Current and future pathological examination in breast cancer

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Introduction

The diagnosis and treatment of breast cancer has rapidly evolved over the past 20 years. In the first 80 years of the 20th century, treatment of breast cancer consisted of radical mastectomy; but adjuvant systemic treatment and adjuvant radiotherapy did not play a major role. Diagnosis of breast cancer was mostly made based on clinical presentation, later aided by mammography and often combined with frozen section pathology confirmation.

Starting in the 1980s, there have been important alterations in the diagnosis and treatment of breast cancer, having an important impact on the diagnostic procedures employed by pathologists and leading to increasingly refined "tailored" treatment, requiring "tailored" diagnosis.

Radiological techniques have greatly improved, and in addition, population-based mammography screening is increasingly offered, especially to women over 50 years of age. These developments have led to the detection of many small non-palpable lesions, including ductal carcinoma in situ.

When an invasive carcinoma is diagnosed, the treatment decisions that need to be taken include:

For local treatment:

Mastectomy versus breast-conserving therapy

For mastectomy patients: whether radiotherapy needs to be given; and to which sites (chestwall; axilla; parasternal lymph node chain)

For patients undergoing breast-conserving therapy: extent of the local excision; the extent of radiotherapy

For adjuvant systemic treatment:

Whether adjuvant treatment is required,

If such treatment is required: hormonal therapy, chemotherapy, or both

For hormonal therapy and chemotherapy: which agents should be given.

Histopathological features play an important role in guiding these decisions. In addition, genetic research is starting to have an increasing impact on guiding therapy by providing predictive and prognostic factors.

In this article, the pathology work-up of breast specimens (including biopsies) is discussed and the minimum requirements for a pathology report are indicated (Table 1).

In addition, the emerging findings from genetic research are described with emphasis on developments with direct clinical relevance.

Table 1
What should be described in a pathology report for invasive carcinoma

Type of surgery
Tumour diameter
Tumour type (WHO classification)
Tumour grade (Elston–Ellis method)
Amount and type of carcinoma in situ component
Status of the resection margins (distance to the margins of invasive carcinoma and carcinoma in situ component in millimetres)
Presence/absence of lymphangio-invasive growth
Oestrogen receptor status
Progesterone receptor status
HER2 status
Sentinel node biopsy:
Number of lymph nodes
Presence/absence of metastases
Diameter of metastases in mm
Axillary lymph node dissection:
Number of lymph nodes with metastases
Total number of lymph nodes
Diameter of the metastases; involvement of resection margins
Status of most distant lymph node

Pre-treatment diagnosis

Diagnosis of breast lesions is based on clinical examination, radiology and pathology. Only by combining the findings for each of these diagnostic disciplines can a reliable diagnosis be given. When abnormalities are found, the diagnostic findings should be discussed among specialists from the three disciplines involved in the diagnostic work-up: the surgeon, the radiologist and the pathologist. The important implication of this multidisciplinary approach for the pathologist is that diagnostic conclusions should always involve the

findings of the other disciplines, most notably the radiological findings.

The following diagnostic procedures are discussed in more detail:
- Fine needle aspiration
- Core needle biopsy
- Incisional/excisional biopsy

Fine needle aspiration (FNA)

This diagnostic approach is very suitable for palpable masses; a thin needle is used to aspirate cells from such a mass. The cells are then placed on a microscope slide and carefully processed into a thin layer of cells, usually by applying a second microscope glass. The cells are then fixed and stained. As fixation and staining can be performed within 5 minutes, this procedure is very suitable for obtaining a rapid diagnosis.

Of course the most important differential diagnosis that has to be solved by fine needle aspiration is between carcinoma and a benign tumour. The most frequently occurring benign tumour in this setting is a fibroadenoma.

If a primary breast carcinoma is present, combining clinical, radiological and cytological features can lead to a final diagnosis in up to 80% of patients. Based on the final diagnosis reached with this triple diagnostics approach definitive treatment can be planned.

While judging a cytological preparation, other differential diagnostic possibilities that should be considered include adenoma of the breast, papilloma, malignant lymphoma, sarcoma, and metastasis of a primary malignancy outside the breast.

