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Incorporating microarray assessment of HER2 status in clinical practice supports individualised therapy in early-stage breast cancer

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ABSTRACT

Accurate determination of human epidermal growth factor receptor-2 (HER2) status is essential for optimal selection of breast cancer patients for gene targeted therapy. The analytical performance of microarray analysis using TargetPrint for assessment of HER2 status was evaluated in 138 breast tumours, including 41 fresh and 97 formalin-fixed paraffin embedded (FFPE) specimens. Reflex testing using immunohistochemistry/in situ hybridization (IHC/ISH) in four discordant cases confirmed the TargetPrint results, achieving 100% agreement regardless of whether fresh tissue or FFPE specimens were used. One equivocal IHC/ISH case was classified as HER2-positive based on the microarray result. The proven clinical utility in resolving equivocal and borderline cases justifies modification of the testing algorithm under these circumstances, to obtain a definitive positive or negative test result with the use of microarrays. Determination of HER2 status across three assay platforms facilitated improved quality assurance and led to a higher level of confidence on which to base treatment decisions.

Introduction

Overexpression of the human epidermal growth factor receptor-2 (HER2) occurs in 15–20% of all invasive breast cancers. Quantification of HER2 status plays an integral role in breast cancer prognostication and prediction of the response to HER2-targeted therapies, shown to result in a 30–50% improvement in disease-free and overall survival when combined with chemotherapy [1]. Assessment of HER2 status is therefore recommended in all patients with invasive breast cancer using immunohistochemistry (IHC) [2]. However, up to 20% of test results may be inaccurate, especially where testing is not centralised.

In an attempt to reduce variability in HER2 testing, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) recommended that laboratories should demonstrate high concordance when comparing their results of HER2 testing with other validated HER2 tests [2]. Adoption of international external quality control measures improved the reliability and standardization of IHC HER2 testing. Fluorescence in-situ hybridisation (FISH) should be performed routinely in equivocal IHC 2 + cases, but small tumour size may be a limiting factor. Complete depletion of the invasive component of the tumour may occur and a high degree of discordance has been reported between different laboratories using FISH [3]. Interpretation of IHC/FISH results may be particularly challenging in cases with tumour heterogeneity or chromosome 17 polysomy. According to the ASCO/CAP revised criteria reflex testing should be performed in equivocal cases using an alternative assay. HER2 equivocal results and variability in

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reporting definitions of the optimal ranges for both IHC and FISH was identified as a gap in the literature as the decision to treat is by nature dichotomous (yes or no). The need for obtaining optimal test results warrants the development of novel methods that may be applied in conjunction with standard pathology to provide a definitive guidance in HER2 targeted therapy.

While determination of HER2 status based on mRNA expression levels has already been introduced into clinical practice, the ASCO/CAP Update Committee is of the opinion that there is insufficient evidence to support the use of genomic tests for this purpose [2]. Reverse transcriptase polymerase chain reaction (RT-PCR) is indeed considered unsuitable for determination of HER2 status [4]. This limitation was highlighted by concerns over the value of assessing HER2 status as part of the RT-PCR method used for the 21-gene Oncotype DX assay, which led to inappropriate HER2 targeted treatment in some patients [5,6]. Determination of HER2 status using multi-gene profiling tests is therefore not recommended due to potential clinical implications of inaccurate results and the impact on cost-effectiveness [6,7]. However, whether this restriction also applies to microarray-based multi-gene assays remains unclear, especially when formalin fixed paraffin embedded (FFPE) tumour tissue is used. Sapino et al. [8] successfully transferred microarray analysis using the 70-gene MammaPrint test from the initial use of fresh tumour to the more convenient use of FFPE tissue; however, a direct comparison between protein expression (IHC) and microarray-based mRNA expression (TargetPrint) for assessment of HER2 status using FFPE specimens has not been reported previously in relation to reflex testing in discordant cases.

HER2 status is reported as a separate read-out (TargetPrint) from the versatile MammaPrint microarray that enables the identification of a subgroup of low-risk patients with HER2-positive breast cancer [9]. In patients classified as low-risk according to the 70-gene MammaPrint profile, chemotherapy can be safely avoided without compromising long-term clinical outcome [10]. MammaPrint has been available in South Africa since 2007, and in 2009 local referral guidelines have been adopted to improve cost-effectiveness [11]. These guidelines referred to as the MammaPrint prescreen algorithm (MPA), exclude hormone receptor negative and HER2 positive patients for reimbursement by medical aid funders. This resulted in a highly selected study population of HER2 negative cases, as well as IHC/ISH equivocal and borderline HER2-positive cases that could be further assessed in relation to the clinical dilemma presented under these circumstances.

