A critical review why assessment of steroid hormone receptors in breast cancer should be quantitative

O. Brouckaert1*, R. Paridaens1, G. Floris1, E. Rakha2, K. Osborne3 & P. Neven1

1Department of Gynaecological Oncology, Multidisciplinary Breast Centre UZ, Leuven, Belgium; 2Department of Cellular Pathology, University of Nottingham and Nottingham University Hospitals NHS Trust, Nottingham, UK; 3Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, USA

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Steroid receptors have been around in the field of breast cancer for decades now. Still, controversy remains on how best to report steroid receptors. In this review, we will convince the reader why benefits outweigh pitfalls, when reporting steroid receptors in a quantitative rather than qualitative way. Summarizing decades of research in this field, we will explore the evidence why quantitative reporting is superior in all settings (neoadjuvant, adjuvant and metastatic settings). Furthermore, we will also summarize different staining methods, definitions and pitfalls that have shown to be important points of discussion in earlier debates. Although the molecular unraveling of breast cancer in the past decade has revolutionized the way we think about breast cancer, we should not easily abandon the classical pathological variables such as steroid receptors in favor of molecular tools.

Key words: breast cancer, ER, PR, qualitative, quantitative, review

introduction

Sex steroids together with growth factors drive the development, growth and differentiation of breast epithelial tissue following activation of the nuclear estrogen (ER) and progesterone receptors (PR) [1–6] and are also critical for breast cancer development and progression. ER and PR are expressed in breast cancer, respectively, in 80% and 60% of cases. Their activation—mainly through ligand binding or growth factor-induced phosphorylation—triggers diverse gene networks, metabolic and cell regulatory pathways, sustaining cancerous breast epithelium [7]. Whilst the PR is induced by an active estrogen–ER pathway, the expression of hormone receptors depends on many host (i.e. age at diagnosis, BRCA-1/2 mutation, body mass index, parity etc.) and tumor characteristics [i.e. histological type and grade, human epidermal growth factor 2 (HER-2) expression] [8].

The qualitative expression (absent/present) of ER and PR is predictive for treatment response and prognostic for outcome but to a different extent for each receptor. Women with an ER-positive breast cancer not receiving endocrine therapy have a better short-term prognosis than women with an ER-negative tumor [9]. This difference is further enhanced with endocrine therapy as ER expression predicts response to anti-estrogen therapeutic strategies [10]. The PR is more complicated: PR expression is predictive for response to tamoxifen in metastatic breast cancer, but prognostic rather than predictive in the adjuvant setting [11–20]. Compared with ER/PR-positive cases, ER-positive/PR-negative cases constitute a more aggressive so-called ‘luminal B’ molecular class of breast cancer [21–25] with lower nuclear ER-activity, greater genomic instability, higher proliferation, more PI3K mutations and more crosstalk between the ER and growth factors, although this might be age- and estrogen level related [26]. With high estrogen levels, the PR can be abundantly present together with alternative growth factor signalling whilst with low estrogen levels, the PR can be absent with an intact estrogen–ER pathway [27]. Loss of PR has several explanations but may reflect treatment resistance or activation of growth factor receptor pathways [28]. The ER and PR should be reported in combination with the HER-2 status, since these clinical phenotypes possess discriminative prognostic information, and are able to challenge gene expression profiling, particularly when carried out in a central lab [29–32].

Semi-quantitative rather than qualitative expression of hormone receptors fine-tunes their predictive and prognostic capabilities. Semi-quantitative subgroups are defined by arbitrarily defined cut-offs of a continuous variable [32–34]. Cut-offs are not based on measurements of functional activity of the ER pathway but response rates to endocrine therapy increase with a higher expression for ER [14, 34–36]. Tumors with strong ER and PR expression most often show an indolent behavior and the benefit from adjuvant chemotherapy is limited [37]. Tumors with a low expression of ER usually lack high PR expression and exhibit features associated with poor prognosis (such as HER-2 expression) and may benefit more from adjuvant chemotherapy. Semi-quantitative PR...
information may refine the 2011 St Gallen classification of luminal breast cancers [37, 38].