When a tumour is not palpable but visible under ultrasound, ultrasound-guided fine needle aspiration is also possible to obtain a diagnosis.

Some investigators have also used fine needle aspiration to obtain a diagnosis when microcalcifications are present. In my personal view, fine needle aspiration is not very suitable for such situations and should be used only when a mass is present in the breast.

In addition to diagnosing a mass as malignant, other features such as histological type and grade, and the amount of ductal carcinoma in situ can be predicted with some degree of certainty based of the cytological findings. Such additional features are, however, usually of limited clinical importance.

Core needle biopsy

Core needle biopsies are taken using thicker needles than those used for FNA, using a device specifically designed to obtain these biopsies. Prior to taking the biopsy, a small incision in the skin is made under local anaesthesia. For breast biopsies, the thickness of the needle is usually 18 or 14 Gauge. Even when a palpable mass is present, the preferred method of obtaining a biopsy is using ultrasound to guide the biopsy device. When a clearly distinguishable mass is present, two or three biopsies are usually sufficient to obtain a definite diagnosis. When the radiological/clinical finding is an architectural distortion or microcalcifications, more biopsies are usually required to obtain a certain diagnosis.

When an abnormality in the breast cannot be detected using ultrasound, most radiology departments have equipment to obtain stereotactical-guided biopsies.

As with FNA, the diagnostic findings in the biopsy should be evaluated in combination with the clinical and radiological findings. The histological diagnosis should fit the clinical and radiological diagnosis.

Incisional/excisional biopsy

The current methods for radiological evaluation in combination with the possibility to obtain fine needle aspirates and core needle biopsies will lead to a diagnosis of most breast lesions, based on which final therapy can be planned. Only in exceptional cases is incisional of excisional biopsy required; frozen sections to obtain a diagnosis have almost become obsolete.

Examination of surgical specimen containing invasive breast cancer

Breast-conserving therapy: wide local excision and lumpectomy specimens

Nowadays, 60–70% of patients undergo breast-conserving therapy. Standardized work up of surgical specimens, obtained as part of breast-conserving therapy, is extremely important. The surgeon should mark the excision specimen so that the pathologist can reconstruct the orientation of the resection margins. Usually it is sufficient to mark the basal resection margin and the resection margin in the direction of the nipple. In combination with the exact location of the tumour within the breast, it can then always be reconstructed where all the resection margins are located.

The resection margins should be marked with ink. At our institute, we use 4 different colours to identify the various resection margins: the resection margins towards the fascia (basal resection margin) is marked with blue ink; margin in the direction of

