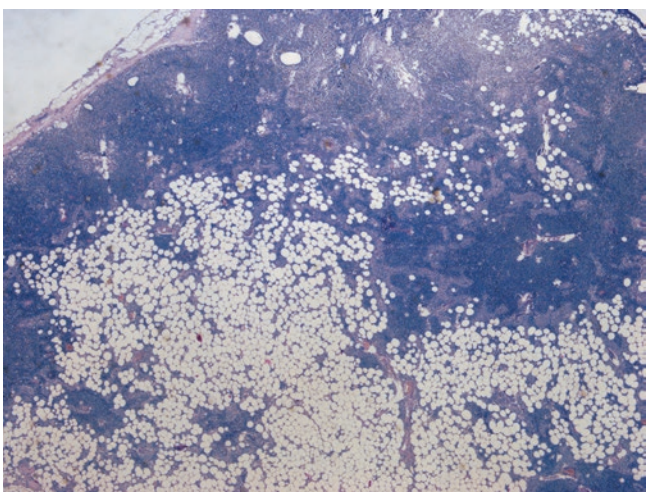

Fig. 18.30 Fat tissue metaplasia involving axillary lymph node: normal lymphoid tissue is replaced by adipose tissue with a variable extent

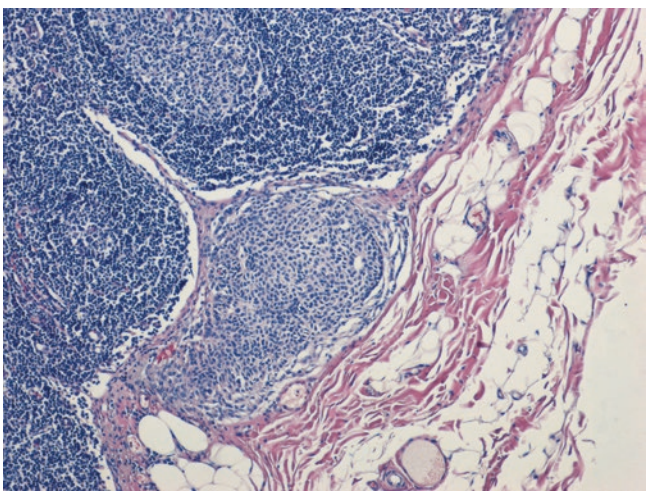

Fig. 18.33 Nevus cells with a capsular location in axillary lymph node: benign-looking cells, oval, with pale cytoplasm, bland nuclei, and indistinct borders; melanin pigment is not present in this case