We here review why semi-quantitative assessment of hormone receptors provides an additional benefit over qualitative reporting in the light of daily clinical decision-making. We first review different methods for measuring hormone receptors and highlight potential pitfalls.

measuring hormone receptors in breast cancer

ER assessment was initially based on standardized ligand binding assays (LBA), but retrospective studies showed that semi-quantitative immunohistochemical (IHC) analysis of ER expression was superior for prognostic and predictive purposes [39–42]. The LBA was therefore abandoned, but prospective studies comparing the predictive value of LBA with IHC are not available [32, 43]. IHC is easy, cheaper, specific, safe and usable for evaluating cytology, fresh frozen tissue and formalin-fixed paraffin-embedded specimens. IHC also allows to discriminate between malignant and benign cells. A lack of standardized protocols in the pre-analytical phase (e.g. choice of specimen, time of fixation, processing of different specimens, type of fixative etc.), staining procedure (e.g. type of antigen retrieval, detection system), assessment methods (scoring, threshold for positivity) and availability of diverse antibodies remain critical issues leading to discordant results in different laboratories [32, 34, 43, 44]. Quality control programs are essential to guarantee reproducibility and comparability between different centres. False-negative/positive results for ER exist, with error rates as high as 20% [32, 43, 45].

Several IHC semi-quantitative scoring systems for ER and PR have been developed. Amongst the first was McCarty’s ‘H’ score, calculated by multiplying the percentage of positive cells by a factor representing the intensity of immunoreactivity (1 for weak, 2 for moderate and 3 for strong), giving a maximum score of 300 (3+) [39]. Another score is the immunoreactive Remmele score (IRS), the product of the proportion score (0–4) and an intensity score (0–3) with a range of 0–12 [46]. Nowadays, the most established score is the Allred (modified quick score) [40]. It uses the same intensity score as the H-score and IRS system (0–3), but adds the proportion of cells staining into six subgroups (0, no staining; 1, <1%; 2, between 1% and 10%; 3, between 11% and 33%; 4, between 34% and 66% and 5, between 67%–100% of the cells staining).

defining hormone receptor positivity in breast cancer

Although the use of clinically validated, reproducible and standardized cut-offs for determining hormone receptor positivity is critical, prospective studies addressing the optimal IHC cut-off for defining positivity based on the efficacy of endocrine therapy are lacking. Retrospective studies previously carried out using the LBA suggested that ER > 3 fmol/mg protein should be considered positive but others suggested >10 fmol/mg and even 4–10 fmol/mg [21, 32, 47, 48]. Harvey et al. compared the LBA with IHC in patients treated with adjuvant tamoxifen [41]. An Allred score of ≥3 is a good cut-off to predict benefit from tamoxifen, but only when using identical standardized staining methods and primary antibody. ER-positivity is considered only when ≥1% (proportion score 2) of the tumor cells stain (intensity score = 1) and the tumor is, as per definition, ER-negative, regardless of the intensity score, when <1% of the tumor cells stain (proportion score 1). Endocrine therapy is not useful in these cases [32, 49].

Although data used to derive the 1% IHC cut-off may be suboptimal (derived from a previous study), there is still an agreement to use it to guide anti-estrogen therapy [32, 50]. In routine practice however, there seems to be a wide range of arbitrary cut-offs in the percentage of stained cells being used (i.e. >0% [51, 52], 1% [53], 5%–10% [54–57] and 20% irrespective of the scoring method used [51]). In view of the great advancement in the IHC technique and the introduction of new highly sensitive antibodies and the wide use of 10% as a cut-off of positivity in many centers, categorization of tumors showing 1%–9% IHC staining remains controversial. Further research in this area is needed to address not only the response rates of these tumors but also their biological features with regard to ER-pathway activation. A recent report in this field states that almost half of tumors staining 1%–9% IHC have molecular characteristic of the basal-like phenotype [58].

