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RESEARCH

Estrogen Receptor Negative and Progesterone Receptor Positive Breast Carcinomas—How Frequent are they?

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Abstract Estrogen receptor (ER) testing has become an important part of breast cancer reporting as the ER status is a predictor of hormonal treatment efficacy. Progesteron receptors (PR) are often tested in parallel, and the best response to hormonal manipulations can be expected in tumors positive for both receptors. The existence of breast cancers with an ER negative and PR positive phenotype is controversial. A series of cases with this phenotype were reevaluated to clarify the existence and the frequency of this entity. A total of 205/6587 (3.1%; range of the rate per department: 0.3–7.1%.) cases reported to have the ER-negative and PR-positive status by immunohistochemistry were collected from 9 Hungarian departments. After careful reevaluation of the tumor slides and control tissues with a 1% cut-off for positivity and restaining of the questionable cases, all but 1 of the reevaluable 182 cases changed their original phenotype. Most cases converted to dual positives (n=124) or dual negatives (n=31) or unassessable / questionable. ER-negative and PR-positive breast cancers are very rare if existing. Such a phenotype should prompt reassessment.

Keywords Breast cancer · Estrogen receptor · Progesterone receptor · Immunohistochemistry · Estrogen receptor-negative progesterone receptor-positive breast cancer

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Abbreviations

Ab	antibody
BF	buffered formaline
ER	estrogen receptor
ER−	estrogen receptor-negative
ER+	estrogen receptor-positive
HIER	heat induced epitope retrieval
IC	internal control
ID	identification
MoAb	monoclonal antibody
PR	progesterone receptor
PR−	progesterone receptor-negative
PR+	progesterone receptor-positive
RT	room temperature
RTU	ready-to-use prediluted antibodies

Introduction

Several risk factors of breast cancer are related to an absolute or relative excess of estrogens. Ovarian ablation has been empirically known to have therapeutic effects in this disease for a long time. However, hormonal manipulations are not effective in all breast cancers. Logically, estrogen receptors (ER) are important predictors of response to anti-estrogenic treatment, although an ER-positive status is not sufficient on its own to reflect a response to hormonal treatment. The synthesis of progesterone receptors (PR) is ER dependent, therefore it is not surprising that the best response to anti-estrogenic treatment can be expected in those ER positive (ER+) breast cancers which are also positive for PR (PR+) [1, 2], and no response can be expected from tumors expressing neither of these receptors (ER-PR-). Traditionally, ER-PR+ carcinomas of the breast were also considered as hormone receptor positive and suitable for endocrine treatment, however this combination seems to be a very rare phenotype, and even its existence has been questioned [3, 4].

In a quality assessment session initiated in September 2009, cases recorded as ER-PR+ by immunohistochemistry were collected for further analysis from eight larger Hungarian pathology departments.

Materials and Methods

The authors were asked to collect 500 to 1,000 breast carcinoma cases with ER and PR status from institutional databases of 8 Hungarian pathology or related oncology departments. These were classified according to their receptor statuses and the ER-PR+ cases were looked at again. The immunostained slides of these cases were reassessed for the adequacy of their internal controls (if applicable), the possibility of being labeled as ER- on the basis of an ER positivity below the formerly used cut-off limit of 10% of the cells. Cases remaining ER-PR+ after reassessment or with no slides available for review had repeat immunohistochemistry for ER and PR locally. Details of the immunostaining processes are listed in Table 1. The proportions of ER-PR+ cases was determined after all steps of reassessment were completed, and the causes of a false ER-PR+ status were analyzed thereafter. No central review of the cases was intended or done.

Results

Finally 495 to 1,399 breast cancer cases were gathered from each institution. The original distribution of their receptor statuses based on the reports based institutional databases are shown in Table 2. There were 205 cases (3.1%; 95% confidence interval 2.7-3.5%) listed with an ER-PR+ status. Although this was the rarest combination of the ER and PR expression, its proportion in a given institution ranged from 0.3% to 7.1%. These cases were reviewed in this study.

