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Tissue confirmation of disease recurrence in breast cancer patients: Pooled analysis of multi-centre, multi-disciplinary prospective studies

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ABSTRACT

Background: Treatment decisions in recurrent breast cancer are usually based on the estrogen (ER), progesterone (PgR) and HER2 receptor status of the primary tumour. Retrospective studies suggest that discordance between receptor expression of primary and recurrent breast cancer exists.
Methods: A pooled analysis of individual patient data from two large prospective studies comprising biopsy of recurrent lesions obtained from consenting patients was undertaken. Tissue was analyzed for ER, PgR by immunohistochemistry and HER2 by FISH. Receptor status of recurrent disease was compared with that of the primary tumour. Recruiting clinicians assessed whether or not receptor discordance affected subsequent systemic treatment.
Results: Two hundred and eighty-nine patients underwent biopsy. Recurrent biopsy specimens were obtained from locoregional recurrence in 48.1% and from distant metastases in 51.9%. Distant sites included skin/soft tissue (25.0%), bone/bone marrow (19.2%) and liver (15.8%). Benign disease or second primary cancer was observed in 7.6% of biopsies. Discordance in ER, PgR or HER2 between confirmed primary and recurrent breast cancer was 12.6%, 31.2% and 5.5%, respectively (all $p < 0.001$). Biopsy results altered management in 14.2% of patients undergoing biopsy (95% confidence intervals 10.4–18.8%, $p \leqslant 0.0001$). The duration between primary and recurrent disease, the site of recurrence and the receptor profile of the primary tumour did not affect discordance rates.
Conclusions: There is substantial discordance in receptor status between primary and recurrent breast cancer. The number needed to biopsy in order to alter treatment was 7.1. Patients with recurrent breast cancer should have tissue confirmation of receptor status of recurrent disease.

Introduction

Intra-tumor heterogeneity at both the genetic and protein levels is well described in breast cancer.[1–3] It is, therefore, not surprising that discordance in tumor characteristics between primary and recurrent breast cancer has been observed.[4,5] Retrospective studies show discordance between expression of estrogen (ER) and progesterone (PgR) receptors in the primary tumor versus recurrent disease in as many as 40% of women. Discordance in human epidermal growth factor-2 (HER2) status is lower[6,7] with pooled estimates showing discordance rates of around 5%.[8]

The majority of studies describing such discordance are retrospective analyses; many used different pathological or laboratory techniques between primary and recurrent tissues and few assessed the clinical impact of discordance in receptor expression. These limitations have led to such data being considered unreliable.[9] Therefore, despite discordance between primary and recurrent breast cancer having been described for almost 30 years, the clinical impact remains unclear, with no guidelines recommending biopsy of recurrent disease. Nonetheless, many have argued that biopsy of recurrent lesions should be carried out, either as standard of care or in the setting of a clinical trial.[10,11]

More recently, two independent, prospective, studies reported on the clinical impact of biopsy of recurrent lesions as well as describing discordance rates between primary and recurrent disease analyzed using consistent and standardized methods.[12,13] Both studies reported substantial changes in clinician's choice of therapy

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after the results of biopsy of recurrent lesions were available. However, relative to retrospective analyses, both these studies had small sample sizes, one multi-centre study[12] predominantly sampled loco-regional recurrent disease while the other[13] focused on distant metastases. Here we report a meta-analysis, using individual patient data, of the Breast Recurrence In Tissues Study (BRITS)[12] and DESTINY[13] studies. The aim of this study was to provide improved accuracy and precision for the estimate of the clinical impact of undertaking biopsy of recurrent breast cancer.

Materials and methods

Study population

This pooled analysis included individual patient data from two prospective studies. The BRITS study[12] was conducted at 20 secondary care sites in the United Kingdom and enrolled 205 patients of whom 168 underwent biopsy of a recurrent lesion. The DESTINY study[13] was a contemporary, single-centre study conducted in Toronto, Canada. A total of 137 patients provided consent and 121 underwent biopsy of recurrent lesion. Eligibility criteria for both studies were similar and included written, informed consent, with availability of archival primary tumor for the purposes of re-analysis. Patients with bleeding diatheses precluding biopsy or those with rapidly progressing disease and/or a life expectancy less than 3 months were excluded. In both studies, treating clinicians determined the choice of therapy based on the primary material and subsequently their choice of therapy based on the ER, PgR and HER2 results of the recurrent tumors. This was to determine prospectively whether or not the biopsy led to a change in therapy.

