ER, PR and HER2 testing in breast cancer

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Abstract

The application of targeted therapies has played important roles in the improvement of breast cancer survival rate during the past two decades. Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) are well established biomarkers for breast cancer prognosis and for guiding treatment. Emerging data furthers our understanding of the biomarkers and their validity as predictive and prognostic indicators. Breast cancer biomarker testing guidelines have been recently updated. There are still several key challenges in the evaluations of these markers, including pre-analytic standards, tissue selection for testing and re-testing, result interpretations, and tumour heterogeneity. In addition to ER, PR and HER2, newer markers and multigene testings may provide additional information in guiding targeted therapy for breast cancer.

Keywords biomarkers; breast cancer; ER; HER2; PR; targeted treatment

Breast cancer remains one of the leading causes of cancer death in women, yet mortality rates have steadily declined over the last decade despite an increasing incidence of breast cancer. One of the major factors behind this improvement in survival rate is believed to be the application of increasingly effective adjuvant treatment and the accuracy in selecting patients to receive appropriate adjuvant treatment. In addition to the traditional pathological parameters, the status of hormone receptors, including estrogen receptor α (ER) and progesterone receptors (PR) as well as the human epidermal growth factor receptor 2 (HER2) amplification status, play important roles in determining prognosis and making treatment decisions. Hormonal therapy and HER2-targeted treatments are among the most widely used targeted cancer therapies. Testing for these biomarkers has become the standard of care in the treatment of breast cancer patients. The accuracy and reliability of these test results are therefore critical in the care of breast cancer patients. Laboratories performing these tests should establish both internal quality assurance program including initial validation of the tests, ongoing proficiency tests of the laboratory and of pathologists interpreting the tests, and external quality assurance program by participating accreditation programs.

ER and PR

Introduction

ER currently remains the most informative biomarker in breast cancer. Approximately 75% of breast cancer is ER positive. These tumours tend to be lower grade and demonstrate better prognosis. They comprise the majority of the luminal type tumours, including both luminal A and luminal B subtypes. ER expression is the main indication for hormonal therapy such as tamoxifen and aromatase inhibitors, which reduces the annual breast cancer death rate by over 30% in ER positive cancer. ER expression also has been associated with poorer response to chemotherapy. ER negative tumours are found to be more likely to show pathologic complete response in neoadjuvant settings.[1]

The expression of PR is strongly associated with the expression of ER, and is found to be expressed in more than half of the ER positive cancers. Tumours expressing both ER and PR have more favourable pathological parameters, prognosis, and response to endocrine treatment. In ER positive patients receiving tamoxifen treatment, those with high levels of PR expression with their tumours have a better outcome than the patients with low PR expression. In ER positive patients, negative or low PR expression is associated with more proliferative and aggressive tumours, with poorer prognosis and higher risk of recurrence. The prognostic and predictive roles of PR alone in breast cancer are less clear. The subgroup of ER negative PR positive disease has been reported as 1–5% of all breast cancer, with newer data suggesting the lower end of the range.[2] ER negative tumours in general do not show benefit from 5-year adjuvant tamoxifen therapy, but some benefit may occur in a small subgroup of ER negative disease with PR positivity.[3]

Historically, ER and PR have been assessed using dextran-coated charcoal/ligand binding assay method. This has been followed by other testing methods such as enzyme immunoassay and enzyme-linked immunosorbent assay. Immunohistochemistry (IHC) on formalin-fixed paraffin-embedded tissue now has replaced those earlier methods and has become the standard testing method widely used since late 1980s to early 1990s. IHC testing on formalin-fixed paraffin-embedded tissue has a number of advantages, including smaller amount of tissue required, no need for fresh or frozen tissue, the ability to correlate results with histology, and the availability of archived material for later use.

Since IHC testing for ER and PR are widely used, there is wide variability in how different laboratories perform the tests and interpret the results. In 2010, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) published Guideline Recommendations for ER and PR IHC testing in breast cancer.[4] In this guideline, it was concluded that up to 20% of IHC determinations of ER and PR testing worldwide may be inaccurate, including false negative and false positive results. The main issues with testing were variation in pre-analytic variables, thresholds for positivity, and interpretation criteria.

Tissue fixation

Based on current recommendations, the time from tissue acquisition to fixation (ischaemic time) should be as short as possible. Samples should be fixed in 10% neutral buffered formalin (NBF) for 6–72 h. Some studies suggest that 10% NBF as a fixative achieves the best results.[4] Even though the upper limit for fixation is recommended as 72 h, with proper analytic methodologies, tissue that has been fixed for several days will continue to immunoreact for ER and PR. Under-fixation on the other hand is more critical than over fixation. The minimum fixation time for reliable IHC ER has been suggested to be 6–8 h, regardless of the type or size of the specimen.

