Original Article

The HercepTest and Routine C-erbB2 Immunohistochemistry in Breast Cancer: Any Difference?

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Abstract

Introduction: The proto-oncogene c-erbB2, located on chromosome 17q21, encodes a 185-kD transmembrane glycoprotein. It is known to be overexpressed, amplified, or both in 20% to 30% of breast cancers. C-erbB2 belongs to the human epidermal growth factor receptor (tyrosine kinase receptor) family that plays an important role in cell cycle regulation and differentiation. Although there are various methods to assess c-erbB2 status in breast cancer, protein overexpression determined by immunohistochemistry and gene amplification using fluorescence in situ hybridisation are most commonly utilised. This study compares the results of the DAKO HercepTest with the immunohistochemical assay (A0485, DAKO), which is routinely used in our pathology laboratory. Materials and Methods: Paraffin-embedded breast cancer tissues from 41 patients operated in a tertiary hospital during the year 2000 were subjected to immunohistochemistry by the above methods. C-erbB2 positivity was defined by cytoplasmic membrane staining of 2+ or 3+ intensity. Results: Overexpression of c-erbB2 protein was present in 36.6% and 41.5% of cases when detected by HercepTest and the DAKO A0485 antibody, respectively. There was almost perfect agreement between both methods (k = 0.898) when positive versus negative results were considered, and moderate agreement in terms of individual staining intensities (k = 0.554). Conclusion: Routine immunohistochemistry using the DAKO A0485 antibody is a reliable, cost-effective alternative to the HercepTest in determining prognosis and suitability of patients for Herceptin therapy.

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Key words: Cost, Fluorescence in situ hybridisation, Overexpression

Introduction

The proto-oncogene c-erbB2 (also known as HER2/neu) is located on chromosome 17q21[1] and encodes a 185-kD transmembrane glycoprotein. It belongs to the human epidermal growth factor receptor (tyrosine kinase receptor) family[1] that plays an important role in the regulation of fundamental processes such as cell growth, survival and differentiation. It is well-known that in approximately 20% to 30% of patients with breast cancer, tumour cells show an amplification and/or overexpression of c-erbB2.[1-3] C-erbB2 overexpression plays a pivotal role as a prognostic marker by itself and in correlation with other markers.[4,5] Positive c-erbB2 status, alone or in association with nodal status, is generally associated with more aggressive disease leading to shortened disease-free survival and overall survival when compared with patients bearing c-erbB2-negative tumours.[3,6-8]

In addition to its prognostic value, evaluation of c-erbB2 (HER2/neu) status is necessary for selection of patients who may benefit from treatment by trastuzumab (Herceptin, Genentech, San Francisco, CA, USA), a humanised anti-HER2/neu monoclonal antibody. Despite the numerous methods available to assess c-erbB2 status, determination of protein overexpression by immunohistochemistry and gene amplification using fluorescence in situ hybridisation (FISH) are the most commonly used.[2,9] The detection rates of these methods, especially immunohistochemistry, reported by different clinical laboratories, differ.[9] This is a potential source of confusion among clinicians when faced with these discrepant results. HercepTest (DAKO A/S,

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Glastrup, Denmark) is an immunohistochemical method that has been approved by the Food and Drug Administration (FDA) of the United States as a clinical test for breast carcinoma,[10] and helps in determining eligibility for trastuzumab therapy.[11] The HercepTest is popular for many reasons, including the similarity in name with the drug Herceptin as well as being FDA-approved. The question remains, however, as to whether the routinely performed immunohistochemical test for c-erbB2 is comparable with the FDA-approved HercepTest.

This study compares the DAKO HercepTest with the immunohistochemical assay (A0485, DAKO), which is routinely used in our pathology laboratory.

Materials and Methods

Paraffin-embedded, archival breast carcinoma tissues from 41 women operated in 2000 at a tertiary hospital were subjected to the 2 different immunohistochemical assays.