Slides were not available for retrospective analysis in 43 cases, and no blocks were available for repeating the assay in 23 of these cases (blocks missing in 39 cases overall). Therefore, 182 cases were further evaluated, as they had either sections or blocks to reinterpret and / or repeat the assay (Fig. 1). Two cases were classified as unsuitable for ER and PR immunohistochemistry due to the poor quality of the tissue specimens. The slides of 162 cases could be reviewed. After review of the slides 70 cases (39%) changed their status (62 to dual positive, 7 to dual negative and 1 to ER-positive and PR-negative; 3 of these changes were due to administrative errors of entering data into databases). More than half of these ER-positive cases had less than 10% of the nuclei staining with the ER antibody, and most of the remaining had only faint staining contributing to the interpretation as ER-negative.

The steroid hormone immunohistochemistry was repeated in the cases unavailable for review (no slides) or interpreted as ER-negative and PR-positive on review. Most cases (123/180; 68%) turned out to be dual positives, false-negative ER result being the most common causes of the ER-negative and PR-positive (Fig. 1). Of the 62 cases (one of them being falsely PR-positive as well)15 had no internal tissue controls, and 23 had internal tissue controls with unacceptable results; the external controls were not always documented in these cases, but 20 of them had adequate external control staining.

False-positive PR status was identified in 24 cases (13%; 1 case associated with false-negative ER status). Eight lacked an internal tissue control and 3 had inadequate internal control reactions. Five cases had lymphocytes

Table 1 Basic details of the methods used for ER and PR status determination

ID	Details	Negativity
A	Manual. Fixation: 8% BF; HIER 45 min pH6 citrate buffer; endogenous peroxidase block with 3% hydrogene peroxide; 6 F11 mouse MoAb for ER (Novocastra, Newcastle, UK) 1:40; PgR636-RTU mouse MoAb for PR (DakoCytomation, Glostrup, Denmark) 1:2; detection with ImPress Universal Kit (Novocastra, Newcastle, UK) 30 min, AEC as chromogen; hematoxylin as counterstain	<10% or <1% (overlap in time)
B	Manual. Fixation: 10% BF, pH7; endogenous peroxidase block with 3% hydrogene peroxide; HIER 20 min pH6 citrate buffer; 1D5 mouse MoAb for ER, PgR 636 mouse MoAb for PR (both DAKOCytomation, Glostrup, Denmark), detection with ENVISION (DAKOCytomation, Glostrup, Denmark) polymer 30 min, DAB as chromogen; hematoxylin as counterstain	0% (i.e.<1%)
C	Automated (Ventana NexES autostainer, Tucson, AZ) HIER 20 min, pH6 citrate buffer; PR/SP2 rabbit MoAb (Labvision, Thermo Fisher Scientific, Fremont, CA) 1:400, ER/SP1 rabbit MoAb (Labvision, Thermo Fisher Scientific, Fremont, CA) 1:100, 30 min, 30–32°C; biotynilated secondary Ab 8 min; strepavidin peroxidase 8 min; DAB as chromogen; hematoxylin as counterstain or Automated (Leica Bond autostainer, Wetzlar, Germany) HIER same; SP2/1:800;followed by 1:1,800, SP1: 1:50, 30 min, RT; biotynilated secondary Ab 20 min; strepavidin peroxidase 20 min; DAB as chromogen; hematoxylin as counterstain	<1%
D	Manual. Fixation: 8% BF, pH7; endogenous peroxidase block with 3% hydrogene peroxide; HIER 20 min pH6 citrate buffer; 1D5 mouse MoAb for ER, 1:400; PgR 636 mouse MoAb 1:500 (both from DAKOCytomation, Glostrup, Denmark), 30 min, detection with SPO (Vector Laboratories, Burlingame, CA), DAB as chromogen; hematoxylin as counterstain.	<10% or <1% (overlap in time)
E	Fixation: 10% BF. Automated (Ventana NexES autostainer, Tucson, AZ) HIER ER: 30 min TRIS-EDTA pH9 and PR: Vector H3300 antigen retrieval solution (Vector Laboratories, Burlingame, CA); NCL-ER-6 F11 mouse MoAb for ER (Novocastra, Newcastle, UK)1:200; NCL-PGR-312 mouse MoAb (Novocastra, Newcastle, UK) 1:200; iVIEW DAB Detection Kit, hematoxylin counterstain.	0% (i.e.<1%)
F	Automated (Benchmark XT, Ventana, Tucson, AZ). Fixation: 8% BF, pH 7; endogenous peroxidase block with 3% hydrogene peroxide; HIER: 30 min pH 6 citrate buffer; SP1 rabbit MoAb for ER (NeoMarkers/Labvision, Astmoor Runcorn, UK) 1:100; NCL-L-PGR-312 mouse MoAb for PR (Novocastra, Newcastle, UK) 1:180; Detection with Ultra-View multimer kit (Ventana, Tucson, AZ) and DAB as chromogen.	<1%
G	NCL-ER 6 F11 mouse MoAb for ER (3:100), NCL-PGR-312 mouse MoAb for PR (1:100)(both from Novocastra Laboratories, Newcastle, UK); detection with Vectastain Universal Quick KIT (Vector Laboratories, Burlingame, CA) or universal ImmPRESS KIT (Novocastra Laboratories, Newcastle, UK); VIP as chromogen (Vector Laboratories, Burlingame, CA); Methyl green or hematoxylin as counterstain.	<10%
H	Automated (DAKO 480 autostainer) HIER 20 min 98°C, pH9 citrate buffer, SP1 MoAb for ER (Hisztopatologia KFT, Pécs, Hungary) 1:50; RB-9017 for PR (Labvision, Fremont, CA, USA) 1:100; detection with Envision (DAKO Cytomation, Glostrup, Denmark) 30 min, chromogen DAB 2×7 min, hematoxylin as counterstain	0% (i.e.<1%)

