Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: http://www.elsevier.com/locate/rpor

Original article

Comparison of *in situ* hybridization methods for the assessment of HER-2/neu gene amplification status in breast cancer using a tissue microarray

Anna Malicka-Durczak, Konstanty Korski, Matthew Ibbs*

Department of Tumour Pathology, Chair of Oncology, Poznan University of Medical Sciences, Greater Poland Cancer Center, Poznan, Poland

ARTICLE INFO

Article history:
Received 22 June 2010
Received in revised form
21 September 2011
Accepted 7 October 2011

Keywords:
Breast cancer
CISH (duo-CISH)
FISH
HER-2
Tissue microarray

ABSTRACT

Background: This project compared HER-2/neu gene status in breast cancers, as demonstrated by FISH (fluorescent *in situ* hybridization) and CISH (chromogenic *in situ* hybridization) and using a tissue microarray (TMA). The study also aimed to show whether the TMA technique could be used in clinical diagnostics, rather than remain a scientific tool.
Materials and methods: A TMA was constructed using 121 breast cancer specimens, 6 cores from each specimen. Demonstration and assessment of HER-2/neu gene status was by FISH (Vysis Path) and CISH (DAKO Duo CISH).
Results: The 121 breast cancer specimens were divided into 3 groups by HER-2 status, as determined by immunohistochemistry. In the HER-2 negative group no amplification was observed in 36 out of 40 cases. 3 cases showed amplification by both methods and one by CISH alone. The equivocal HER-2 group showed no amplification in 30 out of 41 cases and amplification in 9 cases. One case was FISH negative CISH positive and one was discarded. In the HER-2 positive group, amplification was confirmed in 37 of the 40 cases by both methods. 3 cases were unsuitable for assessment.
Conclusions: This study indicated that CISH is a sensitive alternative to FISH in detecting HER2 gene amplification and may replace FISH in HER2 testing. Good agreement was observed between methods (98.5% – 119 out of 121 cases).

Furthermore, as only 4 out of 121 cases were unsuitable for assessment (no signal or missing TMA cores) – it may be feasible to use TMA in diagnostics.

1. Background

One of the most important prognostic and predictive markers in breast cancer is Human Epidermal Growth Factor Receptor 2 (HER2), whose overexpression is associated with an aggressive disease course. In many Polish oncology centres, including the Greater Poland Cancer Centre in Poznan, HER2 status testing has been included in a routine diagnostic procedure applied to all new cases of breast cancer since 2001.[9] The assessment is carried out according to an algorithm (Fig. 1). A routinely obtained result is verified, using the *in situ* hybridization method, only in the event when the result is borderline positive (15–30% of all cases),[4] that is the result

* *Corresponding author at*: Department of Tumour Pathology, Greater Poland Cancer Centre, 15 Garbary Str., 61-866 Poznań, Poland.
E-mail address: anna.malicka@wco.pl (A. Malicka-Durczak).

1507-1367/$ – see front matter © 2012 Greater Poland Cancer Centre, Poland. Published by Elsevier Urban & Partner Sp. z.o.o. All rights reserved.
doi:10.1016/j.rpor.2011.10.005