The aim of the study was to determine the level of agreement between HER2 status based on TargetPrint compared to standard IHC/FISH performed at various local laboratories in South Africa. To our knowledge, the clinical utility of TargetPrint using FFPE tumour specimens for the majority of samples tested has not previously been investigated at the interface between the laboratory and the clinic. Analytical validation of TargetPrint in the South African population is important due to the significant impact of HER2 status on treatment decision-making. Quality assurance may also be improved as a result of this study by obtaining a second opinion of HER2 status based on objective microarray analysis.

Subjects and methods

A central genomics database was established to collect data from South African breast cancer patients using an ethically approved protocol. MammaPrint became commercially available in South Africa after approval of the test by the Food and Drug Administration (FDA) in 2007. TargetPrint was added as a separate read-out from the MammaPrint microarray platform from 2009, providing quantitative estrogen receptor (ER), progesterone receptor (PR) and HER2 status.

Study population

From a total of 157 early-stage breast cancer tumours successfully analysed using the 70-gene MammaPrint microarray profiler, 19 cases were excluded from this study as TargetPrint was not performed prior to 2009. Of the remaining 138 tumours, RNA was extracted for microarray analysis from 41 fresh tumours and 97 FFPE tissue biopsies. The TargetPrint mRNA expression levels were compared with routinely performed IHC and in situ hybridisation (ISH) assessments of HER2 status. As outlined in Fig. 1, HER2 assessments based on protein expression (IHC), DNA amplification (FISH) and mRNA expression (TargetPrint) were performed in a total of 127 samples. A FISH result was not available for 11 of the 138 specimens subjected to TargetPrint.

Analysis of HER2 status using immunohistochemistry (IHC)

Assessment of HER2 status by protein expression using IHC was performed during routine analysis according to local laboratory procedures. Resected surgical specimens or needle core biopsies were fixed in 10% neutral buffered formalin for 6–48 h as specified in the 2013 ASCO/CAP guidelines for HER2 testing. Tissue blocks were processed according to analytically validated protocols and 3 μm paraffin embedded tissue sections were mounted on Histo-Bond® (Marienfield GmbH & Co. KG, Lauda-Königshofen, Germany) positively charged slides. Slides were baked at 60 °C for 30 min in an incubator. Dewaxing and staining of slides were performed on the Leica Bond III™ automated IHC/ISH instrument (Leica Biosystems Pty Ltd, Melbourne, Australia) using the Bond Polymer Refine Detection kit (Cat.DS9800) with diaminobenzidine (DAB) chromogen. The primary antibody, Novocastra™ HER2 (Leica Biosystems, Newcastle, UK) clone 10A7 (Cat. NCL-L-CBE) targeting the external domain of the cell membrane, was diluted 1:50 using Bond™ Primary antibody diluent (Cat.AR9352). Pre-treatment was performed at a pH of 6 for 10 min at room temperature using the Bond™ Epitope Retrieval 1 solution (Cat. AR9961). The primary antibody was incubated for 20 min at room temperature. Positive control sections containing known positive 2+ and 3 + tumour sections were added to each slide for quality control purposes. Non-specific staining for each detection kit was performed omitting the primary antibody. The manufacturer's diaminobenzidine (DAB) detection kit was used that includes the biotin-free polymerase

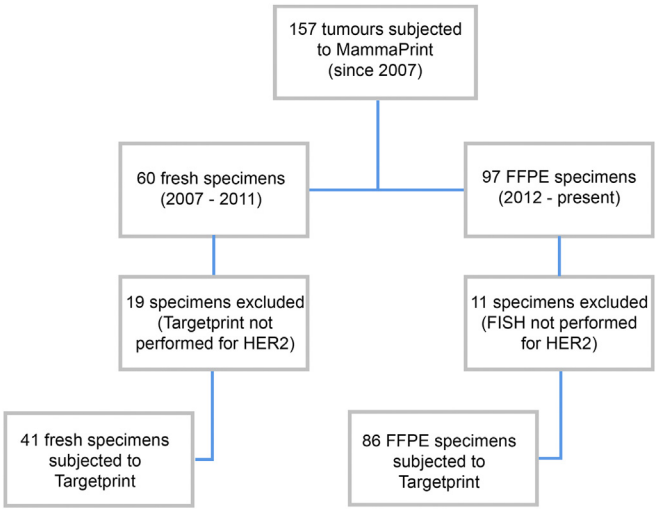

Fig. 1. Selection of 41 fresh and 97 FFPE tumour specimens for comparative analysis of HER2 status between microarray analysis (TargetPrint) and standard IHC/FISH.