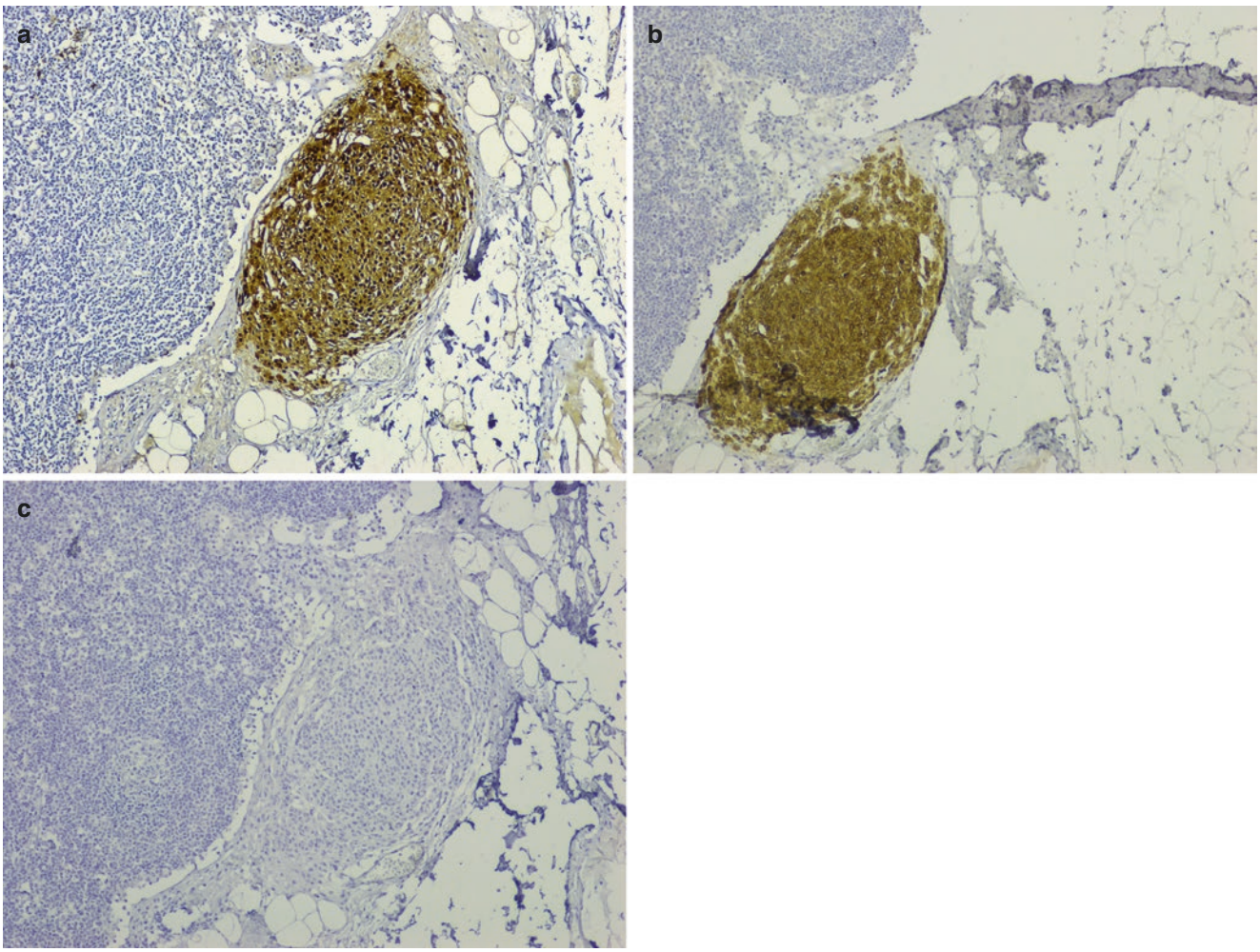

Fig. 18.34 Nevus cells with a capsular location are positive for (**a**) S-100 protein and (**b**) Melan A, and (**c**) negative for Cytokeratin

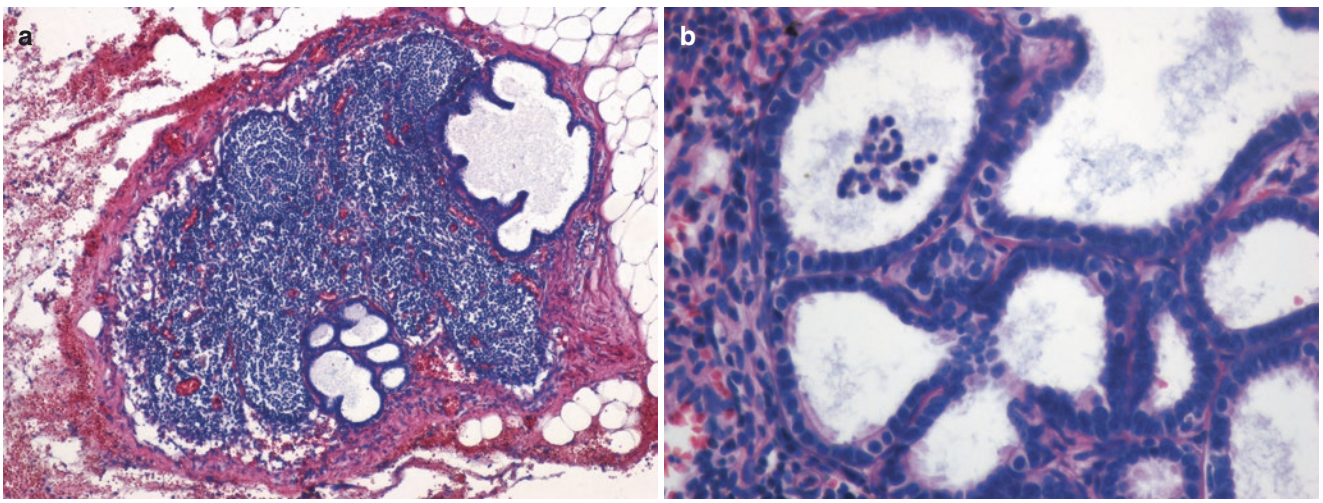

Fig. 18.35 Endosalpingiosis involving axillary lymph node: (**a**) Small glandular inclusions; (**b**) Lined by epithelial ciliated cells resembling the fallopian tube epithelium, without atypia

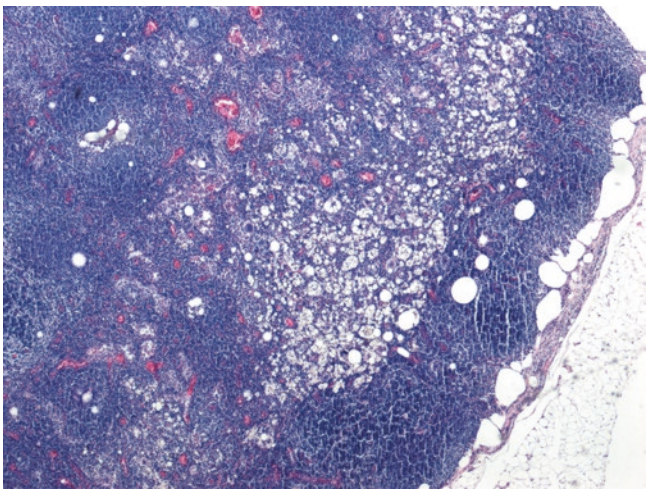

Fig. 18.36 Silicone lymphadenopathy: clear round spaces associated with inflammatory infiltrate, vacuolated histocytes, and multinucleate cells

