Pathological and molecular diagnosis of triple-negative breast cancer: a clinical perspective

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Triple-negative breast cancer (TNBC) is a heterogeneous disease diagnosed by immunohistochemistry and is characterized by tumours that do not express estrogen receptor (ER) or progesterone receptor (PR) at all, and do not overexpress human epidermal growth factor receptor 2 (HER2). Prototypical TNBC is aggressive in nature and associated with a poor prognosis, making the accurate diagnosis of the disease vitally important for ensuring optimal therapy for patients. Morphological and biological analyses can identify subtypes of TNBC, which can have different prognoses, and (in the case of the latter) may eventually be used to predict response to treatment. This mini-review focuses on clinically relevant issues in the diagnosis of TNBC, including the importance of adherence to international guidelines for the detection of ER/PR/HER2 status, and the relationship between TNBC and the overlapping (yet distinct) intrinsic subtype of ‘basal-like’ breast cancer. In addition, we review the potential use of emerging biomarkers as surrogates for molecular subtypes and as a means of identifying potential responders to new therapies.

Key words: biomarkers, diagnosis, molecular, pathological, triple-negative breast cancer

introduction

Breast cancer is a heterogeneous disease that can be subdivided into clinical, histopathological and molecular subtypes [1]. ‘Triple-negative’ breast cancer (TNBC) accounts for ~15% of all breast cancer cases and is characterized by tumours that do not express estrogen receptor (ER), progesterone receptor (PR) or overexpress human epidermal growth factor receptor 2 (HER2) [1]. TNBC is associated with a poor prognosis and a high risk of distant recurrence and death within the first 3–5 years of follow-up [2, 3]. Given the aggressive nature of TNBC, accurate diagnosis of the disease is important for determining prognosis and ensuring optimal therapy for patients.

TNBC is diagnosed based on immunohistochemistry (IHC); however, in clinical practice, a two-step process of morphological imaging and IHC is often employed [1]. Although ultrasound and mammographic techniques can reveal the smooth borders of some TNBC tumours, they do not always efficiently image intratumoural characteristics, such as necrosis and fibrosis, which are typical of TNBC [4]. As such, standard IHC techniques employed in most pathology laboratories are essential for identifying patients with TNBC tumours. Historically, IHC methodologies to detect ER, PR and HER2 status have varied among laboratories and clinical trials [1]; however, recent guidelines by international expert groups are now helping us to standardize these techniques in the hope of improving their reliability and reproducibility.

determination of ER, PR and HER2 status by IHC: clinically relevant issues

In the past few years, joint guidelines by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) on the determination of ER, PR and HER2 status by IHC have been published [5, 6]. These detailed guidelines have also been supplemented by recommendations made during the recent meetings of the San Gallen expert group [7, 8]. Following the publication of these guidelines, it is apparent that several issues are of particular importance to the classification of TNBC tumours in the clinic [5, 6, 8].

samples should be considered ER/PR-positive if at least 1% of the tumour cells are immunoreactive

Prior arbitrary thresholds for discriminating between positive and negative ER and PR status have varied, and it is possible that many physicians are continuing to define ER/PR-negative IHC using the threshold of <10% immunoreactive cells which was previously used in many laboratories [5]. Confusion over this issue may be due to the fact that clinical trials frequently use a <10% threshold when identifying ER/PR-negative tumours. Recent guidelines dictate that a threshold of <1% of cells should be used [5], with the recommendations of the San Gallen expert group indicating adjuvant endocrine therapy in ‘almost all patients whose tumours show evidence of endocrine responsiveness, now defined as the presence of any detectable estrogen receptor’ [8]. The disease-free survival of patients enrolled in the BIG 1–98 trial and treated with endocrine therapy was significantly different according to centrally
assessed ER expression (ER-negative versus 1%–9% versus 10% or more immunoreactive tumour cells) [9]. Furthermore, a more recent study confirmed that the safest clinical approach would be not to deny adjuvant endocrine therapy to the subgroup of patients with 1%–9% ER-positive cells [10].

