The role of the pathologist in the decision-making process

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1. Introduction

During the last two decades the pathological classification of breast carcinoma has evolved rapidly. Starting from the pure assessment of conventional morphology, it has gradually been integrated with immunphenotypic evaluation of the hormone receptor, HER2, and Ki67 status. In addition, molecular genetic testing (mostly by fluorescence in situ hybridisation, FISH) for Her2 immunohistochemically ‘equivocal’ cases has become a standard. Pathological evaluation of breast specimens has shifted rapidly from a mere diagnostic process, aimed at establishing the biological potential of a breast ‘lump’, to a far more complex integration of diagnostic, prognostic and predictive parameters. The current landscape has been further complicated by the relatively recent introduction of a ‘molecular’ classification of breast cancer[1]. Since then pathologists and clinicians have struggled in the attempt to translate (or maybe to force) the classic morphological approach into a molecularly based scheme (Table 1).

Whatever the approach, the role played by the pathologist in the clinical decision-making process has never been so central. Establishing the correct diagnosis, as well as accurately evaluating key prognostic/predictive biomarkers, represent the core of the breast cancer pathology report. Even acknowledging the current complexity of personalised treatments, it is broadly accepted that the information mandatory for inclusion in the pathology report represents a milestone for optimal therapeutic planning.

2. Pathological diagnosis

The pathological diagnosis of breast carcinoma still represents the key step. Before considering the complex integration of predictive and prognostic markers, it should not be overlooked that the diagnosis of breast cancer is not always straightforward. The presence, within the breast cancer multidisciplinary team, of a skilled breast pathologist represents a fundamental prerequisite in order to achieve optimal therapeutic planning.

The World Health Organisation (WHO) has recently updated its breast cancer classification, separating invasive breast carcinoma into two broad categories: invasive carcinoma of no special type (formerly known as invasive ductal carcinoma) and special subtypes (Table 2). The recognition of special subtypes is relevant as distinct morphologies often correlate with distinct clinical outcomes [2].

Once the correct diagnosis of invasive carcinoma is made, pathologists are asked to provide a set of morphological parameters representing important clues to prognostic stratifications. These include the size of the lesion, the presence of lymphatic and blood vessel invasion, the status of lymph nodes and the histological grading (Table 3). The currently adopted grading system is that devised by Elston and Ellis, and represents a powerful prognostic tool that represents a key factor in clinical decision-making [3]. The so-called Nottingham system is based on the evaluation of differentiation (as expressed by the amount of tubule formation), nuclear pleomorphism (by comparing neoplastic cell nuclei with adjacent normal breast epithelial cells) and mitotic activity (as expressed by number of mitoses counted per 10 high-power fields). Of course the dimension of a ‘high-power field’ depends on the size of the microscope. The WHO, in its most recent classification, has therefore provided a conversion table aimed at minimising inter-observer variability [2].

As shown, pathological evaluation of haematoxylin-and-eosin-stained slides still represents the cornerstone of breast cancer diagnosis. Even though molecular testing is playing an increasingly key role in several fields of cancer, it is extremely important that morphological expertise is not lost, and that educational efforts are supported in order to maintain diagnostic skills to the highest possible standard.

3. Evaluation of predictive/prognostic markers

The three main biomarkers used in the routine clinical management of invasive breast carcinoma are represented by the oestrogen receptor (ER), progesterone receptor (PR) and HER2. More recently, the evaluation of the Ki67 labelling index has

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The expression of the ER has been abolished as even 1% of positive cells variably expresses the ER. Any cut-off the help of digital imaging tools. Approximately 80% of invasive breast carcinoma variably expresses the ER and response to hormonal therapy [8]. The ER regulates PR expression, and therefore the presence of the latter gives testament to the functional integrity of the ER pathway [9]. Expression of the PR is detected in approximately 60–70% of invasive breast cancers, and as with the ER there is direct correlation between its level of expression and response to hormonal therapy [8,10].

The best estimation of response to hormone therapy is generated by the combination of both ER and PR expression [11]. The combination ER+/PR+ accounts for approximately 70% of invasive breast cancers and correlates with the best anti-oestrogen response (60%). Approximately 25% of patients exhibit an ER-/PR- phenotype which predicts unresponsiveness to hormone therapy. The ER-/PR- cases are associated with intermediate levels of response, whereas the very existence of true ER-/PR+ cases is still the source of sharp debate.

HER2 (ERBB2) represents a proto-oncogene located on chromosome 17 and is amplified in approximately 15% of breast invasive carcinomas [12]. HER2 amplification strongly correlates with protein over-expression that can therefore be detected immunohistochemically. HER2 represents both a prognostic and a predictive biomarker. HER2 amplification not only correlates with poorer outcome [13] but also predicts response to molecular targeted therapies aimed specifically against HER2 (i.e. trastuzumab and lapatinib) [14,15]. HER2 status is primarily determined immunohistochemically on FFPE tissue and scored according to broadly accepted guidelines [16]. Cases with strong complete membrane staining in more than 30% of neoplastic cells (so-called 3+) are candidates for anti-HER2 therapy. Negative or weakly positive cases (so called 0 and 1+) are generally excluded, whereas cases with continuous but less strong than 3+ membrane staining undergo FISH to assess the presence of HER2 gene amplification. The best response is seen in cases showing strong HER2 over-expression and/or HER2 gene amplification. Lack of accuracy in HER2 testing represents a major obstacle to correct selection of patients and (analogously to ER/PR testing inaccuracy) may impact on clinical outcomes [17].

