Quality and application of oestrogen (ER) and progesterone receptor (PgR) and HER2 analyses

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Oestrogen (ER) and progesterone receptor (PgR) status of breast carcinomas and HER2 over-expression are accepted prognostic/predictive parameters with undisputed clinical implications. Based on the recommendations of the 2005 St. Gallen Consensus [1], which have recently been re-emphasised during the 2007 Consensus, breast carcinomas may be clinically classified in three main categories according to their endocrine responsiveness. Highly endocrine responsive tumours are characterised by extensive expression of both ER and PgR in more than 10% invasive tumour cells, lack of HER2 over-expression and low (less than 20%) Ki-67 labelling index (LI). Patients with highly endocrine responsive tumours may be safely treated with a pure endocrine intervention, tuned according to the risk evaluation. Non endocrine responsive carcinomas may be identified by the lack of any ER and PgR expression. Patients with these tumours exquisitely benefit from a chemotherapeutic intervention, again modulated according to risk. Improper prescription of endocrine therapy to these patients may be detrimental, leading to a reduced disease-free and overall survival [2].The third category includes tumours with uncertain or partial endocrine responsiveness, as documented by a low expression of both ER and PgR, or by the lack of PgR expression, or by the over-expression or amplification of the *HER2* gene, or by a Ki-67 LI higher than 20%. These patients most likely benefit from a combined intervention, including endocrine therapy, chemotherapy, and an anti-HER2 targeted treatment (if appropriate). The hormone receptor status is currently evaluated by means of immunohistochemistry, using specific monoclonal antibodies. The immunohistochemical assay has been shown to predict the response to hormonal intervention better than ligand binding assays [3].

Unfortunately, the immunohistochemical assays are still biased by lack of standardisation in both the technical performance of the test and the evaluation of the results. As a consequence, we have to face a poor reproducibility of the assays, with significant inter- and intra-laboratory disagreement. Recent results of the UK-NEQAS external quality assessment have shown that the rate of unacceptable staining for ER on centrally provided test sections was 18% of 366 participants [4]; in a German trial for quality control in immunohistochemistry, a correct score was assigned to ER status only by 30% of the participants, with 11% false-negative results [5].

To achieve better standardisation and reproducibility of the assessment of hormone receptor status, guidelines and recommendations for a proper performance and interpretation of immunohistochemical assays should be developed and followed, as we have done within the International Breast Cancer Study Group (IBCSG) [3].

A correct assessment of HER2 status in all primary breast carcinomas is necessary to better predict the risk of tumour progression (prognostic factor), to design the most effective adjuvant treatment (predictive factor) and to identify patients most likely to benefit from targeted anti-HER2 interventions. HER2 over-expression in breast carcinoma is most commonly due to *HER2* gene amplification, so that HER2 status may be assessed with either immunohistochemical (IHC) and/or *in situ* hybridisation assays using fluorescent or chromogenic procedures (FISH or CISH). Despite the commercial availability of standardised kits and reagents for the assays, HER2 status assessment is liable to provide inconsistent results, with a high rate of false-positive assays. This has been recently emphasised by the results of the central revision of the HER2 status of the tumours of candidate patients to the enrolment in the NCCTG N9831 trial of adjuvant trastuzumab based on local assessment of *HER2* over-expression or amplification [6]. The mandatory central re-evaluation of HER2 status documented a false-positive rate of almost 21% for the immunohistochemical assays and of 12% for the FISH assays. Even higher rates of false-positive results have been encountered during the central re-evaluation of the samples from the candidate patients to the HERA trial.

Again, to reduce the risk of inaccurate assessments of HER2 status, guideline recommendations have been recently issued by an *ad hoc* joint committee of ASCO/CAP [7]. These recommendations include guidelines for the optimisation of the algorithm for HER2 testing, of the testing requirements for IHC and FISH, of the tissue handling requirements, of the internal validation procedure, of the internal quality assurance procedures, of the external proficiency assessment, and of the laboratory accreditation.

Most remarkably, the guidelines recommend a modification of the scoring criteria for a case to be considered positive either by immunohistochemical or FISH assays. A positive immunohistochemical result now requires more than 30% of invasive tumour cells to show intense circumferential membrane staining. If 30% or less cells are immunostained or if circumferential membrane staining is less intense, then the assay results should be considered equivocal, and the sample should be retested with FISH (or CISH) for the final assessment of HER2 status. A positive test by FISH requires a *HER2* gene/chromosome 17 copy number ratio higher than 2.2 (or a gene copy number higher than 6 for CISH), whereas a ratio of 1.8–2.2 (or a gene copy number between 4 and 6) is considered an equivocal result, to be further evaluated by repeating the assays or by assessing the over-expression of the protein using immunohistochemistry.

Standardisation of the pre-analytical and analytical steps of the assays and of the interpretation criteria of the results is an essential prerequisite for improving the accuracy of the assessment of hormone receptor and HER2 status of breast carcinomas, but this is not enough. Pathologists should become more and more aware of the clinical implications of the assessment of these biological parameters for patients with breast carcinoma, becoming more and more conscious of their unique role in the process of clinical decision making.

Conflict of interest statement

None declared.

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