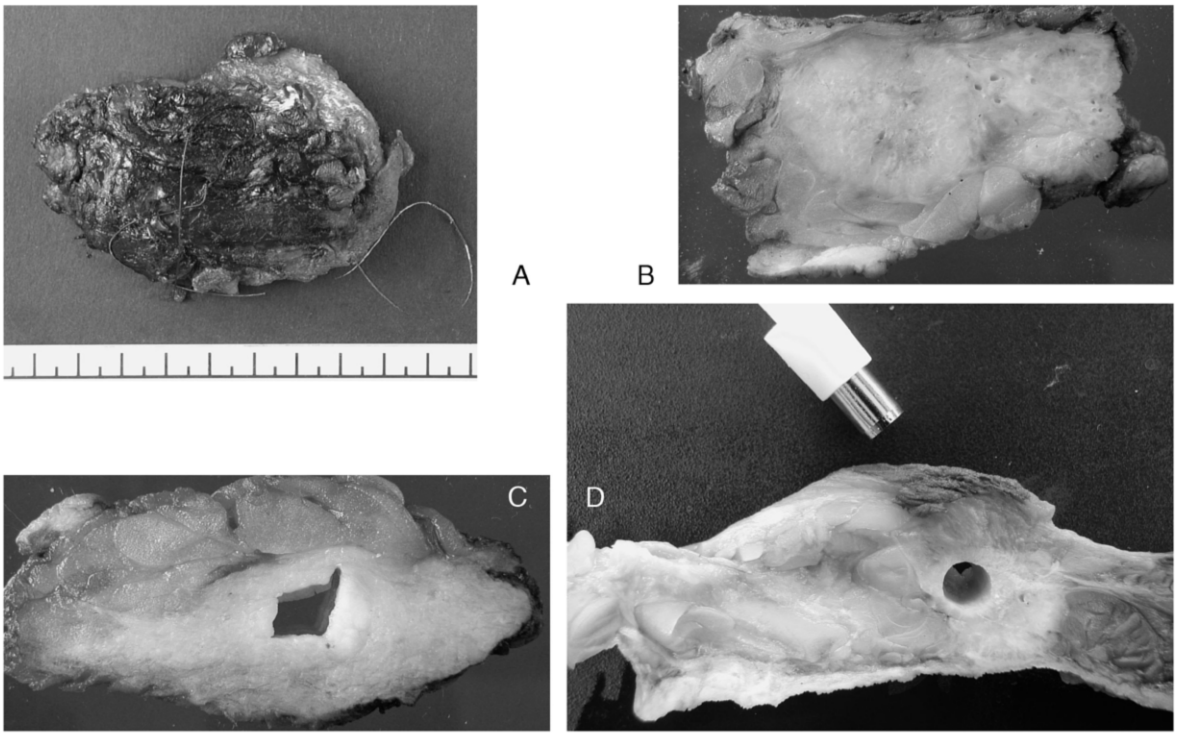

Fig. 1. Work-up of a wide local excision specimen from the breast. The specimen should be sent to the pathology department directly following surgery. (A) Wide local excision specimen from the breast. The sutures have been placed by the surgeon to mark the basal resection margin; and the resection margin towards the nipple. The resection margins have been inked prior to cutting 0.5 cm thick slices. (B) Slice from the specimens shown in (A); centrally, an invasive breast carcinoma is present. (C) Example of central slice similar to the one described in (B), after part of the tumour has been removed for freezing. (D) By using a 0.6 cm core biopsy device, removing part of the tumour for freezing can be done in a more standardized fashion.

the nipple with yellow ink, the specimen is then placed with the basal side downwards and the nipple facing the pathologist; the left resection margin in now marked with green ink; the right margin with red ink (Fig. 1A).

To obtain optimal morphology in the histology sections, and to obtain optimal immunohistochemical staining results (see below), the resection specimen should be cut into thin slices immediately after surgery. At our institute we store the specimen at –20°C, wrapped in aluminum foil. After freezing for 15–30 min, the specimen is then cut into 0.5 cm thick slices (Fig. 1B). This cooling of the specimen is performed to make possible the cutting of thin slices using a fresh specimen. Freezing of the specimen should be avoided to preserve morphology.

The following measurements and information should be recorded: size of the resection specimen in three dimensions; presence and dimensions of skin; presence of localization wires if present.

It is very likely that an increasing number of factors can only be determined using fresh frozen tumour material (see below). It is, therefore, advised to freeze fresh tumour for all invasive breast carcinomas. This can be done by excising a $0.5 \times 0.5 \times 0.5\,cm^3$ piece of tumour tissue (Fig. 1C), preferentially from the periphery of the tumour since some tumours have central sclerosis/fibrosis. Alternatively a biopsy device can be used (Fig. 1D) enabling the pathologist to obtain a standardized piece of tumour tissue. For all tumours larger than 1 cm, it is possible to obtain frozen tumour material in such a way that histological evaluation of the tumour is not affected, and without compromising evaluation of the resection margins.

For microscopic examination the following parts of the specimen should be obtained and processed for paraffin sections: full diameter of the tumour and its surroundings (usually resulting in one to four sections); small part of the tumour to perform immunohistochemistry (see later); if there are macroscopical or radiological abnormalities in the tissue surrounding the invasive tumour, these areas should be sampled, if there are no abnormalities surrounding the invasive tumour mass, at least two sections of macroscopically normal breast tissue should be obtained.

For the invasive tumour, the macroscopic diameter and microscopic diameter should be combined, to provide the final diameter in millimeters for the invasive tumour. Assessing the tumour diameter is extremely important, since therapy decisions, especially decisions on adjuvant systemic treatment, are based on the exact tumour diameter. Often tumours have a stellate configuration, in those cases the core of the tumour should be measured. Thin fibrous projections of the tumour that often contain a small amount of tumour cells, should not be included in the tumour diameter.

