Pathol. Oncol. Res. (2018) 24:881–884
DOI 10.1007/s12253-017-0304-5

ORIGINAL ARTICLE

Re-Appraisal of Estrogen Receptor Negative/Progesterone Receptor Positive (ER−/PR+) Breast Cancer Phenotype: True Subtype or Technical Artefact?

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Received: 29 May 2017 / Accepted: 1 September 2017 / Published online: 11 September 2017

Abstract Expression of the ER and PR receptors is routinely quantified in breast cancer as a predictive marker of response to hormonal therapy. Accurate determination of ER and PR status is critical to the optimal selection of patients for targeted therapy. The existence of an ER−/PR+ subtype is controversial, with debate centred on whether this represents a true phenotype or a technical artefact on immunohistochemistry (IHC). The aim of this study was to investigate the true incidence and clinico-pathological features of ER−/PR+ breast cancers in a tertiary referral symptomatic breast unit. Clinico-pathological data were collected on invasive breast cancers diagnosed between 1995 and 2005. IHC for ER and PR receptors was repeated on all cases which were ER−/PR+, with the same paraffin block used for the initial diagnostic testing. Concordance between the diagnostic and repeat IHC was determined using validated testing. Complete data, including ER and PR status were available for 697 patients diagnosed during the study period. On diagnostic IHC, the immunophenotype of the breast tumours was: ER+/PR+ in 396 (57%), ER−/PR- in 157 (23%), ER+/PR- in 88 (12%) and ER−/PR+ in 56 (8.6%) patients. On repeat IHC of 48/56 ER−/PR+ tumours 45.8% were ER+/PR+, 6% were ER+/PR- and 43.7% were ER−/PR- None of the cases were confirmed to be ER−/PR+. The ER−/PR+ phenotypic breast cancer is likely to be the result of technical artefact. Prompt reassessment of patients originally assigned to this subtype who represent with symptoms should be considered to ensure appropriate clinical management.

Keywords Pathology · Immunohistochemistry · Receptors, estrogen · Receptors, progesterone · Breast neoplasms

Introduction

Breast cancer is a heterogeneous disease which exhibits distinct phenotypes, with therapeutic responsiveness and prognosis based on differing gene expression patterns. Defining hormone status in breast cancer carries, not only important prognostic information, but also critical data, which informs tailored treatment decisions regarding neo-adjuvant and adjuvant therapies. Accurate determination of ER and PR status is crucial. ER status is strongly linked with tumour grade and histology [1], and is reported positive in up to 70% of breast cancers [2]. The Early Breast Cancer Trialists Group discovered that PR status did not independently predict response to adjuvant Tamoxifen, only ER status did this, with no benefit in wholly negative ER disease [3]. ER−/PR+ breast cancer is a poorly defined entity, with conflicting reports in the international literature debating its merits as a genuine independent entity [4], and some reports describing it as a technical failure of immunohistochemistry [5]. The aims of this study were twofold: 1. To ascertain the rate of ER−/PR+ breast cancer at this institution and 2. Repeat immunohistochemistry (IHC) testing was undertaken in ER−/PR+ tumours to confirm

Authorship
Each author contributed equally to this work. All authors were involved in manuscript preparation and editing of the final draft.

Key Messages
ER−/PR+ breast cancer is a disputed entity in the published literature.
We could not confirm the distinct presence of ER−/PR+ breast cancer on retesting.
Repeat testing is recommended, to rule out a false negative ER result.

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hormone receptor status. We hypothesized that a proportion of ER−/PR+ tumours would be re-classified into a different subgroup following retesting.

Methods

Data were collected from a prospectively maintained institutional database on patients who were diagnosed with invasive breast cancer at Galway University Hospital from 1995 to 2005. The inclusion criteria for analysis were availability of ER and PR reporting data and complete treatment and follow up information. The study was carried out with ethical approval from the University College Hospital Galway Research Ethics Committee.

Patients were divided into 4 categories based on immunophenotype/expression of the ER and PR receptors into ER+/PR+, ER+/PR-, ER−/PR- and ER−/PR+. Clinicopathological features were compared across the subgroups including patient age, histological subtype, tumour grade, disease-free and overall survival, in addition to the use of adjuvant therapy. Treatment decisions ensued from rule based selection, following a shared decision making model of discussion with the patient.