Study endpoints

The primary endpoint of this meta-analysis was the proportion of patients in whom results of the recurrence biopsy led to a change in management. The secondary goals were to define the discordance rates in ER, PgR and HER2 between primary and recurrence samples and to assess the proportion of biopsies from recurrent lesions that yielded sufficient material for analysis of receptor status (technical success rate). A pre-planned, exploratory analysis to determine if factors such as location of recurrence, primary tumor receptor profile or time from primary to recurrent disease influenced the likelihood of change in therapy was also carried out.

Pathological analysis

The pathological analysis of the individual studies is described elsewhere.[12,13] Both studies included in this analysis determined estrogen (ER) and progesterone receptor (PgR) expression by immunohistochemistry. The BRITS study assessed ER using the Novocastra 6F11 antibody and PgR using Novocastra Clone 16. A positive result for ER or PgR was an Allred score of 3 or higher.[14] In the DESTINY study, ER staining was carried out using the Ventana SP1 antibody and PgR using Novocastra Clone 16.[15] A positive result was defined as $\geqslant$1% of tumor cell nuclei staining positively with any intensity. A sensitivity analysis utilizing a uniform cut-off as used in the DESTINY study was also carried out. For both the BRITS and DESTINY studies, primary and metastatic tissues were analyzed using the same standardized methodology at a central laboratory. Primary tumor tissue that was not reported in a central, university-affiliated laboratory or which did not use the latest antibodies and/or hybridization techniques was retrieved and re-analyzed. Both laboratories applied rigorous analytical standardization techniques for hormone receptor testing.[16] These included assessment of external controls and internal normal epithelial elements to ensure that all reagents were appropriately dispensed and that the assay performed adequately. HER2 amplification was assessed by immunohistochemistry and/or fluorescent in situ hybridization (FISH). In the BRITS study samples were initially assessed by immunohistochemistry using the Novocastra CB11 antibody.[17] All equivocal (2+) and positive (3+) samples were then re-analyzed by FISH using the PathVysion HER-2 DNA Probe Kit (Vysis). FISH was also carried out on both the primary and the recurrence in all cases where there was discordance between the two samples. HER2 and CEP17 signals were enumerated from 20 non-overlapping tumor nuclei by 2 independent observers. In borderline cases 60 nuclei were enumerated by 3 independent observers. In the DESTINY study, HER2 was assessed exclusively by FISH using the same platform as the BRITS study. HER2 and CEP17 signals were enumerated from 60 tumor nuclei by 2 independent observers. In borderline cases a total of 120 nuclei were enumerated by 3 independent observers. For this analysis, a HER2/CEP17 ratio of >2.2 was used as the threshold for HER2 gene amplification.[18] A sensitivity analysis using a cut-off of 2.0 was also undertaken. For the purposes of analysis, the status of all receptors was dichotomized into positive and negative utilizing cut offs described above. Quantitative change in the determination of ER and PgR status was assessed independently for each study.

Statistical analysis

Individual patient data were combined and assessed as a single cohort of patients. Data were presented descriptively as medians or proportions. Accuracy of sample proportions was assessed by the test of one proportion and was compared to a hypothetical population proportion using the *Z*-test. Two sub-group analyses were pre-specified: the likelihood of change in therapy based on whether the recurrence was loco-regional or distant and the likelihood of change in therapy based on the receptor profile of the primary tumor. Differences between these subgroups were tested by an interaction test.[19] An analysis of the differences in the likelihood of change in therapy based on the duration between primary diagnosis and recurrence biopsy was also pre-planned and assessed using independent samples *t*-test. For all analyses, two-sided tests with p-values <0.05 were considered statistically significant. Corrections were not made for multiple comparisons.

Results

The pooled analysis comprised a total of 342 consented participants, 289 of whom underwent biopsy of a recurrent lesion. A CONSORT diagram is shown in Fig. 1. Characteristics of patients with sufficient material for analysis of receptor status are shown in Table 1 and locations of satisfactory biopsy are shown in Table 2.

Technical success rate

In total, 267 of the 289 biopsies (92.4%) confirmed recurrent breast cancer. In 21 women (7.3%), biopsies showed benign disease. In one participant (0.3%) a second malignancy (basal cell carcinoma) was discovered. Determination of ER and PgR by immunohistochemistry was possible in 231 of 267 (86.5%) women with confirmed breast cancer recurrence: 137 of 150 patients (91.3%) for the BRITS study and 94 of 117 patients (80.3%) for the DESTINY study. Determination of HER2 status was possible in 220 (82.4%) patients with confirmed breast cancer recurrence (in 91.3% and 70.9% of patients in the two studies, respectively).