Ab antibody; *BF* buffered formaline; *HIER* heat induced epitope retrieval; *MoAb* monoclonal antibody; *RT* room temperature; *RTU* ready-to-use, prediluted antibodies;

stained with the SP2 anti-PR antibody which disappeared when the assay was repeated. Cytoplasmic staining was misinterpreted in at least one case.

Altogether 25 cases (14% of the analyzed cases, 0.4% of all cases) could have had an ER-negative and PR-positive phenotype, but 16 of these should currently be categorized as uninterpretable due to the lack or improper staining of the internal tissue control and 6 further cases with this phenotype had no blocks available for controlling the results by repeating the immunostaining assay leaving doubt about the credibility of the results. Therefore 3 cases (<2% of the analyzed cases, 0.05% of all cases) had an ER-negative and PR-positive phenotype after reevaluation of the original slides, repeating the assay with an adequate internal tissue control. The corresponding patients' ages were 70, 57 and 55. All three tumors were grade 3, poorly

Table 2 Distribution of breast cancer cases by ER and PR status

ID	n	ER+PR+	ER+PR-	ER-PR-	ER-PR+
A	1000	690 (69.0%)	117 (11.7%)	186 (18.6%)	7 (0.7%)
B	1081	761 (70.4%)	95 (8.7%)	192 (17.7%)	27 (2.5%)
C	500	401 (80.2%)	40 (8.0%)	43 (8.6%)	16 (3.2%)
D	495	311 (62.8%)	66 (13.3%)	98 (19.8%)	20 (4.0%)
E	1399	711 (50.8%)	324 (23.2%)	340 (24.3%)	24 (1.7%)
F	526	412 (78.3%)	39 (7.4%)	72 (13.6%)	2 (0.3%)
G	607	326 (53.7%)	77 (12.7%)	161 (26.5%)	43 (7.1%)
H	986	655 (66.4%)	107 (10.9%)	158 (16.0%)	66 (6.7%)
All	6587	4267 (64.8%)	865 (13.1%)	1250 (19.0%)	205 (3.1%)

ID identification of data providing institution; *n* number of cases; *ER+* estrogen receptor positive, *ER-* estrogen receptor negative; *PR+* progesterone receptor positive; *PR-* progesterone receptor negative.