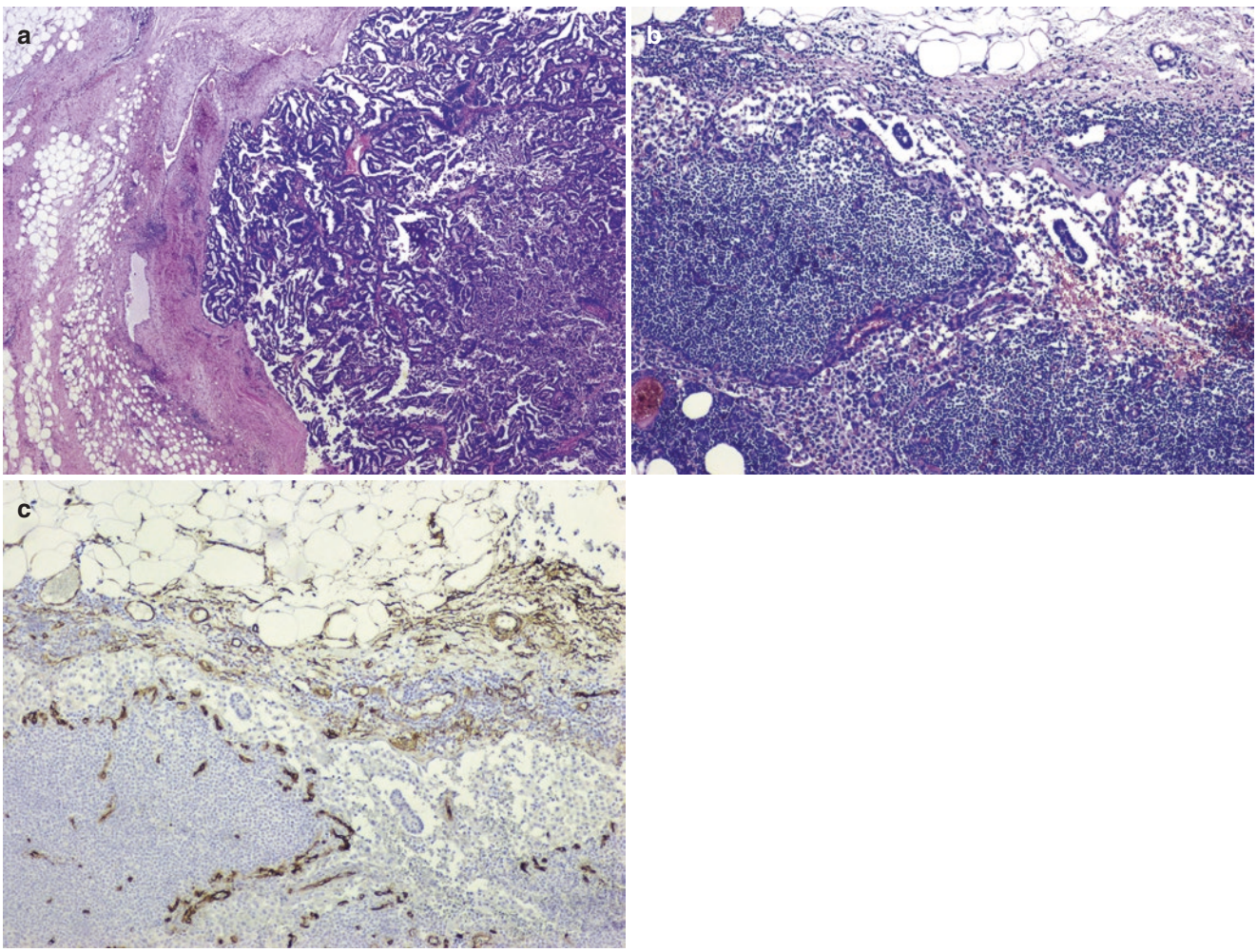

Fig. 18.37 Displaced epithelium involving axillary lymph node: (**a**) Primary breast tumor of encapsulated papillary breast carcinoma; (**b**) After Tru-Cut biopsy of the primary tumor, small groups of cells with papillary architecture are found in the subcapsular sinus of the removed sentinel lymph node; (**c**) CD34 is negative within the surrounding space

18.2.10 Nottingham Prognostic Index

A group of pathologists and oncologists in Nottingham formulated a prognostic indicator called the Nottingham prognostic index (NPI), the calculation of which takes into account the following parameters: tumor size, stage of axillary and internal mammary lymph nodes, and histological grade of the tumor in question [56]. Index calculation is made using the following formula:

$$\text{NPI} = 0.2 \times \text{tumor size} + \text{lymph node stage} + \text{histological grade}.$$

Tumor size is assessed in centimeters. The appearance of lymph nodes is estimated by 1, 2, or 3 points, depending on the presence of metastases in axillary or internal mammary lymph nodes.

Depending on the Nottingham prognostic index, patients can be classified into three categories: good prognosis (index below 3.4), moderate (index between 3.41 and 5.4), and poor prognosis (index higher than 5.41) [56]. The NPI has been validated by both retrospective and prospective studies. The NPI may assist the clinician in selecting which patients should receive systemic adjuvant therapy and what type of therapy.

18.3 Molecular and Genetic Prognostic Factors

Three molecular biomarkers are routinely used in the diagnosis and management of breast carcinoma: estrogen receptors, progesterone receptors, and HER2, all of which are indicators of effectiveness of therapies in breast carcinoma. Thus, correct assessment of these parameters is very important, and the pathologist dealing with these investigations has a very important role. Also, every laboratory performing these tests is responsible for providing accurate and reproducible results.

