The Breast 22 (2013) 974–979

Contents lists available at SciVerse ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

An evaluation of the impact of technical bias on the concordance rate between primary and recurrent tumors in breast cancer

Jose A. Pérez-Fidalgo [a,*,f], Pilar Eroles [b,f], Jaime Ferrer [c], Ana Bosch [a], Octavio Burgués [c], Francisco Martínez [d], Begoña Bermejo [a], Ana Lluch [a,*], Ana M. González-Angulo [e]

[a] Department of Oncology and Hematology, Research Institute INCLIVA, Hospital Clinico Universitario Valencia (HCUV), Avda Blasco Ibañez 17, 46010 Valencia, Spain
[b] Research Institute INCLIVA, University of Valencia, Spain
[c] Department of Pathology, Breast Cancer Unit, Research Institute INCLIVA, HCUV, Spain
[d] Department of Statistics, University of Valencia, Spain
[e] Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center Houston Texas, USA

ARTICLE INFO

Article history:
Received 3 December 2012
Received in revised form
16 April 2013
Accepted 23 May 2013

Keywords:
Metastatic breast cancer
Estrogen receptor
Progesterone receptor
HER2
Technical bias

ABSTRACT

Purpose: Whether or not to biopsy the metastasis in recurrent breast cancer has become mired in controversy. Several studies have shown an important discordance of the immunohistochemical (IHC) determinations for ER, PR and HER2 between primary (PT) and recurrent tumors (RT). Yet it remains unknown within this what impact technical issues have. The aim of our study was to assess whether technical variability might have an impact on the concordance between PT and RT.
Methods: IHC determinations in paired biopsies from PT and RT were compared under routine vs study conditions. In the former, pathological analysis reproduced the conditions used in the routine of a University Pathology Department. In the latter, in a technical bias-minimizing manner, samples were re-assessed at the same timing and by two independent observers.
Results: 128 paired biopsies from 64 patients were analyzed under both conditions. Concordance under routine vs study conditions for ER was 66% vs 93.4% ($p = 0.001$), for PR 58.7% vs 80.3% ($p = 0.064$) and for HER2 86.8% vs 96.8% ($p = 0.25$). Kappa index under routine versus study conditions for ER was 0.27 vs 0.79 ($p = 0.002$), for PR 0.26 vs 0.39 ($p = 0.47$) and for HER2 0.67 vs 0.9 ($p = 0.14$).
Conclusions: Although discordance rate between PT and RT decreased under conditions minimizing technical issues, some discordant cases appeared not to be subjected to this confounding factor. Either for clinical practice or for future studies reassessment of PT in recurrent breast cancer should be encouraged.

Introduction

Metastatic breast cancer (MBC) remains an incurable disease but correctly targeted treatment can improve outcomes [1,2]. Nowadays, among other considerations, clinicians base the treatment strategy on the characterization of hormone receptor (HR) status and HER2 expression in order to determine those patients who may benefit from HR and HER2 blockage strategies. Knowing HR and HER2 status is therefore essential, for it not only provides prognostic information but it is also used to predict response to specific treatment.

Many retrospective series and some prospective studies [3–15] have reported changes in HR status and HER2 expression between PT and RT in breast cancer. To our knowledge, however, changes in the determination of these receptors have not been biologically explained even though these changes may have an impact on the clinical management of MBC patients [15]. Previous studies have also shown that patients with changes in HR and HER2 expression have worse prognosis than those without them [16]. This may be because of inappropriate use of targeted therapies, actual changes in tumor biology or a selection of cells having become resistant to previous targeted therapies. Whether these changes may also be attributed to either suboptimal reproducibility in determination techniques, such as IHC, or to biological changes in receptor expression [17,18] remains a matter of high concern.

In order to address this query we performed a retrospective study in patients with recurrent breast cancer in which paired samples of PT and RT were available. Our principal objective was to

* Corresponding authors. Department of Medical Oncology, Hospital Clinico Universitario, Avda Blasco Ibanez s/n, 46010 INCLIVA, Valencia, Spain. Tel.: +34 645317947, +34 963862625.
E-mail address: japfidalgo@msn.com (J.A. Pérez-Fidalgo).
[f] JAPF and PE contributed equally to the development of this manuscript.

0960-9776/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.breast.2013.05.014

evaluate the impact of technique bias in the concordance rate in HR and HER2 status between PT and RT under two different technique conditions: diagnostic routine conditions and study reassessment conditions. Two secondary objectives were to examine the concordance rate between PT and RT in our series and to explore the potential influence of other variables.

Patients and methods

Patients

We performed a retrospective analysis of patients diagnosed between July 1999 and June 2010 with an early breast cancer that presented a metachronic recurrence and for whom we had available paired biopsies of their PT and RT. All metastatic locations were included. In patients with more than one metastatic site, location of biopsy was decided by the clinician according to feasibility and safety criteria. Patients diagnosed up-front with a stage IV breast cancer were excluded from the study.