The histologic type of the tumour should be assessed according to World Health Organisation (WHO) guidelines. Grading should be performed according to the adaptation by Elston and Ellis of the Bloom and Richardson grading system [1].

The distance of the invasive component of the tumour to the nearest resection margin should be provided in millimetres.

It has been shown that an extensive component of intraductal carcinoma (EIC) which has been incompletely excised, is an important risk factor for local recurrence after breast-conserving therapy [2]. For this reason, the ductal carcinoma in situ (DCIS) component in and around the tumour should be assessed.

The reason that EIC is a risk factor for local recurrence is that a large amount of DCIS may be left behind in the breast after excision in some patients [3]. For this reason, the most important task for the pathologist in this respect is to estimate the likelihood that a large amount of DCIS is left behind in the breast.

In most published series, only poorly differentiated DCIS has been evaluated as a risk factor for local recurrence. Much less is known about residual well differentiated DCIS left behind in the breast; it is likely that an extensive component of well differentiated DCIS is a risk factor for local recurrence, but after a much longer interval.

Lobular carcinoma in situ (LCIS) adjacent to invasive disease has not been associated with an increased risk of local recurrence [4].

In the original publications, an extensive intraductal component has been defined as an area >25% of the invasive tumour being involved with DCIS; in combination with any component of DCIS surrounding the invasive tumour. In these published series, most of the tumours were excised with only a small amount of surrounding normal breast tissue. When a breast tumour is excised with a large amount of normal breast tissues surrounding the tumour (and in most institutes this is the recommended treatment), it should be assessed how much of the normal breast tissue is involved with DCIS. The amount of DCIS should be categorized as: a small amount, a moderate amount, or a large amount of DCIS (Fig. 2).

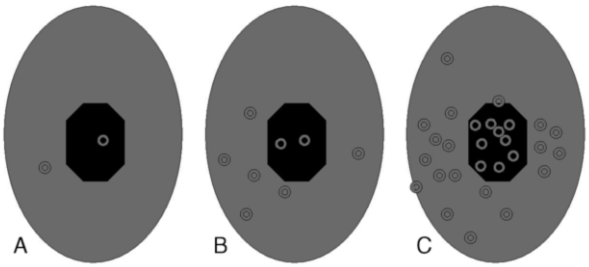

Fig. 2. Illustration of the various amounts of ductal carcinoma in situ (DCIS) that can accompany an invasive breast carcinoma. The grey area is the resection specimen; the black oval structure is the invasive carcinoma; the small round structures are ducts/lobules, involved with DCIS. (A) Small amount of DCIS. Almost every invasive carcinoma is accompanied by DCIS; (B) Moderate amount of DCIS; (C) Large amount of DCIS; this presentation has also been described as "extensive intraductal component". Note that DCIS is also present at the resection margin.

In addition, it should be recorded whether the DCIS component is well differentiated, moderately differentiated or poorly differentiated. When a large amount of poorly differentiated DCIS is present, it should be indicated whether the margins are involved. The margins can be defined as not involved (distance to the resection margins should be provided in millimetres); focally involved; of extensively involved. In our institute, when there is a small or moderate amount of DCIS, the status of the resection margins for DCIS is considered as unimportant for the risk of local recurrence and is not reported.

The presence of tumour emboli in lymph vessels of blood vessels surrounding the invasive tumour should be evaluated and reported.

When satellite foci of invasive breast cancer are present, this should also be recorded.

Mastectomy specimens

Mastectomy specimens should also be sent to the pathology department directly after surgery. The resection specimen should be cut into 5 mm thick lamellae. The specimen is cut at the site of the thoracic fascia with the skin facing downwards, so that the lamellae stay connected by the skin. The distance of the tumour to the skin and to the basal fascia should be recorded. It should be recorded whether the fascia is involved with tumour. When there is tumour involvement of the fascia (which is very rare) the resection margin should be evaluated by inking the margin.

Sections of tumour and surrounding tissue should be taken in the same way as described for breast-conserving therapy specimens. Reporting of the microscopic findings is also similar to that described for breast-conserving therapy specimens.