18.3.1 Hormonal Receptor

Determination of hormonal receptor status in breast carcinoma is a routine examination and is performed in all infiltrating breast carcinomas as well as in some *in situ* types. Estrogen receptor (ER) is a nuclear transcription factor involved in the breast developments as well as in tumorigenesis, and it regulates expression of genes such as progesterone receptor (PR). ER and PR levels are strongly and inversely correlated with other prognostic parameters. Also, ER and PR are prognostic and predictive factors, and their presence or absence is routinely assessed in all patients with breast cancer as a predictive factor for response to therapeutic and adjuvant hormonal therapy. This examination identifies patients who will respond to hormonal treatment. Approximately 55–65% of primary breast tumors and 45–55% of metastases present ER and PR. Studies have shown that 55–65% of patients with positive ER and PR respond to hormone therapy, compared to 8% of patients with negative ER responding to this treatment. Well-differentiated tumors usually have positive ER and a better prognosis. Approximately 45–60% of primary breast carcinomas and their metastases contain PR. If a breast tumor has both positive ER and PR, the response of these tumors to hormonal therapy increases from 55–60% to 75–80%. The presence of positive PR within a breast cancer is associated with a favorable prognosis. Approximately 46% of the ER-negative tumors, but positive PR, respond to hormonal therapy. Estrogen and progesterone receptors can be determined by immunohistochemical methods or molecular methods. Studies have shown that immunohistochemical determination correlates much better with assessing prognosis than conventional biochemical methods. Determination of hormone receptors by immunohistochemical methods can be done on fresh or paraffin-embedded tissue. The determination is made both on the *in situ* hormone receptor component, as well as the invasive one, and reporting of the results must indicate the percentage of tumor cells positive or

negative for both receptor components; however, in invasive breast carcinomas, the positivity of the infiltrative component is taken into account when deciding the hormonal treatment. Also, for the cases of DCIS, the percentage of the tumor cells positive for ER will indicate the hormonal treatment. Assessment of positivity is only done on the nuclei of tumor cells, and cytoplasmic positivity is not taken into account (Figs. 18.38 and 18.39). For this purpose, the tumor must be properly fixed in formalin, immediately sectioned, and immersed in the suitable amount of fixation substance. Positive and negative control must be performed in every case. In most cases, normal breast tissue adjacent to the tumor represents a very good internal control because it normally contains ER and PR. A simpler method of assessment is to determine the hormone receptor positive tumor cell percentage. Different cutoffs have been used for the positivity of ER and PR. Recent studies have shown, however, that a 1% cutoff is advisable since there are convincing data that patients with even a few cells positive will benefit from hormonal therapy [57].

Some laboratories use the H score to report immunohistochemical results. H score is calculated by taking into account both the percentage of positive nuclei and the intensity of the reaction. H score calculation is as follows: (1× % of weakly positive cells) + (2× % moderately positive cell) + (3× % of cells strongly positive). Percentage is calculated on 500–1000 tumor cells. By this calculation four grades are obtained:

Negative H Score: 0–50
Weakly positive H score: 51–100
Moderately positive H score: 101–200
Strongly positive H score: 201–300.

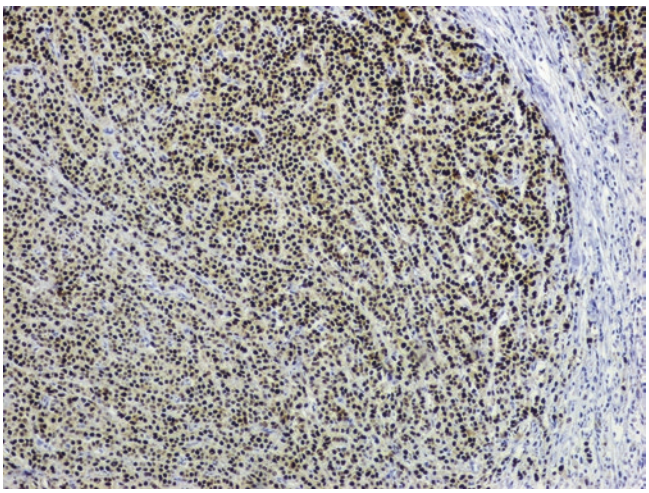

Fig. 18.38 Estrogen receptor is positive in more than 90% of the tumor cells in this case

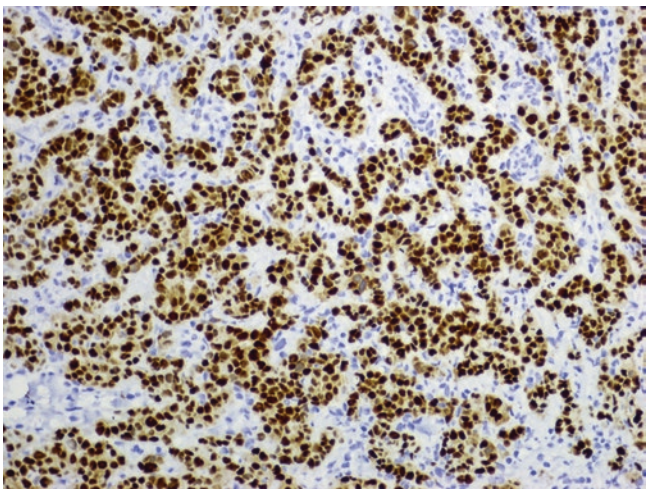

Fig. 18.39 Progesterone receptor is positive in more than 90% of the tumor cells in this case

18.3.2 Oncogene c-ErbB2

Oncogene c-ErbB2 or HER2/neu belongs to the ErbB oncogene family and is closely correlated with epidermal growth factors. Studies have shown amplification of this oncoprotein in about 15% of breast cancers. It is an independent factor for assessing the prognosis of patients with axillary lymph node metastases [57, 58]. The HER2 amplification is associated with poorly differentiated tumors with metastases in the axillary lymph nodes, hormone receptor negativity, and a poor prognosis [59]. HER2 testing should be performed in all newly diagnosed invasive breast cancers and for first recurrences of breast cancers. Amplification of c-ErbB-2 oncoprotein may be demonstrated by immunohistochemical methods on the cell membrane or *in situ* hybridization (measuring the number of HER2 gene copies). Multiple studies have shown, however, that the accuracy of HER2 assay used in clinical practice is a major concern owing to false-positive and false-negative results. This is why it is advised to perform the HER2 testing only in accredited laboratories. Intracytoplasmic positivity is non-specific by diffusion from the membrane, and it is not considered if it is not associated with membranous positivity. The score performed using the immunohistochemical method is calculated as follows:

Score 0 (negative): no staining is observed, or membrane staining is observed in less than 10% of the tumor cells.