Hormone Receptor Expression

Determination of hormone receptor status by IHC is undertaken in the routine pathologic evaluation of breast cancers at Galway University Hospital. During the study period the antibody clones 1D5 (Dako Ltd. Denmark) and PgR 636 (Dako Ltd. Denmark) were utilised to determine ER and PR status respectively, on formalin-fixed paraffin embedded tissue blocks. Cut-off scores for ER/PR positivity were determined using the Allred scoring system [6] and a score of >/= 3 was considered positive for steroid hormone receptor expression.

Validation of ER−/PR+ Immunophenotype

For all cases considered ER−/PR+, the clinical pathology report, H&E and IHC slides were reviewed by two independent pathologists. A representative block of tumour was selected and the paraffin embedded blocks were used to re-evaluate ER and PR status with IHC using current gold standard techniques. Tumours were re-evaluated on the same paraffin embedded blocks that were utilised for diagnostic IHC. 3 μm sections were cut and mounted on charged superfrosted slides. These were then oven dried at 60° for 2 h and placed in automated Ventana benchmark ST machines. Antigen retrieval was performed using heat induced epitope retrieval (HIER) by placement on heated thermopads on the Ventana Immunostainer.

FDA approved ER rabbit monoclonal antibody clone SP1 (ThermoScientific) and PR mouse monoclonal antibody clone SAN 16 and 27 (Leica) were used and multimer technology was utilized by the ventana uview kits (Ventana Medical Systems Inc.). Counterstaining was achieved using Ventana Haematoxylin 2-Mayers haematoxylin. This complies with recent ASCO/CAP guidelines [6], and local and internationally validated guidelines. An Allred cut-off score of 2 or less was used to determine negativity which correlates with less than 1% of cells displaying weak positivity. For cases diagnosed with breast carcinomas that were ER−/PR+, the clinical history, pathology report and IHC slides were reviewed.

Results

The characteristics of ER−/PR+ and ER−/PR- breast cancers were compared (Table 1). In total, 66 patients had ER−/PR+ disease. Histological samples were unavailable for 8 patients and 10 patients were excluded from the analysis on pathological grounds, due to inadequate tissue samples being available for retesting. Therefore, 48 patients were successfully retested. Of those, 22 were false negatives with respect to ER status (ER+/PR+); 21 were ER−/PR-; and 3 were ER+/PR-. We could not confirm the presence of any ER−/PR+ tumours for the period outlined. For those who were found to be ER+, clinical review elucidated that most of these patients were treated with hormone therapy (n = 6). For those who were found to be both ER and PR negative, only a small number were treated with adjuvant endocrine therapy (n = 4). Information on disease free and overall survival is outlined below in Table 1.

Discussion

Rakha et al., in a case series of almost 2000 primary breast cancers, found an ER−/PR+ rate of 3.4% (n = 60), and a trend towards outcomes associated with a triple negative phenotype [7]. De Maeyer et al. found a rate of 1.5% (n = 32) in their cohort of patients, however when they re-stained the samples they found that previously identified ER−/PR+ tumours, were in fact either ER+/PR+, or ER−/PR- [8]1. Colditz et al., identified 3.8% (80/2096) tumours as being ER−/PR+ [9]. Park et al. grouped ER−/PR+ into the luminal A subtype of breast cancer in their case series and also compared the rates of two datasets, the Severance Hospital Breast Cancer Registry dataset and the

Table 1 Characteristics of ER −/PR+ compared with ER−/PR- tumours

	ER−/PR+ (n = 48)	ER−/PR- (n = 157)	P-value
Disease free survival (months)	79.9 (+/− 42.7)	60.6 (+/− 42.4)	0.006
Overall survival (months)	83.9 (+/− 40.7)	70.9 (+/− 41.3)	0.057
Age at diagnosis (years)	53.6 (+/− 11.7)	56.5 (+/− 13.6)	0.185
Tumour size (mm)	26.3 (+/− 15)	28 (+/− 19.2)	0.571
Nottingham Prognostic Index (NPI)	17 (+/− 3.2)	10 (+/− 4)	0.266