**internal positive controls for ER/PR staining are of paramount importance to avoid false-negative assays**

When performing IHC staining for ER/PR status, slides should not only be representative of the invasive component of the tumour, but also include normal epithelial elements that have been obviously handled and fixed in a manner that is identical to the tumour tissue [5]. This internal control must display a heterogeneous staining pattern of the luminal cells, with a suitable mixture of cells exhibiting weak, moderate and intense immunoreactivity, together with cells devoid of any immunoreactivity [5]. Assays that only exhibit staining of normal breast epithelial cells with intense immunoreactivity may indicate that the staining reaction was too weak to enable the detection of tumour cells with weak or moderate immunoreactivity [5]. These assays present an increased risk of false-negative assessment and should therefore be discounted. Myoepithelial cells and stromal cells from normal tissue should always be negative for ER/PR; therefore, the use of normal breast tissue also provides an important control for the specificity of the reaction, which is critical when evaluating a negative classification of IHC, such as in TNBC samples.

**additional confirmatory analyses are required in cases of equivocal HER2 status**

The ASCO/CAP guidelines published in 2007 highlighted the importance of additional confirmatory analysis by fluorescent in situ hybridisation (FISH) for IHC samples with equivocal HER2 status [6]. Such samples are defined as exhibiting complete membrane staining that is either non-uniform or weak in intensity but with obvious circumferential distribution in at least 10% of cells (corresponding to a score of 2+). Retrospective analysis of the early trials of trastuzumab demonstrated that only 24% of tumours identified as HER2-overexpressing with IHC 2+ staining had HER2 gene amplification when tested by FISH [11]. Employing routine FISH in equivocal cases of anti-HER2 staining helps ensure that the detection of ‘false-positives’ and ‘false-negatives’ is avoided, alongside the corresponding administration of ineffective yet potentially toxic targeted therapy, or the denial of potentially useful treatment. The ASCO/CAP guidelines define an unequivocally positive HER2 result as uniform intense membrane staining (3+) of >30% of invasive tumour cells [6]; however, this cut-off was not intended to replace the original Food and Drug Administration-approved scoring system (3+ staining of 10% of cells), but rather to improve the correlation between IHC and FISH assays. Newly available types of brightfield in situ hybridisation, such as chromogenic in situ hybridisation and silver-enhanced in situ hybridisation, can also be considered when determining the HER2 status [6, 12]. These assays may facilitate the scrutiny of the hybridised tissue sections, by allowing examination at lower magnification and by making easier the identification of the invasive components of the tumours.

**confirmatory biopsy of secondary tumours could be necessary for effective patient management**

Confirmatory biopsy of relapsed or metastatic lesions is rarely carried out at most centres, even if many years have passed since the initial diagnosis. Nevertheless, it is known that relapsed or metastatic lesions can exhibit different phenotypes when compared with their corresponding primary tumours. Retrospective reviews estimate that the rate of this discordance is around 15%–40% for ER and PR status and 7%–26% for HER2 status [13]. In a prospective study of 258 patients with breast cancer, biopsies of relapsed tumours showed that ER, PR or HER2 status was discordant with the corresponding primary tumour in 13%, 28% and 5% of the samples, respectively [14]. Loss of ER/PR/HER2 expression in relapsed or metastatic lesions is known to be more common than gain of expression, and patients with ER-positive or HER2-overexpressing primary tumours may have triple-negative secondary carcinomas [13, 15]. Importantly, patients with discordant triple-negative biopsies have been shown to have worse post-recurrence survival compared with patients with concordant triple-negative biopsies [16]. The therapeutic ramifications of these observations are considerable, and it has been estimated that confirmatory biopsies of metastatic lesions could alter treatment patterns in up to 20% of breast cancer cases [13]. However, it is also possible that a significant proportion of discordant cases may be due to inaccuracies in the assessment of the primary and/or metastatic biopsy, or more rarely, sampling errors in receptor-negative tumours with small pockets of ER/PR/HER2-positivity [17].

**molecular markers for TNBC and basal-like breast cancer**

The pivotal report by Perou et al. [18] in 2000 redefined the classification of breast cancers, utilising microarray analysis to identify five intrinsic subtypes [18, 19]. Basal-like breast cancers make up 27% of these subtypes, are most frequently triple-negative (alongside the more recently described claudin-low subtype) and are associated with a poor prognosis [19]. A common misconception is that all basal-like breast cancers are TNBC; however, only 77% of basal-like breast cancers are triple-negative, with 71%–91% of TNBC being basal-like [1]. Therefore, although TNBC and basal-like breast cancers overlap, they represent distinct classifications. This serves to highlight the heterogenous nature of the TNBC classification and the need to provide subclassifications to elucidate prognosis and identify potential responders to existing and future treatments.