Score 1+ (negative): a faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells.

Score 2+ (weakly positive, equivocal): a weak-to-moderate complete membrane staining is observed in more than 10% of the tumor cells.

Score 3+ (strongly positive): a strong, complete membrane staining is observed in more than 10% of the tumor cells (Fig. 18.40) [60].

Score 0 and 1 are considered as HER2 negative, Score 2 is considered as equivocal; the recommendation is reflex testing using the ISH method on the same specimen or ordering a new test with immunohistochemistry or ISH if a new specimen is available. Of interest, some invasive carcinomas (like the micropapillary carcinoma) are HER2 1+ positive in 10–80% of cases, with intense but incomplete staining (basolateral or U-shaped) found to be HER2 amplified [60]. The pathologist should consider also reporting these specimens equivocal and request reflex testing using alternative test [60].

By using the ISH assay, ISH test is negative if average HER2 copy number is less than 4 signals/cell, it is equivocal if average HER2 copy number is between 4 and 6 signals/cell and it is positive if average HER2 copy number is more than 6 signals/cell. For the equivocal results, reflex test with dual-probe ISH or with immunohistochemistry on the same specimen must be ordered, or a new test with ISH or immunohistochemistry if a new specimen is available.

Evidence from trastuzumab adjuvant trials show that HER2 testing by immunohistochemistry or ISH have similar utility to predict clinical benefit from HER2-targeted therapy.

Of recent drugs, Herceptin is used in patients with c-ErbB-2 amplification and has proven effective in 20% of these patients. Problems still exist with indeterminate IHC cases, which need to be solved by FISH (an expensive method), with a very well-trained pathologist experienced with HER2 interpretation, as well as with rigorous quality control programs.

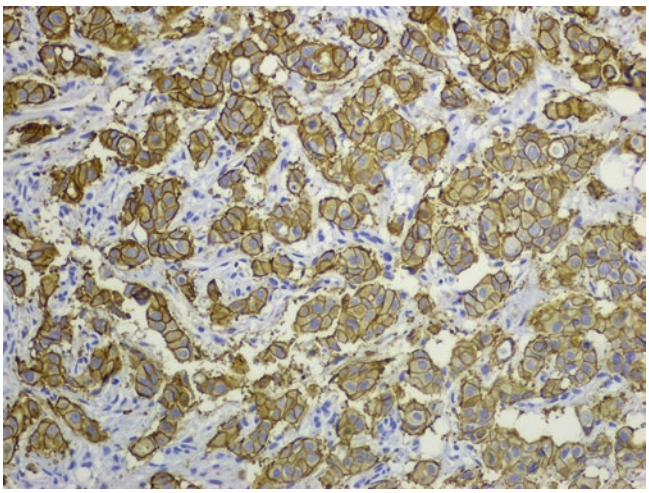

Fig. 18.40 Evaluation of HER2 positivity: score 3+ (strongly positive) is diagnosed when a strong, complete membrane staining is observed in more than 10% of the tumor cells

18.3.3 Proliferation Markers

Proliferation markers are represented by: the number of mitoses, Ki-67, and DNA content of tumor cells, and S-phase fraction (both determined by flow cytometry). Flow cytometry determines the DNA content of tumor cells and the histograms obtained indicate the euploidy or aneuploidy of these cells. Aneuploidy is associated with a poor prognosis. The S-phase fraction, which is proportional to the proliferation rate, can also be determined. The percentage of Ki-67 positive tumor cells allows patients to be grouped in those with good prognosis and those with poor prognosis. A high percentage of Ki-67 positive tumor cells advocates an unfavorable prognosis, but on the other hand it is a good indicator for better response to neoadjuvant chemotherapy [57]. Determination of Ki-67, however, reflects a more significant information about cell proliferation than DNA content, and the assessment of Ki-67 has become a major factor in treatment decisions of breast carcinoma patients, and is used in the routine work in some oncology centers as an additional factor for decision-making on adjuvant/neo-adjuvant treatment strategies. Also, in the 2015 St. Gallen Consensus Conference, the majority of panelists voted in favor of taking into account the Ki-67 index in the administration of adjuvant/neo-adjuvant chemotherapy in individual cases because Ki-67 score carries robust prognostic information and has a high value in predicting the benefit of addition of cytotoxic chemotherapy [61]. Ki-67 index is determined by immunohistochemical stains. Despite efforts within the last decade, however, an international cut-off for the low versus high index is still missing and different medical centers use different cutoffs (15%, 17%, 20%, 25%) (Fig. 18.41) [38, 61–66]. Also, breast intratumoral heterogeneity has been noted in breast carcinomas [66]. Since the Ki-67 index value could have an impact on clinical decisions, it is mandatory to evaluate the whole specimen and not only the core biopsy specimen, and to correlate it with the mitotic count.