Examination of axillary lymph nodes

Sentinel node biopsy

On average, one to three sentinel node biopsies are obtained from the axilla; in some patients, sentinel nodes are also obtained from the parasternal region. In many institutes, frozen section evaluation of the sentinel node biopsy is performed. If the sentinel node is found to be tumour positive, a full axillary dissection can immediately be performed.

For frozen section, usually the sentinel node is bisected and both halves are evaluated. After formalin fixation, the sentinel node can be processed for paraffin sections.

There is agreement that 1 hematoxylin and eosin (H&E) stained section of the sentinel node should always be evaluated. There is much discussion on the value of examining various levels of the sentinel node and on performing immunohistochemistry with antibodies directed against keratin for the evaluation of the sentinel node [5,6].

The size of the lymph node metastasis should be categorized as follows:

>2 mm = a macrometastasis

0.2–2 mm = a micrometastasis

<0.2 mm = isolated tumour cells (the tumour cells can often only be detected by immunohistochemistry)

Axillary dissection

When a full axillary dissection is performed, fatty tissue is removed which contains the lymph nodes. The pathologist should carefully identify all the lymph nodes that are present in the resection specimen, and each of these lymph nodes should be embedded for histological examination. Lymph nodes up to 1 cm can be totally embedded, larger lymph nodes should be bisected or lamellated and fully embedded. The number of lymph nodes containing metastases should be recorded. In addition, the diameter of the largest lymph node metastasis should be recorded.

It should be attempted to identify the most distal lymph node in the resection specimen, usually the medial axillary apex; whether this lymph nodes contains tumour cells or not should be separately recorded.

When there is massive involvement of lymph nodes with tumour, the resection margin of the axillary dissection specimen should also be examined.

Examination of surgical specimen containing ductal carcinoma in situ (DCIS)

In population mammographic screening, 20% of the malignant lesions are DCIS, which are detected as microcalcifications on the mammogram [7]. When a surgical specimen containing DCIS is examined, there are two important histopathological features that should be reported: the absence or presence of an invasive component, and margin status.

To detect possible foci of invasive carcinoma it is important to perform adequate sampling of the area of the breast involved with DCIS. It has been found that small foci of invasive carcinoma are present in up to 20% of patients in which DCIS was detected by mammographic screening and in which only microcalcifications are present [8].

It may be difficult to differentiate between true invasion and distortion of ducts when inflammation is present surrounding the ducts and lobules involved with DCIS. Invasive carcinoma should only be diagnosed when an invasive focus can be detected outside the stroma of the lobules.

The surgical margins should be carefully inked and sections should be taken of the areas with DCIS in relation to the resections margins. For all cases of DCIS identified by the presence of microcalcifications, the sections should be taken guided by an X-ray of the tissue lamellae of the surgical specimen. Because DCIS spreads through the ducts and lobules, when a lobule or duct involved with DCIS is present very close to the resection margin, it should be assumed that there is likelihood that ducts and lobules on the other side of the resection margins can also be involved. In such instances the resection margin should be described as tumour. It is not known with certainty what a save margin is. If normal ducts and lobules are present between the resection margin and the DCIS, it can be assumed that the resection margin is free of tumour. It has been shown that the risk of local recurrence after breast-conserving therapy decreases with increasing margin width [9]. To help in decisions on the optimal treatment of DCIS, the Van Nuys Prognostic Index (VNPI) was developed [10]. The VNPI combines three significant predictors of local recurrence: tumor size, margin width, and pathologic

classification. Scores of 1 (best) to 3 (worst) are assigned for each of the three predictors and then totaled to give an overall VNPI score ranging from 3 to 9. It has been suggested that DCIS patients with VNPI scores of 3 or 4 can be considered for treatment with excision only. Patients with intermediate scores (5, 6, or 7) show a 17% decrease in local recurrence rates with radiation therapy. Patients with VNPI scores of 8 or 9 exhibit extremely high local recurrence rates, regardless of irradiation, and should be considered for mastectomy. It should be noted, however, that at present the VNPI is not widely used.