Discordances between the H-score and the Allred score for defining ER-positivity exist. These are mainly observed when 1%–10% of cells stain, but also in Allred scores 7–8. The two scoring methods remain in use and a modified conversion table, originally suggested by Shousha, making the two systems more equivalent is shown here (Table 1) [59]. Discordances may also result from using different antibodies with variable sensitivity, particularly when the hormone receptor expression is low. The two antibodies used in the first generation IHC studies were mouse monoclonal antibodies (MMabs), MMab 6F11 and MMab 1D5. Recently, rabbit monoclonal antibodies (RabMabs) have become increasingly popular because of higher affinities for the desired epitope [60]. RabMab SP1 is such an antibody exhibiting 8-fold higher affinity than MMab 1D5 for the ER [61]. Both the 6F11 and 1D5 as well as SP1 are correlated with clinical outcomes and outperform LBA [62]. A previous retrospective study comparing the newer RabMab SP1 with the conventionally used MMab 1D5 using mostly previously frozen tissue in 4105 cases reported that 8% of breast carcinomas were SP1+/1D5− (correlating with poorer outcomes) and 2% were SP1−/1D5+ (correlating with poorer outcomes) [63]. This study showed that MMabs have lower sensitivity and specificity when compared with RabMabs potentially misclassifying patients who may benefit or not from endocrine therapy. Another prospective study comparing SP1 and 1D5 stainings in 508 cases identified only 2 SP1+/1D5− and no SP1−/1D5+ cases [64]. Discordance was mainly present in those with <1% staining and therefore of no clinical importance. SP1 was consistently noted as having a greater intensity of staining compared with 1D5, and thus, it was much easier to read positive-low results and negative results. Also, the cytoplasmic staining seen with 1D5 in SP1 ER− cases was never seen with SP1. The use of RabMab SP1 therefore seems to reduce the likelihood of false-positive results for ER. The primary antibodies for the ER supported by the American
activity of ER-positive tumors and classical combined recurrence score is heavily dependent on the proliferative value of the qualitative ER and PR expression is well demonstrated both in patients treated with endocrine therapy and, but to a lesser extent, in endocrine therapy naïve patients [41]. However, the prognostic effect is time-dependent, disappearing after 5–8 years, and survival curves between ER-positive and ER-negative disease cross somewhere between 5 and 10 years, also illustrative for late recurrences typical for ER-positive breast cancer [9, 76–80]. This is probably explained by the strong correlation between (positive) ER status and (lower) rates of tumor cell proliferation, as noticeable also in gene expression studies where lower quantitative ER correlates with higher recurrence score [81]. It takes ER+ tumors longer to recur and ER status may thus not be a marker for metastatic potential.

### Prognostic Value of Quantitative ER and/or PR-Expression in ER-Positive Breast Cancer

A prognostic factor is a clinical, pathological, or biological feature associated with the aggressiveness of (un)treated invasive breast cancer, and if adverse usually results in the use of (more) adjuvant therapies following surgery. The prognostic value of the qualitative ER and PR expression is well demonstrated both in patients treated with endocrine therapy and, but to a lesser extent, in endocrine therapy naïve patients [41]. However, the prognostic effect is time-dependent, disappearing after 5–8 years, and survival curves between ER-positive and ER-negative disease cross somewhere between 5 and 10 years, also illustrative for late recurrences typical for ER-positive breast cancer [9, 76–80]. This is probably explained by the strong correlation between (positive) ER status and (lower) rates of tumor cell proliferation, as noticeable also in gene expression studies where lower quantitative ER correlates with higher recurrence score (which is mainly driven by proliferation) [81]. It takes ER+ tumors longer to recur and ER status may thus not be a marker for metastatic potential.
The prognostic value of quantitative hormone receptor expression in ER-positive endocrine treatment naïve cases might be more important for PR than ER. Data from a placebo-controlled trial have shown that quantitative ER expression in patients with an ER-positive breast cancer is not strongly prognostic [35, 68]. ER expression was quantified by the 21 gene Oncotype DX assay using quantitative qRT-PCR in archived paraffin blocks. ER and PR expressions were measured on a log based 2 scale from 0 to 15 (relative to reference genes) and a one unit increment is associated with a 2-fold change in expression. The Kaplan–Meier plot showed that untreated patients with an ER-positive breast cancer have significant differences in time to distant recurrence by quantitative PR expression ($P = 0.002$). The highest PR tertile had the longest time to distant recurrence and the lowest PR tertile the shortest. Most other studies have confirmed these results in postmenopausal women [82–84]. Interestingly, a small retrospective study including 138 ER-positive, lymph-node negative and stage I and II breast carcinomas recently showed that lack of PR expression associated with mitotic count greater than score 1 is a strong predictor of high Oncotype DX® recurrence score [72].

In patients treated with endocrine agents, high ER expression is associated with better outcome than lower ER expression, which is better than no ER expression. This is mainly due to a better therapy response in patients with higher ER expression reflecting its predictive effect. The same applies to the PR within the ER-positive treated breast cancer patients, but here, the quantitative PR is prognostic rather than predictive [15, 30, 68, 81, 85, 86]. Furthermore, the overall good prognosis of patients with tumors staining strongly for ER and PR is in line with their negative association with other unfavorable prognostic markers like HER-2 receptor expression and high histological grade, although this might be age related [87–89].

**predictive value of quantitative ER and/or PR-expression in ER-positive breast cancer**

**the adjuvant setting**

A factor is predictive if it predicts the likelihood of responding to specific types of therapies in both the adjuvant and metastatic settings.