Korean Breast Cancer Society dataset (9.4 vs 5.3%, n = 63, n = 936). Yu et al. reported that the 11% ER−/PR+ tumours (n = 205) that were encountered in their review were more likely than ER+/PR+ tumours to get adjuvant chemotherapy and less likely to get adjuvant tamoxifen [10]. Kiani et al. in 2006 reported that ER−/PR+ tumours were of a higher grade and were larger and more aggressive [11]. More recently Shen et al. reported a rate of 2.3% in their case series of 5374 consecutive breast cancers [12]. They again noted younger age and higher histological grade as being associated with the ER−/PR+ phenotype. Dunwald et al. in a series noted a rate of 3% ER−/PR+ tumours, which declined over time [13]. Many of these case series have examined the patient characteristics of ER−/PR+ tumours, without examining whether there may be technical factors associated with reporting of hormone receptor status. Of the reports in the literature, where the receptor status was retested, interesting findings have been unearthed. Rhodes et al. reported in 2000, that the variables affecting receptor positivity were patient age and IHC assay sensitivity [14]. In fact, in laboratories with high IHC assay sensitivity, the frequency of ER−/PR+ tumours was as low as 2.9%. Only 63% (n = 43) of laboratories involved in the study were reported to have high assay sensitivity for ER. Mann and colleagues have suggested that up to 9% of women may have false positive ER IHC results [15]. Schnitt, in an article in 2006, postulated reasons for the discrepancies in ER testing over time. These included; a shift from ligand binding assays (LBA), which required fresh tumour, to antibodies that recognise ER in formalin fixed paraffin embedded tissue resulting in a shift to immunohistochemistry analysis [16]. While the LBA method produced a continuum which closely correlated with response to anti-ER therapy, IHC labels >90% of tumours as either unequivocally positive or wholly negative, without a linear relationship to the quantity of cell based ER. Goldstein identified that at least 6–8 h of formalin fixation was required to obtain reliable ER determination by IHC [17].

Technical difficulties are reported as the major factor in the reported ER−/PR+ breast cancer phenomenon. Several factors affect the sensitivity of the IHC assay including the primary antibody used, the effectiveness of antigen retrieval, the secondary detection systems and the quality of tissue fixation [18–20] . Rhodes et al., in an analysis of ER and PR testing in laboratories across Europe, discovered that the disparities in staining may have been due to too short a time at the maximum temperature during the antigen retrieval step [21].

The method used by laboratories in calculating ER positivity is also disparately reported with 70.8% reporting their method of evaluation as the 10% threshold [13]. High cutoff values may result in tumors being misclassified as ER-. A range of arbitrary cutoffs for ER positivity varying between 5 and 10% are currently in practice in different laboratories, however only one cut-off is clinically validated in predicting response to Tamoxifen [22]. Guidelines for immunohistochemistry assays now recommend definition of ER positivity as 1% or more cells staining, but with some initial uncertainty about whether to include the range 1–10%. However, few patients if tested properly have 1–10% cells staining, and a low cutoff minimises life-threatening false negative ER results due to technical error. Up to 20% of IHC ER/PR testing worldwide, is inaccurate and the ASCO/ACP recommended that tumours staining 1% or greater should be classified as ER+ [6].

Chan et al. [23], in 2015, postulated that the ER−/PR+ subtype may result from failed binding of the ER antibody to the receptor, possibly because of structural changes from mutations. Chans group recommend independent testing using two different antibodies. The true existence of an ER−/PR+ is a topic of hot debate. Chan et al. and others have data to support its existence as a true entity. The data generated by our study has not been able to determine its existence in our cohort of patients. Indeed, other authors have found it a very difficult subtype to reproduce and have generated similar data to ours [24].

As assays improve, fewer breast cancers are reported as ER −/PR+ (4% in the early 1990's, but only 1% in recent years in the SEER cancer registry data) [1]. For the few patients still reported as ER−/PR+, repeat testing on another tissue sample has been recommended [21], to rule out a false-negative ER assay in a patient who could benefit from endocrine treatment. These authors wholly agree with this assertion, given the findings reported herein.

Compliance with Ethical Standards

Conflict of Interest The authors have no conflicts of interest to declare.

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