Additional factors: oestrogen receptor, progesterone receptor and HER2

For the treatment of breast cancer, especially the choice of adjuvant systemic treatment, determining oestrogen receptor status, progesterone receptor status, and HER2 status is extremely important. Pathologists should be aware that a false negative or false positive test result can have profound influence on the adjuvant systemic treatment choices, and can possibly lead to decreased survival chances for individual patients.

The detection method for each of these proteins is immunohistochemistry. All laboratories participating in performing immunohistochemistry for the detection of these proteins, should have standardised protocols and internal and external validation of the protocol used.

It is beyond the scope of this article to discuss in detail the various antibodies and immunohistochemical staining techniques used. Some important aspects will be briefly mentioned.

Oestrogen receptor

Staining for oestrogen receptor is always nuclear in localisation. There are several scoring systems to arrive at a semi-quantitative score for oestrogen receptor expression [10,11]. As a general rule, approximately 30% of invasive breast carcinomas do not show any staining, and approximately 50% show strong staining of the majority of the tumour cell nuclei. For these two categories, a semi-quantitative staining score does not appear to be relevant. For the approximately 20% of breast carcinomas that show weak staining and/or staining of part of the tumour cells, a semi-quantitative score may have predictive value for the likelihood of response to hormonal therapy. In most institutes, all patients with a tumour in which more than 10% or more than 1% of the tumour cells show positive staining are candidates for adjuvant hormonal therapy.

Progesterone receptor

The staining pattern for progesterone receptor is very similar to that for oestrogen receptor. The percentage of progesterone receptor positive breast cancers is smaller than that of oestrogen receptor positive breast cancers. Personally, I have only very rarely come across breast carcinomas where the oestrogen receptor was negative and the progesterone receptor was positive (<1% of the cases seen). It is likely that many are progesterone receptor-positive, oestrogen receptor-negative breast carcinomas are the result of false negative oestrogen receptor staining.

When negative staining for oestrogen receptor and/or progesterone receptor is seen, it is important to confirm that the staining of the hormone receptor-negative case has been successful. This can usually be tested, since the majority of normal breast tissues contain some nuclei in ducts and lobules that are positive for oestrogen and progesterone receptor. If no normal breast epithelial cells are found to show positive staining, the hormone receptor assays should be repeated on another tumour block. If no normal breast tissue can be found to show positive staining, it should be noted that the hormone receptor essay is possibly false negative.

HER2

HER2 gene amplification is observed in 15–30% of invasive breast cancers and leads to HER2 receptor overexpression. It is an early event in cancer but is also associated with aggressive disease and poor clinical outcome in most, but not all, studies [12,13]. Taken together, these factors provided the rationale for the development of antibody-based therapy with trastuzumab, which was specifically designed to target HER2 [14]. In addition to predicting response to trastuzumab, data suggest that HER2 positivity may predict relative sensitivity to anthracycline- and taxane-based regimens and indicates decreased sensitivity to tamoxifen and CMF [15].

Several technologies are available for determining HER2 status, but the two most commonly used are immunohistochemistry (IHC), which measures HER2 protein overexpression, and fluorescence in-situ hybridisation (FISH), which detects HER2 gene amplification. IHC scoring provides a semi-quantitative interpretation of HER2 expression, based on the intensity and percentage of stained cells. The most commonly used IHC scoring system considers a score

of ' 0' and ' 1+' as HER2 negative. A ' 2+' result is considered equivocal and should be followed by retesting by FISH. Only ' 3+' unequivocally indicates HER2 positivity. Clinical outcomes data demonstrate similar clinical benefits in patients with IHC 3+ and/or FISH-positive tumours, supporting the diagnostic findings that a score of IHC 3+ and FISH-positivity are highly concordant and can identify patients eligible for trastuzumab. Based on these findings, the testing algorithm for HER2 is as follows: if IHC is performed as the initial HER2 assessment test, women with IHC 3+ tumours are eligible for trastuzumab, while those with IHC 0/1+ tumours are not eligible. Women whose tumours are equivocal (IHC 2+) should be retested with FISH, with a positive result indicating eligibility for trastuzumab. According to the algorithm, FISH may also be conducted as the initial assay for HER2 status determination, with positivity indicating eligibility for trastuzumab.