In the routine conditions, information regarding demographic variables, prognostic pathological factors and treatment as the result of ER, PR and HER2 was obtained from the medical and pathological records of each patient prospectively maintained in our database. In order to assess the impact of discordance on treatment decision for endocrine therapy, results from ER and PR were regrouped in a new variable, named hormone-sensitivity (HS). ER− (negative ER) and PR− (negative PR) cases were considered hormone resistant (or non-HS). Cases that presented either an ER+ (positive ER) or PR+ (positive PR) result, or both (ER+ and PR+), were considered hormone sensitive (HS).

Methods

The samples analysis was performed in the Department of Pathology at the Hospital Clínico Universitario of Valencia, a university-associated institution. The Department laboratory, has been submitted to external Quality Assurance Programs. The procedure consisted of staining and determining HR and HER2 status in both routine and study conditions. Biopsies and surgical specimens from PT and RT had been fixed for 24 h in 10%-buffered formaldehyde and embedded in paraffin. Tissue from all bone metastasis included in the study had been obtained by means of trephine biopsy and decalcified in Bouin's fixative for a maximum of 24 h. For each case, samples consisting of hematoxylin–eosin stained slides were reviewed by two expert pathologists (OB, JF-L) in order to confirm their accuracy. Relevant tumor features (histological type, size, grade, presence and type of in situ component, and lymph node status) were assessed following international guidelines.

Definition of diagnostic routine conditions

IHC (and later fluorescent in situ hybridization [FISH]) technologies and analysis have been subjected to change since they were implemented at the Department of Pathology of our institution in 1989. Automated work has generally involved a stricter adjustment to manufacturers' products, guidelines and protocols. IHC methods and antibodies used at the Department of Pathology between 1990 and 2012 are summarized in Table 1.

For each case, three-micrometer sections from formalin-fixed, paraffin-embedded tumor tissue were set for IHC together with appropriate positive controls. For the principal duration of our study, primary antibodies against ER (clone 6F11) and PR (clone 1A6) were purchased from Novocastra (Newcastle Upon Tyne, UK). In the last two years of the study (2008–2010), monoclonal anti-rabitt antibodies against ER (clone SP1) and PR (clone 1E2) from Ventana (Tucson, AZ) were employed. IHC determination of HER-2 changed from manual (clone CB11; Novocastra; Newcastle Upon Tyne, UK) to semi-automated (Herceptest™ kit, Dako; Glostrup, Denmark) performance, and to fully automated work (Pathway™ antiHER2, clone 4B5; Ventana; Tucson, AZ) (see Table 1). For FISH studies, a HER-2 DNA probe and a α-satellite centromere probe of chromosome 17 (both from Oncor Inc; Gaithersburg, MD) were initially employed [19]. From 2008 onward, FISH analysis was conducted using a HER-2 FISH PharmDx™ kit (Dako; Glostrup, Denmark).

The number of pathologists involved in the diagnosis and IHC assessment of breast pathology has also varied in the our Department from up to eight general pathologists to two specialist pathologists since the Breast Pathology Unit was established in 2008.

Definition of reassessment study conditions

A complete reassessment of ER, PR and HER-2 status was made for the PT and RT samples. Our Institutional Review Board approved the laboratory studies and chart reviews. IHC and FISH were newly conducted in freshly cut 3-μm sections of paraffin blocks. We used the same methods and antibodies as those described above for routine conditions for the 2010–2012 period (see Table 1). Under these conditions, two observers (OB and JF-L) scored the results in a random order, blinded to other paired samples of data. Cases of inter-observer disagreement were reassessed and jointly discussed until an agreement was reached.

Assessment of HR status

In the diagnostic routine conditions ER and PR were assessed by determining the percentage of positive nuclear staining and H-Score [20]. A <10% of positive cells, or H-Score <10, was considered a negative expression of ER/PR. Since 2008 the Allred score, or quick score [21], has replaced H-Scores in pathology reports. Tumors with an Allred score ≥3/8 [21,22], that is with a moderate to intense nuclear staining of at least 1% of nuclei, are considered ER or PR positive. We adopted this latter criterion for our study reassessment conditions.

Assessment of HER-2

HER-2 positivity was defined as 3 + receptor over-expression on IHC staining (strong membranous staining in at least 30% of cells), and/or gene amplification found on FISH. Initially, a gene copy/

Table 1
Methods and antibodies employed for IHC distributed in periods.

		1999–2005	2006–2009	2010–2012
Automation		Dako Autostainer	Dako Autostainer Link48	Ventana Benchmark XT
Antigen retrieval		Pressure cooker, 3 min, 1.5 atm, citrate buffer pH = 6.5	Dako PT Link, Target Retrieval Solution, 20 min pH = 9.0	Ventana CC1, 30 min, pH = 8.4
Developing technique		LSAB Dako	Dako EnVision Flex™	Ventana Ultraview DAB detection kit
Primary antibodies (source, clone, dilution)	ER	Novocastra, 6F11, 1:40	Novocastra, 6F11, 1:200	Ventana, Confirm anti-ER, SP1, prediluted
	PR	Novocastra, 1A6, 1:30	Novocastra, 1A6, 1:50	Ventana, Confirm anti-PR, 1E2, prediluted
	HER2	Dako, HercepTest™ kit	Dako, HercepTest™ kit	Ventana, Pathway anti-HER2, 4B5, prediluted