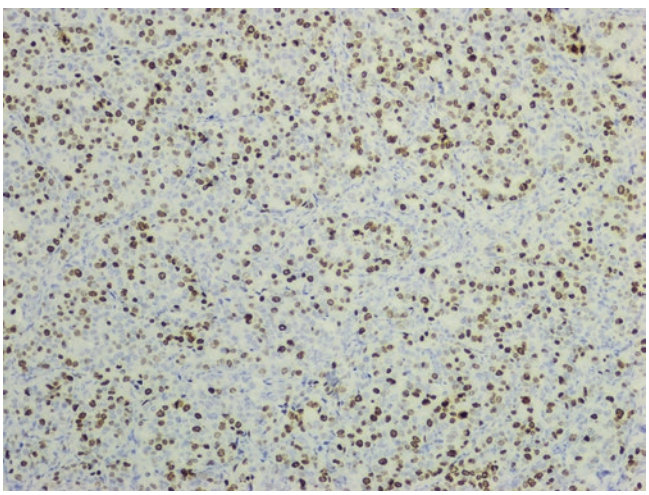

Fig. 18.41 High Ki67 index: more than 70% of the tumor cells are positive with variable intensity for Ki67 in this case

18.3.4 Other Markers

Other markers such as overexpression of *p53* can be demonstrated by immunohistochemical methods in breast carcinoma, and its presence correlates with a poor prognosis. However, evaluation of p53 is not recommended for evaluation of breast carcinoma in routine practice. More recently, genomic, and expression microarray technology has been used to classify breast cancer patients into those with good versus worse prognosis. There are several commercial *multiparameter gene expression analysis tools* (like Oncotype DX, which is done on paraffin sections, or Mamma Print, which is done on fresh tissue) which are discussed in Chap. 19 of this book [67]. Also, several prospective clinical trials (like Tailorx, ONCOTYPE DX, MINDACT) have investigated the usefulness of these tests in the management of patients with breast cancer [66–73].

References

1. Wallack MK, Wolf JA Jr, Bedwinek J, Denes AE, Glasgow G, Kumar B, et al. Gestational carcinoma of the female breast. Curr Probl Cancer. 1983;7(9):1–58.
2. Petrek JA. Breast cancer during pregnancy. Cancer. 1994;74:518–27.
3. Petrek JA, Dukoff R, Rogatko A. Prognosis of pregnancy-associated breast cancer. Cancer. 1991;67:869–72.
4. Ruiz R, Herrero C, Strasser-Weippl K, Touya D, St Louis J, Bukowski A, et al. Epidemiology and pathophysiology of pregnancy-associated breast cancer. A review. Breast. 2017;35:136–41.
5. Fisher ER, Fisher B, Sass R, Wickerham L. Pathologic findings from the national surgical adjuvant breast project (protocol no 4). XI. Bilateral breast cancer. Cancer. 1984;54:3002–11.
6. Haagensen CD. Diseases of the breast. 2nd ed. Philadelphia, PA: WB Saunders; 1971. p. 449–58.
7. Leis HP Jr. Managing the remaining breast. Cancer. 1980;46:1026–30.
8. Robbins GF, Berg JW. Bilateral primary breast cancers; a prospective clinicopathological study. Cancer. 1964;17:1501–27.
9. Wanebo HJ, Senofsky GM, Fechner RE, Kaiser D, Lynn S, Paradies J. Bilateral breast cancer. Risk reduction by contralateral biopsy. Ann Surg. 1985;201:667–77.
10. Karakas Y, Kertemen N, Lacin S, Aslan A, Demir M, Ates O, et al. Comparison of prognosis and clinical features between synchronous bilateral and unilateral breast cases. JBUON. 2017;22(3):623–7.
11. Katz A, Strom EA, Buchholtz TA, Theriault R, Singletary SE, McNeese MD. The influence of pathologic tumor characteristics on locoregional recurrence rates following mastectomy. Int J Radiat Oncol Biol Phys. 2001;50(3):735–42.
12. Yerushalmi R, Kennecke H, Woods R, Olivotto IA, Speers C Gelmon KA. Does multicentric/multifocal breast cancer differ from unifocal breast cancer? An analysis of survival and contralateral breast cancer incidence. Breast Cancer Res Treat. 2009;117(2):365–70.
13. Tot T. The role of large-format histopathology in assessing subgross morphological prognostic parameters: a single institution report of 1000 consecutive breast cancer cases. Int J Breast Cancer. 2012;2012:395415.
14. Fish EB, Chapman JA, Link MA. Assessment of tumor size for multifocal primary breast cancer. Ann Surg Oncol. 1998;5:442–6.
15. Joergensen LE, Gunnarsdottir KA, Lanng C, Moeller S, Rasmussen BB. Multifocality as a prognostic factor in breast cancer patients registered in Danish Breast Cancer Cooperative Group (DBCG) 1996–2001. Breast. 2008;17:587–91.
16. Pedersen L, Gunnarsdottir KA, Rasmussen BB, Moeller S, Lanng C. The prognostic influence of multifocality in breast cancer patients. Breast. 2004;13:188–93.
17. Tot T. Clinical relevance of the distribution of the lesions in 500 consecutive breast cancer cases documented in large-format histologic sections. Cancer. 2007;110(11):2551–60.
18. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti AIII, et al. AJCC cancer staging manual, vol. 7. New York, NY: Springer; 2010.