To ensure the highest possible accuracy, pathology centers must standardise methodologies and testing procedures, regularly validate their testing procedures, implement quality control and quality assurance measures, and have adequate experience of performing the techniques.

FISH can also be replaced by chromogenic in-situ hybridisation (CISH) [16], which is based on similar methodology to FISH, but it uses a chromogenic reaction, similar to that used with IHC.

Prognostic and predictive factors

In the past 20 years, much research has been devoted to identify prognostic and predictive factors, especially to guide adjuvant systemic therapy.

All this research has resulted in thousands of scientific papers, but only recently have some of these assays started to be used in day-to-day clinical decision-making, mainly in the form of incorporation of these assays into clinical trails. It is to be expected that in the coming years the process of bringing this knowledge from scientific research into the clinic will be proceeding at a higher speed than we have seen in the past 20 years.

Genetic alterations

Through the great advances in molecular biology, differences between cancer cells and their normal counterparts can be analysed at the molecular genetic level. These assays have led to an impressive catalogue of genetic alterations in malignancies and also in breast cancer.

The heterogeneity of breast cancer is also reflected by the genetic alterations that have been identified. Most genetic alterations are present in approximately 15–20% of all tumours and there are no common genetic alterations that can be found in the majority of breast carcinomas.

To date, mainly amplification of approximately 10 different chromosomal regions and inactivation of a limited number of tumour suppressor genes have been found. The presence of frequent loss of heterozygosity for many chromosomal regions indicates that more tumour suppressor genes are likely to be present and will be identified in the coming years.

For clinical practice, the following genetic alterations are of importance at present:

- HER2 gene amplification, which has been discussed in the previous section.
- Germ-line mutations in the BRCA1 and 2 genes [17]: these mutations are associated with an 80% lifetime risk of breast cancer. In counselling families with hereditary breast cancer, genetic testing is routinely performed to identify mutations in these two breast cancer predisposing genes.
- Mutations in the E-cadherin gene are specific for lobular breast cancer [18].

At present, no associations of specific genetic alterations with clinical behaviour have led to clinical testing. As some of these genetic alterations may lead to novel targeted therapies, this situation may change in the future. As this chapter is mainly aimed at discussing clinically relevant factors, a more detailed discussion of the genetic alterations in breast cancer is beyond the scope of this chapter.

Gene expression profiles

DNA microarrays

DNA microarrays use gene chip technology to simultaneously measure the mRNA expression of several thousand genes in biological specimens. Using this approach, prognostic subgroups of breast carcinomas have been identified. Using supervised classification, an expression profile of 70 genes has been identified that can predict distant metastasis-free probability in node-negative breast cancer patients younger than 53 years of age [19,20]. More recently, a gene expression profile consisting of 76 genes was identified [21] by a similar approach that led to the identification of the 70-gene profile. Other investigators have also identified subgroups of tumours characterized by specific gene expression profiles, which are associated with outcome [22]. These are recent results and at present

there is not yet a gene expression pattern that integrates all of these studies. Prospective studies are now underway to validate the findings of these retrospective gene expression-profiling studies. Taken together, the data show the feasibility of this approach, but do not yet justify clinical application of microarray-based tests. Despite these promising results, more studies are needed with larger numbers of uniformly treated patients to determine whether adjuvant therapy, or a particular type of adjuvant therapy, improves prognosis in patients with an adverse genetic profile. A prospective randomised trial incorporating the 70-gene prognosis profile [19] is now being planned by the Breast Inter Group (BIG); the acronym for this study is: Microarray for Node-Negative Disease may Avoid Chemotherapy (MINDACT).

The use of DNA microarray technology to identify a prognostically predictive gene expression signature is but one of its many potential applications in breast cancer management. There is also evidence that the technology may be able to define a multigene predictor of complete pathological response to a particular primary systemic chemotherapy regimen [23–25]; and also predict responsiveness to hormonal therapy [26]. Gene expression profiles can distinguish sporadic breast cancer cases from those associated with mutations in the BRCA1 and 2 genes [19,27], and can distinguish novel molecular classes of breast cancer, including luminal and basal epithelial cell subtypes [28]. The differential expression of genes in tumour versus normal tissue may also help in identifying novel therapeutic targets for the development of drugs.