4. Towards a molecular classification of breast carcinoma

Molecular analysis of breast carcinoma using a gene expression array approach has led to the recognition of several genetically distinct forms [1]. Gene expression profile assays measure quantitatively in tumour samples the expression of each gene harboured on the array. These techniques generate great amounts of data that need to be analysed with bioinformatic techniques. Two main approaches are most often used: unsupervised hierarchical cluster analysis and supervised classification. Unsupervised approaches analyse gene expression within a series of tumours without using the clinical and/or pathological information available. Hierarchical cluster analysis then subclassifies tumours into distinct subgroups. If the aim of analysis is to identify gene expression patterns predictive of clinical behaviour then a supervised approach seems to be more appropriate. This technique in fact

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Table 1 – Molecular classification of breast cancer.

| Subtype                        | Clinico-pathological definition |
|--------------------------------|---------------------------------|
| Luminal A                      | ER- and/or PR-positive          |
|                                | HER2-negative                   |
|                                | Ki67 low (<14%)                 |
| Luminal B                      | Luminal B (HER2-negative)       |
|                                | ER- and/or PR-positive          |
|                                | HER2-negative                   |
|                                | Ki67 high                       |
|                                | Luminal B (HER2-positive)       |
|                                | ER- and/or PR-positive          |
|                                | HER2-positive                   |
|                                | Any Ki67                        |
| HER2-positive (non-luminal)    | HER2-positive                   |
|                                | ER- and PR-negative             |
| Basal-like                     | Triple-negative (no special type)|
|                                | ER- and PR-negative             |
|                                | HER2-negative                   |

Table 2 – WHO classification of breast cancer.

| Invasive carcinoma of no special type | Special types: |
|--------------------------------------|----------------|
|                                      | • Invasive lobular carcinoma |
|                                      | • Tubular carcinoma |
|                                      | • Cribriform carcinoma |
|                                      | • Carcinoma with medullary features |
|                                      | • Metaplastic carcinoma |
|                                      | • Carcinoma with apocrine differentiation |
|                                      | • Salivary gland/skin adnexal type tumours |
|                                      | • Adenoid cystic carcinoma |
|                                      | • Mucoepidermoid carcinoma |
|                                      | • Polymorphous carcinoma |
|                                      | • Mucinous carcinoma (including signet ring variant) |
|                                      | • Carcinoma with neuroendocrine features |
|                                      | • Invasive papillary carcinoma |
|                                      | • Invasive micropapillary carcinoma |
|                                      | • Inflammatory carcinoma |
|                                      | • Exceptional rare types and variants |
specifically correlates gene expression with key clinical parameters such as overall or disease-free survival as well as response to a given therapy.

The unsupervised hierarchical cluster analysis of breast carcinoma has led to a broad division into ER+ and ER- cases [18,19]. If the set of genes expressed by the two categories is examined closely, ER+ cases are linked to breast luminal cells, whereas ER- cases are associated with myoepithelial cells. The next step is the correlation of these subgroups with clinical outcomes. This approach has led to the definition of the entities (intrinsic subtypes) listed in Table 1: namely types luminal A and B, HER2 and basal-like [1,20].

The attempt to correlate gene expression profiles with clinical outcome has generated several gene signatures. The most popular is represented by a 70-gene signature that may determine prognosis in stage-1 or -2 node-negative patients affected by breast carcinomas smaller than 5 cm. The 70-gene signature separates patients into two groups with good and poor prognoses, and appears to work as an independent predictor of metastatic spread [1]. The 70-gene signature has been popularised with the commercial label Mammaprint which has been cleared by the FDA as an in vitro diagnostic multivariate index assay.

An alternative approach is represented by the 21-gene recurrence score [21]. This is a qRT-PCR-based signature commercially named Oncotype DX, that predicts the likelihood of recurrence at 10 years for ER-positive, lymph-node-negative patients. The test provides a continuous recurrence score (RS) and risk category: low (RS < 18), intermediate (RS 18–30) and high (RS > 30). The 21-gene recurrence score apparently may also correlate with benefit from chemotherapy in ER-positive breast cancer patients [21].

The clinical utility of gene expression profiling in breast cancer has generated a lively and still ongoing debate. Even if there is a strong pressure (particularly in the US, United States) towards a broader use of such approaches, their potential benefit seems until now to be restricted to a minority of breast cancer patients. Nonetheless, also in consideration of the rapid evolution (and cost reduction) of molecular genetic techniques, it has to be expected that molecular assays will be implemented increasingly in clinical practice.

5. Conclusions

Pathological evaluation of breast cancer specimens plays a key role in planning the best therapeutic options. In addition to accurate diagnosis of malignancy and cancer subtype, pathologists are central in helping clinicians in the selection of patients for both endocrine therapy as well as for anti-Her2 targeted approaches. Precise evaluation of breast cancer biomarkers still represents a key issue not yet entirely resolved, and it has been shown to impact on clinical decision-making as well as on patient outcome. It is vital that pathology laboratories systematically implement External Quality Control policies aimed at achieving and maintaining the highest diagnostic standard.

The rapid evolution of molecular techniques has in part changed the landscape of breast cancer prognostic biomarkers. The advent of genomic signatures certainly represents a step forward, but their clinical utility is being still debated; complete agreement regarding their clinical as well as their cost-effectiveness is still to be achieved.

Conflict of interest statement

The authors have no conflict of interest relating to this article.

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