In the adjuvant setting, placebo-controlled tamoxifen studies showed that the benefit of tamoxifen increases with increasing ER expression, independent of menopausal status [14, 35, 36, 68]. Tumors with low ER expression are less sensitive to tamoxifen. Although there is a gradient of increasing response to endocrine agents with increasing levels of ERα, the gradient is skewed such that tumors expressing even very low numbers (e.g. between 1% and 10%) of positive cells show a significant benefit far above that of ERα-negative tumors, which are essentially unresponsive. The predictive value of quantitative PR expression for response to tamoxifen is less clear (in the adjuvant setting). As indicated by the recently updated EBCTCG study, the benefit of endocrine adjuvant therapy seems not to be affected by quantitative PR expression in ER-positive breast cancers [36]. A Swedish study randomizing between tamoxifen and placebo in women under age 50 reported the benefit of tamoxifen to be restricted to ER-positive breast cancers with a strong expression of PR (>75%) [90]. Strong PR expression can indeed be interpreted as a surrogate marker of stronger ER expression.

There is little information on the prognostic value of quantitative expression of ER or PR from placebo-controlled trials using aromatase inhibitors (AIs) as such trials are almost nonexistent. A subgroup analysis of the MA 17 study showed that the clinical benefit of extended letrozole versus placebo following 5 years of tamoxifen therapy was limited to the subgroup expressing PR but quantitative levels were not studied. The benefit of tamoxifen in ER-negative breast cancer is confined to PR-positive cases and these cases may need to be restated to rule out a false-negative ER staining [32, 91]. As might be predicted from biology, ER-negative/PR-positive tumors are rare and may well represent a false-negative ER assay [31].

PR expression was originally thought to discriminate the benefit of tamoxifen compared with an AI but these data did not hold up over time [15, 86, 92, 93]. A prospectively planned pathology substudy in the TEAM trial reported that patients with ‘strong’ ER expressing tumors may derive an additional benefit from an oral AI over tamoxifen although this was not statistically significant [15].

HER-2-positive breast cancers expressing ER and/or PR belong to the so-called luminal B tumors [24]. Quantitative expression of ER and/or PR is lower than seen in so-called luminal A tumors [24]. The lower levels of ER in HER-2-positive tumors may contribute to the lower benefit from endocrine therapy seen in patients with these tumors. Such tumors may also be less responsive to trastuzumab. Tumors expressing both ER/PR and HER-2 may be driven by both pathways and may thus be resistant to a drug targeting only one of them. Combination therapy may be more beneficial in these tumors as suggested by Neosphere trial [94] and the TBCRC 006 trial [95]. To our knowledge however, no data are available for a predictive role of quantitative expression of hormone receptors in response to trastuzumab or endocrine therapy in HER-2 overexpressing breast cancers. The statistical significance of the benefit from adjuvant trastuzumab is dependent on PR’s presence in ER-positive breast cancers in HERA [96].

There is a direct correlation between the likelihood of clinical response to hormonal therapies and the level of ER expression [41]. Tumors with high levels of ER and PR are highly sensitive to endocrine therapy. The 2011 St-Gallen consensus was that benefit of adjuvant chemotherapy is small here, irrespective of menopausal status [37, 97, 98]. It remains unclear whether the lack of benefit from chemotherapy in these patients is due to an excellent outcome with endocrine therapy or due to genuine lack of biological effect. ER-positive tumors have lower rates of tumor cell proliferation and numerous prior studies have shown a relationship between the measures of cell proliferation and the benefit from chemotherapy [99, 100]. Most postmenopausal patients with tumors expressing very high levels of ER can be optimally treated with adjuvant hormonal therapy alone, and can safely
forego the rigors of chemotherapy [99, 101–105]. A recent retrospective study confirms this [106]. This stresses the need for robust quantitative methods for analyzing hormone receptor expression. The IBCSG study IX investigated the role of CMF chemotherapy before tamoxifen therapy in postmenopausal women with a lymph node-negative ER-positive breast cancer. There was a survival benefit only in the tumors with low ER expression [107]. IBCSG study VIII in premenopausal women demonstrated that little benefit was to be expected from chemotherapy in patients with tumors expressing PR [108]. The fact that AdjuvantOnline? does not incorporate quantitative ER receptor expression and no PR expression at all suggests that the benefit of chemotherapy is therefore overestimated [109]. Many other studies describe the link between hormone receptor levels and breast cancer survival with or without chemotherapy. The studies are in line with the consensus that patients with strong hormone receptor expression have a good prognosis and that there is little benefit from adjuvant chemotherapy [99,110–112].

the neoadjuvant setting

In menopausal women, there is a linear relationship between the quantitative expression of ER and the chance of responding to endocrine therapy, both for tamoxifen and AI. Patients with a lower expression for ER had little benefit from tamoxifen but more benefit from an AI. This effect decreased with increasing expression for ER [113].