With so much focus on the investigation of intrinsic breast cancer subtypes, it can be difficult to identify the practical relevance of gene-expression profile-based definitions in the clinic, where such experiments are rarely carried out. Some have questioned whether such approaches offer additional prognostic, and to some extent, predictive information compared with conventional IHC [20]. An early study in 2004
suggested that ‘good old’ clinical markers provide a level of prognostic power comparable with microarray gene-expression profiliers [21]. More recently, a four-component algorithm that uses ER, PR, HER2 and Ki67 to generate a single predictor, known as IHC4, was shown to have a similar predictive value to a commercial microarray assay, OncotypeDX®, in terms of demonstrating efficacy [29–33]. Another emerging strategy for treating TNBC concerns the targeting of androgen receptor (AR), with recent studies suggesting that around 10%–35% of TNBC tumours harbour an AR-positive gene-expression profile [34, 35]. Initially identified by microarray analysis, these tumours are negative for ER, but exhibit a gene-expression profile that is similar to ER-positive tumours [36].

A phase II trial of bicalutamide (an AR antagonist) in TNBC disease is ongoing, and early evidence suggests that this treatment is capable of disease stabilisation in ER/PR-negative patients [37, 38]. Alterations in BRCA1 and other genes involved with DNA repair are also associated with TNBC. It has been estimated that up to 90% of BRCA1-mutated tumours are TNBC or have a basal-like phenotype [39]. Assays that identify dysfunctional DNA repair may eventually be used to identify patients with TNBC who are likely to benefit from DNA-targeted therapy, such as anthracyclines, platinum and poly(ADP-ribose) polymerase inhibitors. Lastly, the number of circulating tumour cells and/or circulating endothelial cells in blood has been proposed as an independent prognostic indicator in breast cancer, and may help predict the effectiveness of neoadjuvant chemotherapy and antiangiogenic drugs, such as bevacizumab [40, 41].

The recent emergence of these new treatment-guiding biomarkers means that there is a lack of agreed thresholds and techniques for their use, which, to date, has been limited to clinical trials. Further work will be required to establish the precise prognostic significance of these biomarkers and to determine whether their use is of clinical relevance to the treatment of patients.

surrogate biomarkers for intrinsic breast cancer subtypes

Approximately half of all new breast cancers are diagnosed in the developing world, where the analysis of prognostic factors needs to be inexpensive and easy to replicate [21]. Even in the developed world, microarray analysis has yet to fully replace classical IHC. Thus, in the absence of routine gene-expression profiling, surrogate IHC markers for molecular breast cancer subtypes have emerged as a more practical means of characterising TNBC tumour types according to prognosis and/or differential response to specific agents [26]. For example, a five-marker method, which examines ER, PR, HER2, cytokeratin 5/6, and the epidermal growth factor receptor (EGFR) has been proposed as a surrogate system for identifying basal-like breast cancer [27, 28]. Such an approach could have practical benefits for clinical trials seeking to enrol patients with the basal-like molecular subtype. Moreover, it is becoming increasingly apparent that the success of new anticancer therapies is likely to be dependent upon the use of new biomarkers to detect patients who will benefit from a particular treatment.

There are currently several therapies under development that target biomarkers of TNBC or the basal-like subtype. The EGFR is frequently expressed in basal-like breast cancer and has emerged as a potential target for therapy with the biological agents such as cetuximab and panitumumab, although mixed results have been obtained in clinical trials in terms of demonstrating efficacy [29–33]. Another emerging strategy for treating TNBC concerns the targeting of androgen receptor (AR), with recent studies suggesting that around 10%–35% of TNBC tumours harbour an AR-positive gene-expression profile [34, 35]. Initially identified by microarray analysis, these tumours are negative for ER, but exhibit a gene-expression profile that is similar to ER-positive tumours [36].

conclusion

There is currently no substitute for well-conducted clinicopathological examination and IHC staining; however, careful adherence to standardised guidelines is of crucial importance, given the subjective nature of these analyses. New assays that evaluate surrogate biomarkers for subtypes of TNBC are emerging; however, from a clinical perspective, these new assays for TNBC will only be useful if they present additional prognostic information and are able to provide further information on how patients can be treated effectively.

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references