Multigene RT-PCR

Quantitative real-time polymerase chain reaction (RT-PCR) has been used to develop a prognostic gene expression classifier in node-negative, ER-positive breast cancer after adjuvant therapy with tamoxifen. This multigene RT-PCR assay allows quantitation of gene expression from fixed, paraffin-embedded tissue samples [29]. Candidate genes were selected from the literature, including published results of DNA microarray studies, and then tested in three independent breast cancer studies to identify an optimal gene panel for clinical validation. The resulting 21-gene panel comprised 16 cancer and 5 reference genes. The cancer genes were associated mainly with proliferation, invasion, and the estrogen and HER2 pathways. The gene panel was then prospectively validated in the tamoxifen arm of the large, multicenter NSABP B-14 clinical trial.

The assay was successful in 99% of the patients tested, indicating the feasibility and reproducibility of the technique. Regression analysis was used to calculate a recurrence score (RS), predicting the probability of disease- and relapse-free survival (DRFS) up to 16 years after diagnosis. The test for the 10-year DRFS comparison between the low- and high-risk groups was highly statistically significant ($p < 0.00001$). These findings indicate that the RS accurately and precisely predicts the likelihood of distant recurrence.

A prospective adjuvant clinical trial incorporating this assay is now planned by the North American Breast Intergroup.

Proteins: from immunohistochemistry to proteomics

The term proteome describes all the proteins expressed by a tissue. Proteomics includes the characterization of proteins and their post-translational modifications, differential display for comparison of proteins in different disease states, and studies of protein–protein and protein–DNA/RNA interactions. It employs high throughput techniques, including protein arrays, two-dimensional gel electrophoresis, and mass spectroscopy. These techniques are currently being used to analyse serum from breast cancer patients, and protein isolated from breast carcinomas. At present, there are no results from these high throughput proteomics studies that can already be applied in the clinic. In the coming years, it is likely that important developments will take place in this research area.

Immunohistochemistry (IHC) is one of the most convenient methods to study protein expression in paraffin embedded clinical samples. IHC for the detection of ER, PR and HER2 has been discussed in the previous section. IHC has also been used to study many potential prognostic factors, but none of these is presently in clinical use.

An important prognostic factor is the combined determination of urokinase-type plasminogen activator (uPA) and its inhibitor, activator inhibitor type 1 (PAI-1), by ELISA of protein lysates from breast carcinomas. The prognostic impact of both factors in primary breast cancer has been substantiated by pooled analysis of large series of patients and in a multicentre prospective randomised therapy trial in node-negative breast cancer [30]. Patients with node-negative breast cancer with low antigen levels of uPA and PAI-1 in their primary tumour tissue have a very good prognosis and, therefore, may be spared the burden of adjuvant chemotherapy, whereas those with elevated uPA/PAI-1 antigen levels carry an increased risk of disease recurrence.

Discussion

Histologic tumour assessment is used to determine tumour size, type and grade, and serves to evaluate the resection margins and lymph node status. These pathological features play a key role in providing optimal therapy tailored to individual patients. The assessment of these factors should be standardized and subjected to quality control in every institute. Assessment of oestrogen receptor, progesterone receptor, and HER2 status should be performed for every breast carcinoma. The methods to assess these factors should also be standardized and subjected to quality control.

Genetic tests to identify mutations in the BRCA1 and 2 genes are available to evaluate individuals with a family history of breast cancer.

Recent developments in molecular technologies have resulted in greater understanding of the processes/pathways involved in tumourigenesis and cancer development, and it is now apparent that tumours with similar phenotypes can actually be genetically quite distinct. The application of molecular diagnostics is beginning to show improvements over existing clinicopathologic marker assessment. Single-marker diagnostic tests are already a reality in the clinical setting and the best examples are ER status and HER2 status.

Data suggest that gene expression profiling have exciting potential in the development of new diagnostic tests to predict prognosis and response to specific therapy regimens.

Taken together, there is an ongoing increase in the therapy choices that have to be made for individual patients. This is paralleled by an ongoing increase in the diagnostic possibilities to provide optimal patient tailored treatment of breast cancer.

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