19. Sobin LH, Gospodarowicz MK, Wittekind C, editors. TNM classification of malignant tumors 7. Oxford: Wiley-Blackwell; 2009.
20. Boros M, Marian C, Moldovan C, Stolnicu S. Morphological heterogeneity of the simultaneous ipsilateral invasive tumor foci in breast carcinoma: a retrospective study of 418 cases of carcinomas. Pathol Res Pract. 2012;208(10):604–9.
21. Boros M, Ilyes A, Nechifor Boila A, Moldovan C, Eniu A, Stolnicu S. Morphologic and molecular subtype status of individual tumor foci in multiple breast carcinoma. A study of 155 cases with analysis of 463 tumor foci. Hum Pathol. 2014;45(2):409–16.
22. Boros M, Voidazan S, Moldovan C, Georgescu R, Toganel C, Moncea D, et al. Clinical implications of multifocality as a prognostic factor in breast carcinoma: a multivariate analysis study comprising 460 cases. Int J Clin Exp Med. 2015;8(6):9839–46.
23. Tavassoli FA. Pathology of the breast. 2. Appleton and Lange: Stamford, CT; 1999.
24. Lakhani SR, Ellis IO, Schnitt S, Tan PH, van de Vijver MJ. World Health Organization classification of tumors of the breast. 4th ed. Lyon: IARC Press; 2012. p. 10–71.
25. Ellis IO, Galea M, Broughton N, Locker A, Blamey RW, Elston CW. Pathological prognosis factors in breast carcinoma. II. Histologic type. Relationship with survival in a large study with long-term follow-up. Histopathology. 1992;20:479–89.
26. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 1991;19:403–10.
27. Roylance R, Gorman P, Harris W, Liebmann R, Barnes D, Hanby A, et al. Comparative genomic hybridization of breast tumors stratified by histological grade reveals new insights into the biological progression of breast cancer. Cancer. 1999;59:1433–6.
28. Sotiriou C, Wirapati P, Loi S, Harris A, Fox S, Smeds J, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst. 2006;98:262–72.
29. Ellis IO, Elston CW. Histologic grade. In: O'Malley FP, Pinder SE, Mulligan AM, editors. Breast pathology. Philadelphia, PA: Elsevier; 2006.
30. Ro JY, Silva EG, Gallager HS. Adenoid cystic carcinoma of the breast. Hum Pathol. 1987;18(12):1276–81.
31. Rosen PP. Adenoid cystic carcinoma of the breast. A morphologically heterogeneous neoplasm. Pathol Annu. 1989;24Pt2:237–54.
32. Pinder SE, Ellis IO, Galea M, O'Rourke S, Blamey RW, Elston CW. Pathological prognostic factors in breast cancer. III. Vascular invasion: relationship with recurrence and survival in a large series with long-term follow-up. Histopathology. 1994;24:41–7.
33. Carlomagno C, Perrone F, Lauria R, de Laurentiis M, Gallo C, Morabito A, et al. Prognostic significance of necrosis, elastosis, fibrosis and inflammatory cell reaction in operable breast cancer. Oncology. 1995;52:272–7.
34. Ishihara A, Tsuda H, Kitagawa K, Yoneda M, Shiraishi T. Morphological characteristics of basal-like subtype of breast carcinoma with special reference to cytopathological features. Breast Cancer. 2009;16(3):179–85.
35. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015;26:259–71.
36. Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Lawrence N, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials. ECOG 2197 and ECOG 1199. J Clin Oncol. 2014;32(27):2959–66.
37. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Onco. 2014;25:1544–50.
38. Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28:105–13.
39. Schnitt SJ, Connolly JL, Harris JR, Hellman S, Cohen RB. Pathologic predictors of early local recurrence in stage I and II breast cancer treated by primary radiation therapy. Cancer. 1984;53(5):1049–57.
40. Schnitt SJ, Connolly JL, Khettry U, Mazoujian G, Brenner M, Silver B, et al. Pathologic finding on re-excision of the primary site in breast cancer patients considered for treatment by primary radiation therapy. Cancer. 1987;59(4):675–81.
41. NCCN. NCCN clinical practice guideline in oncology (NCCN guidelines). NCCN.org: Breast Cancer; 2017.