Quantitative expression of ER and PR can be used to determine the benefit from preoperative chemotherapy [114, 115]. Pathological complete remission in patients with ER-positive breast cancer treated with neoadjuvant chemotherapy decreases with increasing ER expression [116]. This relation is also true for quantitative PR expression in ER-positive breast cancer, but apparently only in HER-2-negative cases [115].

Pathological complete response increases progressively in HER-2-positive patients treated with combined chemotherapy–trastuzumab with decreasing semi-quantitative ER and PR expression. The benefit from adding trastuzumab is highest in ER- and PR-negative cases [94, 117].

The prognostic value of pathological complete response also depends on the ER and PR status and is only significant in luminal B/HER-2-negative, HER-2-positive (nonluminal) and triple-negative disease [118].

the metastatic setting

Metastatic postmenopausal breast cancer patients have a clinical benefit from first-line tamoxifen or AIs of 38%–59% [119] depending on the quantitative ER expression [120–122]. In ER low/intermediate/high expression tumors, the response rates of tamoxifen are 25% / 46% / 66% respectively [41]. For PR, there is a linear response ratio with increasing PR expression [20], but endocrine therapy can be useful even in patients with absent PR expression [123]. Surprisingly, until recently, there were no studies assessing the relationship between the use of the recent third generation AIs in the metastatic setting and quantitative hormone receptor expression [124].

Response rates for third generation AI’s in first-line metastatic setting are 30%–50% [125]. However, in ER-positive tumors, higher PR expression is associated with increased time to treatment failure and this is not dependent only on the higher proliferation rate seen in PR-negative tumors [124, 125]. This parallels the decreased benefit from tamoxifen in the metastatic setting with the absence of PR, although in the adjuvant setting PR expression does not predict clinical benefit from AI’s over tamoxifen or vice versa. For ER expression, there was only a nonsignificant trend for clinical benefit with higher ER expression, consistent with the previously described findings of Ravdin with tamoxifen. Even in the lower quartile of ER expression, clinical benefit (complete/partial response/stable disease for ≥6 months) is 50% but no effect on overall survival for different quantitative hormone receptor expression was found.

Reports in the early 1980’s suggested that lack of ER expression was associated with an increased response to chemotherapy in metastatic breast cancer but others found a high ER expression to be associated with higher response rates [126, 127]. Until today, the impact of quantitative and certainly qualitative hormone receptor expression on chemotherapy response rates in patients with ER-positive metastatic breast cancer remains unclear. The level of ER expression could be predictive of lower response to chemotherapy in the (neo)adjuvant setting [102] and some guidelines extend this knowledge into the metastatic setting [124, 128]. A major problem in metastatic breast cancer is that few studies looked at the level of ER on a metastatic biopsy but only at expression on the primary tumor. Quantitative changes in ER/PR/HER-2 between primary tumor and metastatic lesion occur in up to 16%/40%/10% of patients, respectively, leading to a change in management in 14% [129]. Qualitative ER expression remains concordant between primary tumor and metastatic lesion in 77% and the qualitative changes in the remaining 23% are small [130].

Single-agent first-line trastuzumab achieves response rates of up to 41% in patients with a HER-2 FISH-positive tumor [131, 132]. Benefit from trastuzumab with chemotherapy is similar when hormone receptor expression is assessed qualitatively [133], but decreases with (quantitatively) higher levels [8].

conclusion

Strong IHC expression for ER/PR correlates with better prognosis and higher chance of responding to endocrine therapy. The (semi)quantitative assessment of hormone receptor expression rather than qualitative assessment estimates the benefit of (neo)adjuvant chemotherapy, since this benefit decreases with increasing hormone receptor expression. This is known for over >30 years now and we daily implement this knowledge when we consider (neo)chemotherapy in patients of certain age with certain hormone receptor expression. Receptor assays showing a range of ER and PR expression levels will provide the most help to clinicians and their patients.

disclosures

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References 61 to 134 are available as Supplementary data in Annals of Oncology online.