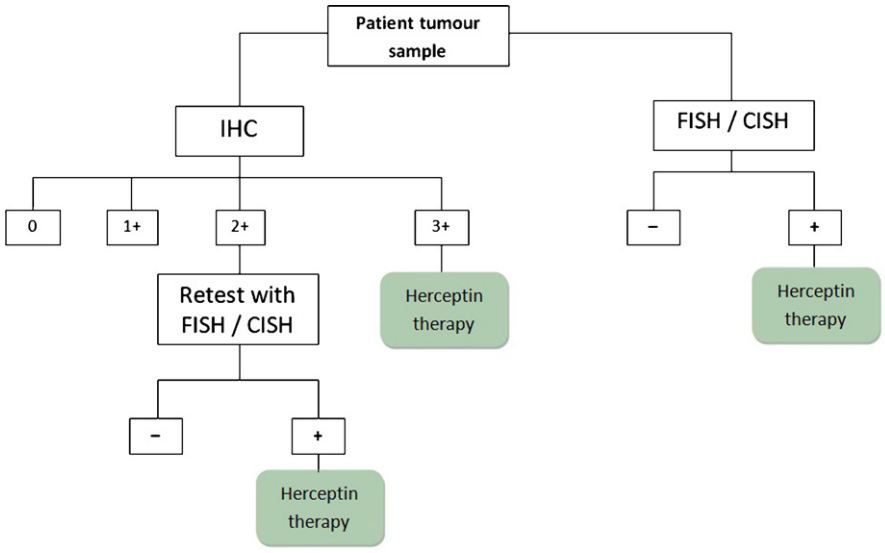

Fig. 1 – The algorithm for the determination of HER2 status in breast cancers.

of immunohistochemical HER2 assessment is graded 2+. Amongst patients with metastases to the lymph nodes, overexpression of HER2 is associated with a median survival period of 3 years, while a 6–7 year survival period is an average for patients without overexpression of HER2 in their cancer cells.[7,10,18]

The value of HER2, both as a prognostic and as a predictive factor, requires practical, repeatable, reliable and easily accessible assessment methods. In practice, methods for the assessment of HER2 status include immunohistochemistry (IHC) and *in situ* hybridization (fluorescent – FISH and chromogenic – CISH). Their value arises from the possibility of assessing the parameter of interest within preserved cancer specimens (IHC) or within the nuclei of cancer cells (FISH, CISH) by means of an optical microscope (or a fluorescence microscope in the case of the FISH method). Besides, these methods allow a simple testing of archival neoplastic tissues stored in paraffin blocks. HER2 testing should be performed routinely in the case of patients with a diagnosis of invasive breast cancer. Some controversy exists, however, as to the best method for the determination of HER2 status. This concerns not only the choice of test, but also the optimal method for the application of that test to every case.[11,16–18]

In spite of efforts on the part of the international pathology community aimed at improving the status of HER2 testing in routine practice,[6] inadequacies in immunohistochemical and *in situ* hybridization tests remain a serious problem.[16,17]

For the purpose of saving both time and money, it seems reasonable to test as many samples as possible on a single slide. The introduction of the tissue microarray (TMA) technique in 1986, with further subsequent improvements, helped achieve this goal. This approach allows the assessment of many sections at a time by putting tens of tissue cores into one paraffin block (up to 200 per block) and simultaneously reduces the amount of reagent required to test each specimen.[1–3,13]

2. Aim

The aim of this study was to confirm that:

1. The CISH method is concordant with the currently most widely used FISH method, for the assessment of HER2/neu status in breast cancer cells.
2. The TMA technique is a reliable and inexpensive approach to the simultaneous assessment of HER2/neu status in breast cancer samples taken from many different patients.

3. Materials and methods

The study group comprised of 121 women diagnosed in the Greater Poland Cancer Centre in 2009, aged between 34 and 87 years, with a clinical diagnosis of invasive breast cancer.

Cases were excluded from the study if the originally diagnosed material had been obtained by fine needle aspiration biopsy or if the fixation of the tissue had been insufficient. On the basis of a histopathological assessment of immunohistochemically stained slides (stained for overexpression of the HER2 receptor), the group of 121 breast cancer cases was subdivided into 3 groups (by IHC):

1) negative (IHC based HER2 receptor status: score 0/1)
2) equivocal (IHC based HER2 receptor status: score 2)
3) positive (IHC based HER2 receptor status: score 3)

Subsequently, on the basis of a histopathological assessment of traditionally stained haematoxylin and eosin (H&E) stained slides, areas of tissue containing cancerous cells were selected. These areas defined source material for the collection of tissue cores to be used in the construction of tissue microarray (TMA) blocks.