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Original Research

Can breast cancer patients with HER2 dual-equivocal tumours be managed as HER2-negative disease?

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KEYWORDS
Breast cancer;
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Outcome

Abstract ***Background:*** Increasing human epidermal growth factor receptor 2 (HER2) immunohistochemistry (IHC)/fluorescence *in situ* hybridisation (FISH) dual-equivocal breast tumours are reported after the 2013 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guideline update. The aim of this study is to investigate the clinico-pathologic characteristics, treatment patterns and disease outcome of these patients with HER2 dual-equivocal tumours.

Patients and methods: Patients with HER2 IHC 2+ and available FISH results were retrospectively analysed from the Comprehensive Breast Health Center, Shanghai Ruijin Hospital. The 2013 ASCO/CAP guideline was applied to define HER2-positive, dual-equivocal and -negative groups. Patient characteristics, systemic treatment patterns and survival were compared among these groups. Reverse transcriptase-polymerase chain reaction-based assays were applied to test *HER2* mRNA expression level.

Results: Among 691 patients included, 133 (19.25%) were HER2 positive, 25 (3.62%) were HER2 dual-equivocal and 533 (77.13%) were HER2 negative. Univariate and multivariate analyses stated that HER2 dual-equivocal tumours shared more similarity with HER2-negative tumours, whereas HER2-positive tumours had rather different clinico-pathologic features. HER2 dual-equivocal tumours had similar *HER2* mRNA levels compared with HER2-negative tumours ($P = 0.26$), which were much less compared with HER2-positive breast cancer. Besides, adjuvant systemic treatment patterns were comparable between HER2-negative and

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dual-equivocal tumours, and none of HER2 dual-equivocal patients received anti-HER2 treatment. There was no survival difference among these three groups ($P = 0.43$).
Conclusion: HER2 dual-equivocal tumours share more similarity with HER2-negative disease in terms of clinico-pathologic features, *HER2* mRNA levels, adjuvant systemic treatment patterns and disease outcome, which deserves further clinical evaluation.

1. Introduction

Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor family, encoded by the gene *ERBB2* (also as *HER2/neu*) located on chromosome 17q12. *HER2* gene amplification and/or HER2 protein overexpression presents in 15–20% of breast cancer cases and have been well established as both prognostic and predictive factor [1]. Over the past decades, the application of HER2-targeted therapy has significantly improved the prognosis of HER2-enriched diseases in adjuvant, neo-adjuvant and metastatic settings [2,3]. Therefore, it is critical to accurately determine HER2 status to maximise the benefit derived from HER2-targeted therapy while minimising unnecessary adverse effects in the management of invasive breast cancer patients.

In clinical practice, when initial HER2 immunohistochemistry (IHC) result is equivocal (IHC HER2 2+), a reflex HER2 test, fluorescence *in situ* hybridisation (FISH) for example, on either the same specimen or an alternative one should be ordered for an accurate determination of *HER2* status. Routine FISH testing has raised the question whether *HER2* gene copy number or *HER2*/control probe ratio better determines *HER2* status and predicts potential benefit from anti-HER2 therapy. To ensure the safety and efficiency of targeted therapy, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) has updated the guideline for HER2 testing in 2013 [4]. Thereafter, the rate of HER2 IHC/FISH dual-equivocal tumours was increasing compared with 2007 ASCO/CAP guideline, which now is defined as *HER2*/CEP17 ratio <2.0 with average *HER2* gene copy number 4.0–5.9 signals/cell [5–7].

Different methods have been tailored to resolve such uncertainty. Shah *et al.* [5] used alternative control probe D17S122 in the initially dual-equivocal patients and managed to reassign 61.2% of them to FISH categorical subset. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)-based tests were also applied in several studies because *HER2* gene amplification is highly associated with mRNA overexpression [8–10].

Although alternative testing methods may help identify HER2 status in HER2 dual-equivocal breast diseases, there were little data about the biological behaviour and treatment selection in this subset. Therefore, in this study, we aimed to investigate the clinico-pathologic characteristics, adjuvant systemic therapies for these HER2 dual-equivocal breast cancer compared with HER2-negative or -positive tumours. Besides, we will also evaluate the clinical outcome of these breast cancer patients with different HER2 statuses, thus, to guide further optimal treatment decision for patients with HER2 dual-equivocal tumours.

2. Patients and methods

2.1. Patient population

We retrospectively analysed patients who underwent surgical procedure from January 2009 to December 2014 in the Comprehensive Breast Health Center, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China. Clinical details were achieved from Shanghai Jiaotong University Breast Cancer Database. Patients who met the following criteria were enrolled for further analysis: (1) female gender, (2) invasive breast cancer proven by core needle biopsy or open excision biopsy, (3) HER2 IHC 2+ with accessible FISH result and (4) complete clinical and follow-up data. Those who receive neo-adjuvant therapy or diagnosed with *de novo* stage IV breast cancer were excluded. This approach was approved by the independent Ethical Committees of Ruijin Hospital, Shanghai Jiaotong University School of Medicine.

2.2. HER2 evaluation algorithm

Tumour biological and histo-pathologic assessments were all performed in the Department of Pathology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China. HER2 IHC staining was first performed on 4-μm slices of formalin-fixed paraffin-embedded (FFPE) specimens of invasive breast cancer and was stained with primary antibody against HER2 (4B5, Roche, Switzerland) at 42 °C for 16 min in Ventana BenchMark XT system (Ventana Medical Systems, Tucson, AZ). All HER2 statuses were re-evaluated and determined according to 2013 ASCO/CAP guideline. The methods and positivity criteria for IHC assessment of oestrogen receptor (ER), progesterone receptor (PR) and Ki-67 were as described in our previous reports [11,12].