differentiated ductal carcinomas. These three cases were also retested with an alternative antibody for ER: SP1 was substituted by 6F11, whereas 6F11 was replaced by SP1. With adequate internal tissue control, the ER status of the tumor cells in the tested block remained negative on the retest done in a third laboratory also taking part in the study, except for one case where the tumor showed 3 cells, which comprised more than 1% of the small invasive carcinoma associated with an ER-negative high grade in situ ductal cancer. The same respective area contained also

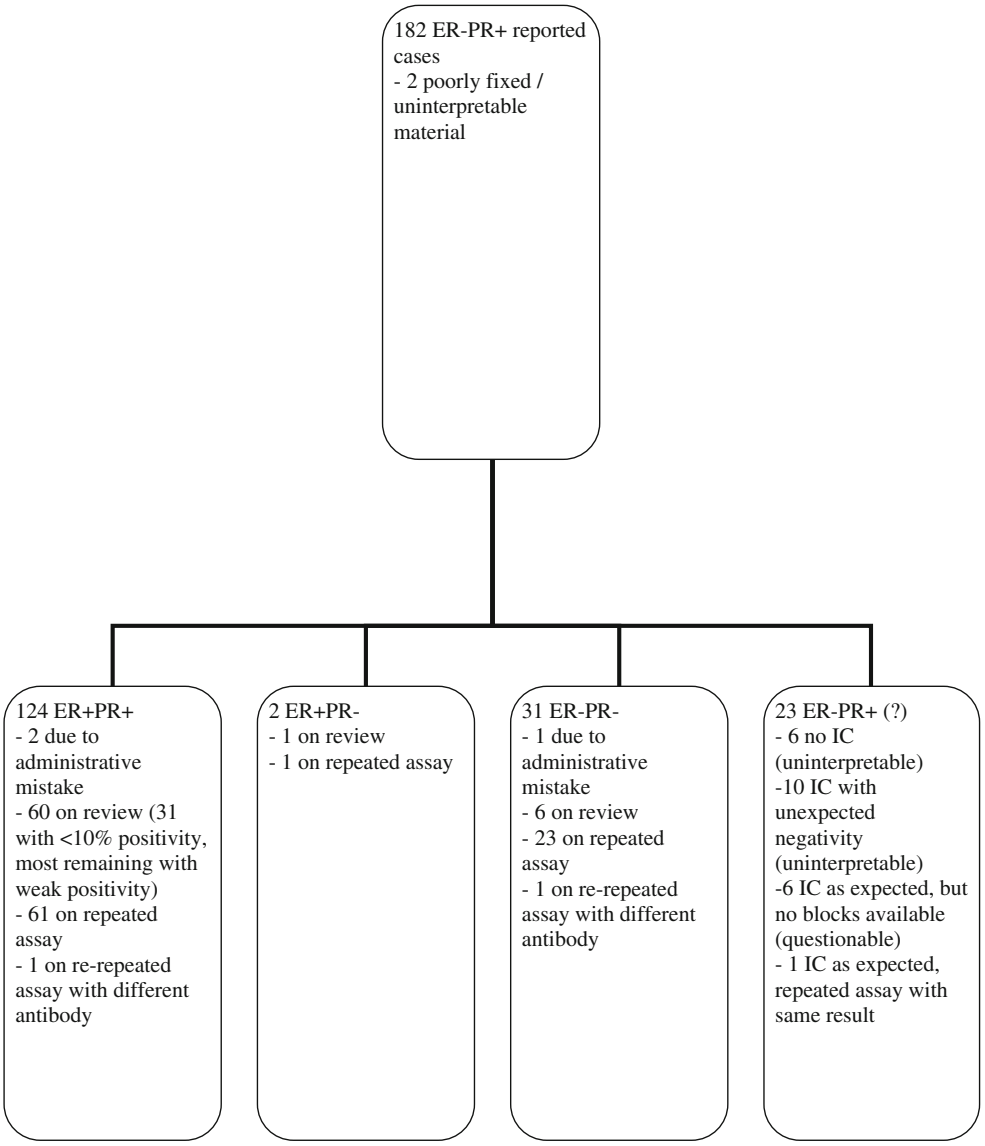

Fig. 1 Redistribution of cases identified as ER negative PR positive

three PR positive cells (retest done with PgR636 antibody), and therefore this tumor turned out to be weekly positive for both receptors. A second tumor was found to be PR negative too on the retest at alternate laboratory and therefore only one tumor kept its ER- PR+ phenotype.