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IHC scoring system

Scoring methods for ER and PR IHC are generally based on the proportion of positive tumour cells and the intensity of the staining, as well as the combination of these two parameters. Studies have shown a high reliability when comparing different scoring methods. Fisher and colleagues reported high interobserver agreement for ER and PR using percentage of positivity, intensity or binary scoring methods for positive and negative.[5] Review of studies also indicated that Allerd's proportion score was a better predictive indicator than was the intensity score or the Allerd's total (combined) score.[6] Overall, it is believed that the percentage of tumour cell nuclear staining or a binary score of positive or negative, are adequate in predictive and prognostic validity and probably are superior to an intensity score or a combination of percentage and intensity score for reliability.

Cutpoints for optimizing predictive or prognostic validity of ER and PR is not without controversial. While some authors suggested a 10% staining as the threshold, others suggested a cutpoint as low as 1%.[6] Current ASCO-CAP guideline recommendations use $\geq$1% as the cutpoint for ER and PR positivity, regardless of intensity. Recent molecular studies, however, suggested that tumours with 1–10% ER staining show molecular subtype that is more basal like or HER2 enriched, and have pathological features similar to ER negative tumours.[7] Clinically the majority of these tumours were also found to behave more like ER negative tumours.[8] While the binary scoring system may be adequate, the percentage score may provide additional information in treatment decision making, as ER and/or PR levels have been demonstrated to be associated with patient outcome. Staining of >50% of tumour cells was viewed as indicating highly endocrine-responsive tumours.[9]

Image analysis systems including computer assisted image analysis system, automated quantitative analysis, photoshop, and other types of imaging systems have being used to assess ER/PR IHC results and the results have been compared with the standard IHC results assessed manually. Image analysis is highly concordant to manual estimation and shows good reliability.[6] There is, however, no prospective data to suggest the replacement of manual assessment by image analysis.

Receptor status change

Metastasis from just less than 10% of ER negative tumours will be ER positive. These patients have been reported to have a better prognosis than those patients whose metastases remains ER negative. Patients with ER negative tumour prior to neoadjuvant chemotherapy, who are ER positive post treatment, also appear to have a better prognosis.[10] On the other hand, neoadjuvant chemotherapy may results in a significant shift to more ER/PR negative results on excision compared to no neoadjuvant chemotherapy group.

Testing core needle biopsy vs. re-testing on excision

Core needle biopsy has increasingly being used as the initial material for ER and PR IHC testing. Studies have compared ER or PR IHC values taken from core biopsies and comparing them to IHC on surgical specimens. By systematic review of multiple studies, ER is found to have concordance values greater than 83% with the median being 95%.[6] PR concordance values are greater than 69% with the median being 88.5%. The majority of the studies have found higher ER positive and/or PR positive rates in core biopsies compared with surgical specimens. This may be due to better fixation of core biopsy material compared to surgical excision specimen. There are also high concordance rates between core biopsies taken from different parts of the same tumour (ER 100%, PR 85%),[11] as well as between core biopsies taken from the different foci of a multifocal disease.[12] Situations where repeated ER/PR testing should be conducted on subsequent excision are listed in Table 1.

Uninterruptable, sample rejection and pitfalls in interpretation

False positive results for ER and/or PR IHC are relatively uncommon. One potential cause for false positive results is to interpret ductal carcinoma *in situ* (DCIS) or entrapped non-neoplastic epithelium as invasive carcinoma. In patients with invasive carcinoma and DCIS, ER/PR should be reported only for the invasive component. DCIS staining pattern can be provided in a comment. Evidence has shown that adjuvant Tamoxifen reduces subsequent breast cancer in women with ER positive DCIS, which is about 75% of all DCIS.[13] ER/PR for DCIS only cases may be scored and reported.

False negative results for ER and/or PR IHC are more common in practice. The potential causes for a false negative result include poor tissue quality, assay problem, interpretation of cases with positivity at the lower end of the spectrum, and tumour heterogeneity. Careful evaluation is required to avoid false negative results caused by pre-analytical issues. For example, a sample should be considered as uninterpretable when the IHC assay controls are not as expected or the pre-analytic conditions do not follow the standard guidelines and tumour cells are negative for the staining in the absence of normal epithelial elements to serve as internal control. Test results should be rejected if normal ducts and lobules do not show obvious staining of some cells with variable intensity in the event of negative tumour cells. Samples with obscuring artifacts should also be rejected (Table 2).

Repeat biomarker testing on excision

Situations require repeat biomarker testing on excision after initial testing on core biopsy

Tumour characteristics in the excision are different from the core biopsy (type or grade)
Multifocal tumour when only one focus was previously tested and the tumours appear morphologically different
Area of invasive tumour is small on core biopsy
Post -neoadjuvant treatment
Core biopsy result is indeterminate for any of the markers
ER IHC negative on core biopsy, regardless of the other markers
Core biopsy result is equivocal for HER2 after testing by both IHC and ISH
High nuclear grade (score 3) and HER2 negative on core biopsy if appropriate
Any discordance that the pathologist concerns about inaccurate results

Table 1