42. Houssami N, Macaskill P, Marinovich ML, Dixon JM, Irwig L, Brennan ME, et al. Meta-analysis of the impact of surgical margins on local recurrence in women with early-stage invasive breast cancer treated with breast-conserving therapy. Eur J Cancer. 2010;46(18):3219–32.
43. Moran MS, Schnitt SJ, Giuliano AE, Harris JR, Khan SA, Horton J, et al. Society of Surgical Oncology-American Society for Radiation Oncology consensus guideline on margins for breast-conserving surgery with whole-breast irradiation in stages I and II invasive breast cancer. J Clin Oncol. 2014;32(14):1507–15.
44. Vinh-Hung V, Nguyen NP, Cserni G, Truong P, Woodward W, Verkooijen HM, et al. Prognostic value of nodal ratios in node-positive breast cancer: a compiled update. Future Oncol. 2009;5(10):1585–603.
45. Martin FT, O'Fearraigh C, Hanley C, Curran C, Sweeney KJ, Kerin MJ. The prognostic significance of nodal ratio on breast cancer recurrence and its potential for incorporation in a new prognostic index. Breast J. 2013;19(4):388–93.
46. Wilson RE, Donegan WL, Mettlin C, Natarajan N, Smart CR, Murphy GP. The 1982 national survey of carcinoma of the breast in the United States by the American College of Surgeons. Surg Gynecol Obstet. 1984;159:309–18.
47. Hurvos AG, Hutter RV, Berg JW. Significance of axillary macrometastases and micrometastases in mammary cancer. Ann Surg. 1971;173(1):44–6.
48. Chen SL, Hoehne FM, Giuliano AE. The prognostic significance of micrometastases in breast cancer: a SEER population-based analysis. Ann Surg Oncol. 2007;12:3378–84.
49. Weaver DL. Pathology evaluation of sentinel lymph nodes in breast cancer: protocol recommendations and rationale. Mod Pathol. 2010;23(Suppl 2):S26–32.
50. Weaver DL, Ashikaga T, Krag DN, Skelly JM, Anderson SJ, Harlow SP, et al. Effect of occult metastases on survival in node-negative breast cancer. N Engl J Med. 2011;364:412–21.
51. Weaver DL. Sentinel node biopsy and lymph node classification in the 6th edition staging manual. In: O'Malley FP, Pinder SE, Mulligan AM, editors. Breast pathology. Philadelphia, PA: Elsevier; 2006. p. 257.
52. Daien CI, Monnier A, Claudepierre P, Constantin A, Eschard JP, Houvenagel E, et al. Sarcoid-like granulomatosis in patients treated with tumor necrosis factor blockers: 10 cases. Rheumatology (Oxford). 2009;48(8):883–6.
53. Stolnicu S, Preda O, Kinga S, Marian C, Nicolau R, Andrei S, et al. Florid papillary endosalpingiosis of the axillary lymph nodes. Breast J. 2011;17(3):268–72.
54. Takhar AS, Ney A, Patel M, Sharma A. Extramedullary haematopoiesis in axillary lymph nodes following neoadjuvant chemotherapy for locally advanced breast cancer. BMJ Case Rep. 2013;pii:bcr2013008943. https://doi.org/10.1136/bcr-2013-008943.
55. Hoda SA, Resetkova E, Yusuf Y, Cahan A, Rosen PP. Megakaryocytes mimicking metastatic breast carcinoma. Arch Pathol Lab Med. 2002;126(5):618–20.
56. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham prognostic index in primary breast cancer. Breast Cancer Res Treat. 1992;22:207–19.
57. Mohsin SK. Molecular markers in invasive breast cancer. In: O'Malley FP, Pinder SE, Mulligan AM, editors. Breast pathology. Philadelphia, PA: Elsevier; 2006. p. 267.
58. Köninki K, Tanner M, Auvinen A, Isola J. HER2 positive breast cancer: decreasing proportion but stable incidence in Finnish population from 1982 to 2005. Breast Cancer Res. 2009;11:R37.
59. Ménard S, Fortis S, Castiglioni F, Agresti R, Balsari A. HER2 as a prognostic factor in breast cancer. Oncology. 2001;61(Suppl 2):67–72.
60. Wolff AC, Hammond EH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer. American Society of Clinical Oncologu/College of American Pathologists clinical practice guideline update. J Clin Oncol. 2013;31:3997–4013.
61. Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, et al. Tailoring therapies – improving the management of early breast cancer: St Gallen international expert consensus on the primary therapy of early breast cancer 2015. Ann Oncol. 2015;26(8):1533–46.
62. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the international Ki67 in breast cancer working group. J Natl Cancer Inst. 2011;103:1656–64.
63. Denkert C, Liobl S, Müller BM, Eidtmann H, Schmitt WD, Eiermann W, et al. Ki67 levels as predictive and prognostic parameters in pretherapeutic breast cancer core biopsies: a translational investigation in the neoadjuvant GeparTrio trial. Ann Oncol. 2013;24:2786–93.
64. Denkert C, Budczies J, von Minckwitz G, Wienert S, Loibl S, Klauschen F. Developing Ki67 as a useful marker. Breast. 2015;24(Suppl 2):S67–72.
65. Polley MY, Leung SC, Gao D, Mastropasqua MG, Zabaglo LA, Bartlett JM, et al. An international study to increase concordance in Ki67 scoring. Mod Pathol. 2015;28(6):778–86.
66. Boros M, Moncea D, Moldovan C, Podoleanu C, Georgescu R, Stolnicu S. Intratumoral heterogeneity for Ki-67 index in invasive breast carcinomas: a study on 131 consecutive cases. Appl Immunohistochem Mol Morphol. 2017;25(5):338–40.
67. van de Vijver MJ, He YD, Van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med. 2002;347(25):1999–2009.
68. Zujewski JA, Kamin L. Trial assessing individualized options for treatment for breast cancer: the TAILORx trial. Future Oncol. 2008;4(5):603–10.
69. McVeigh TP, Hughes LM, Miller N, Sheehan M, Keane M, Sweeney KJ, et al. The impact of Oncotype DX testing on breast cancer management and chemotherapy prescribing patterns in a tertiary referral center. Eur J Cancer. 2014;50(16):2763–70.
70. Aalders KC, Kuijer A, Straver ME, Slaets L, Litiere S, Viale G, et al. Characterisation of multifocal breast cancer using the 70-gene signature in clinical low-risk patients enrolled in the EORTC 10041/BIG 03-04 MINDACT trial. Eur J Cancer. 2017;79:98–1.
71. Cardoso F, Piccart-Gebhart M, Van't Veer L, Rutgers E, TRANSBIG Consortium. The MINDACT trial: the first prospective clinical validation of a genomic tool. Mol Oncol. 2007;1(3):246–51.
72. Cardoso F, Van't Veer L, Rutgers E, Loi S, Mook S, Piccart-Gebhart MJ. Clinical application of the 70-gene profile: the MINDACT trial. J Clin Oncol. 2008;26(5):729–35.
73. Mook S, Van't Veer L, Rutgers EJ, Piccart-Gebhart MJ, Cardoso F. Individualization of therapy using mammaprint: from development to the MINDACT trial. Cancer Genomics Proteomics. 2007;4(3):147–55.