Discussion

The steroid hormone receptor status of breast carcinomas is an important predictive factor of this disease, and has a major impact on the selection of systemic treatment [5]. Endocrine treatment is traditionally available for patients being ER+ and/or PR+.

After the collection of ER-PR+ cases from departmental/institutional databases, there were 205/6587 (3.1%) cases recorded with this phenotype. Although there was a variation in the frequency of this combination of hormone receptors by institution, the overall rate is very similar to the 3.2% (131/4053) mentioned by Rhodes et al [6] or the 3.4% reported by Rakha et al, who found 60/1944 of primary breast cancers from the Nottingham Tenovus Study to have this phenotype by using <1% nuclear immunostaning rate to define the negative categories [7]. Although most departments used this cut-off for defining their negative categories, a few used the 10% limit (Table 1), and 31 cases (17%) turned to be ER+ simply by applying the new cut-off of 1% [8].

After reviewing the cases which had slides available for repeated reading or after reperforming the immunostaining of the ER-PR+ cases, the majority turned to be either ER+PR+ (68%) or ER-PR- (17%). Of the 25 cases keeping their ER-PR+ phenotype, about two thirds should be labeled uninterpretable, due to the absence or inadequate staining of internal tissue controls [8], and only 3 cases tumors (0.05% of all cases, and 1.7% of the reassessed cases) demonstrated the ER-PR+ combination, but reassessment at a different laboratory with alternative ER antibody spilt these 3 cases into ER+PR+, ER-PR- and ER-PR+, leaving only a single tumor in this questioned category. It has been suggested that this phenotype is more likely to occur in patients younger than 51 [6] and is associated with more aggressive tumor behavior [7], but the few cases left, were not suitable for statistical analysis; neither of the tumors occurred in young patients, but all three were high grade.

Although data on the actual therapy of individual patients was not available due to the nature of the study, ER-PR+ tumors were generally considered as steroid hormone receptor positive carcinomas, and this was considered a subset of hormone-sensitive tumors. Therefore, hormonal treatment must have been considered and/or recommended for most of them. In the light of a few cases turning to a double negative phenotype due to a false-positive progesterone receptor status, these patients might have received hormonal treatment without benefit.

Review of the ER-PR+ breast carcinomas has resulted in a sharp decrease of the numbers assignable to this phenotype in several previous reports. Of 105 cases reviewed in the context of the BIG 1-98 study, 81 displayed at least 1% ER positivity, leaving only 23% with the ER-PR+ status. The details of the review were not fully given, but seemingly the review consisted of both reviewing slides and repeating the immunostains centrally [9]. A locally performed review of all 32 ER-PR+ cases among 2,013 primary breast cancers from Leuven made all of these tumors transformed to either dual positive (27 cases, 85%) or to dual negative (5 cases, 15%). Our study similarly demonstrated a significant reduction of the ER-PR+ phenotype with review of the original slides or newly stained slides, and most cases turned to be dual positive (due to false-negative ER status) and fewer to dual negative (due to false-positive PR status).

The occurrence of downstream functions in the absence of upstream signaling path activation is not unknown in biology and carcinogenesis, therefore the existence of an ER-PR+ phenotype cannot be excluded on theoretical grounds. However recent evidence suggests that this combination of steroid hormone receptors is extremely uncommon (if existing at all). An ER-PR+ phenotype should urge the repetition of the test on the same or alternative material. Both false-negative ER status or false-positive PR status can lie behind the finding of an ER-PR+ immunohistochemistry result. To avoid false results, a 1% cut-off value should be used to separate receptor-negative and –positive statuses and internal (and/or external) tissue controls should be considered with sufficient caution as suggested by the newly published ASCO/CAP guidelines. The use of an uninterpretable category for cases with inadequate staining of the controls is also recommended.

Although no clinical validation was possible, the results reported are in keeping with several recent publications and stress the need to reevaluate any cases that show to be ER-PR+, and here an alternate antibody, tissue block or laboratory should also be considered.

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