Sections of 4 μm were cut from appropriately selected paraffin blocks containing lesional tissue using a rotary microtome (Leica RM 2135, Meyer Instruments, Houston, Texas, USA). These were mounted on glass slides coated with silane (3-aminopropyltriethoxysilane; Cat. #A3648, Sigma Immunochemicals, St Louis, MO, USA), and dried overnight at 37ºC. The sections were deparaffinised in xylene and rehydrated via a series of graded alcohols (absolute alcohol to wash away the xylene, followed by 95% and then 70% alcohol, with a 5-minute duration each for rehydration). Endogenous peroxidase activity was blocked by incubating the sections in 12 mL of methanol containing 200 μL of 30% hydrogen peroxide for 10 minutes, followed by washing under running tap water. Appropriate unmasking was carried out in the microwave oven using citrate buffer at pH 6, after which antibody to c-erbB2 (A0485, DAKO) was applied. Non-specific binding sites were blocked by 10% normal swine serum for 10 minutes. Sections were then incubated with primary c-erbB2 antibody at 1:1500 dilution in a humid chamber for 30 minutes at room temperature. The primary antibody was rinsed off with Tris-buffered saline at pH 7.4 and incubated with linking biotinylated antibody (Dako LSAB2 kit, K0675, DAKO A/S, Glastrup, Denmark) for 20 minutes. This was then rinsed off with TBS and followed by incubation with peroxidase-conjugated streptavidin complex (Dako LSAB2 kit, K0675, DAKO A/S, Glastrup, Denmark) for 20 minutes. Freshly prepared DAB solution (3, 3'-diaminobenzidine tetrahydrochloride, Sigma D5637, dissolved in 10 mL Tris/hydrochloric acid buffer at pH 7.6, to which 100 μL of 1% hydrogen peroxide was added just before use) was applied for 10 minutes after the tertiary layer was rinsed off with TBS. DAB was removed by rinsing with distilled water. The slides were counterstained with haematoxylin, dehydrated in increasing grades of ethanol, cleared in xylene and mounted in depex.

For the HercepTest, after sectioning, deparaffinising and rehydrating, sections were taken to water. Epitope retrieval was carried out in the microwave oven using epitope retrieval solution (ERS) at 98ºC for 40 minutes. Then the sections were rinsed under running water and taken to TBS/Tween. Endogenous peroxidase activity was blocked by incubating the sections in 12 mL of methanol containing 200 μL of 30% hydrogen peroxide for 30 minutes, followed by washing in TBS/Tween. Then prediluted HER2/neu antibody was applied for 30 minutes followed by rinsing in TBS/Tween. Visualisation reagent was applied for 30 minutes and rinsed with TBS/Tween, followed by DAB solution for 10 minutes. DAB was removed by rinsing with distilled water. The slides were then counterstained with haematoxylin, dehydrated in increasing grades of ethanol, cleared in xylene and mounted in depex.

A breast tumour known to react with c-erbB2 antibody was used as positive control; staining of slides with TBS without primary antibody was used as negative control in each staining batch for both methods.

The DAKO HercepTest Protocol system[12] was used to grade the degree of membrane staining. No staining or membrane staining observed in <10% of tumour cells was given a score of 0; faint/barely perceptible membrane staining detected in >10% of tumour cells was scored as 1+; a weak to moderate and strong complete membrane staining observed in >10% of tumour cells were graded as 2+ and 3+, respectively. A score of 0 and 1+ was considered negative; 2+ and 3+ were considered positive.

SPSS 11.5 statistical software (SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis. The staining intensity was compared using chi-square test. The level of inter-test agreement was quantitated using pairwise kappa statistics.[13] The kappa values were interpreted following the guidelines described by Landis and Koch.[14] Briefly, the greater the kappa value, the stronger the agreement between the tests. If the kappa value ranges from 0.81 to 1, the strength of agreement is considered almost perfect. When it ranges from 0.61 to 0.8, it implies substantial agreement; if it ranges from 0.41 to 0.6, 0.21 to 0.4 or 0 to 0.2, the strength of agreement is moderate, fair or slight, respectively.

Results

Overexpression of c-erbB2 protein was present in 34.1% and 39% of cases when detected by HercepTest and DAKO A0485 antibody, respectively (Fig. 1 and Table 1). When positive versus negative results were compared, 95% (39/41) of cases showed good concordance; 2 cases showed discordant results, even after repeating the tests. Overall variation in staining intensities were 31.7% in all cases (13/41), and 26.8% in 39 concordant cases (11/39; 6 negative

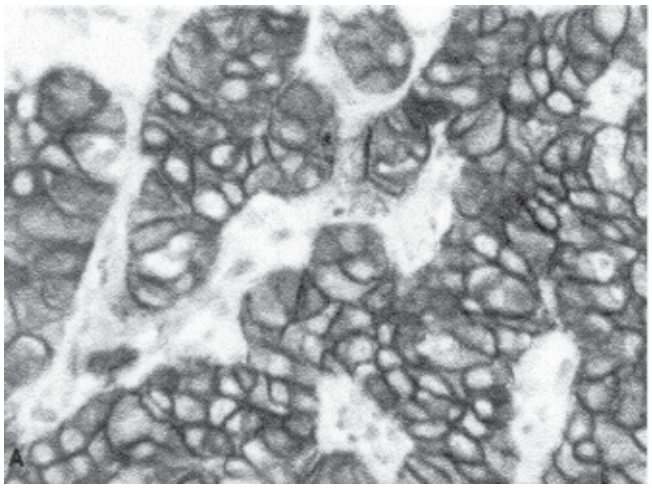

Fig. 1a.

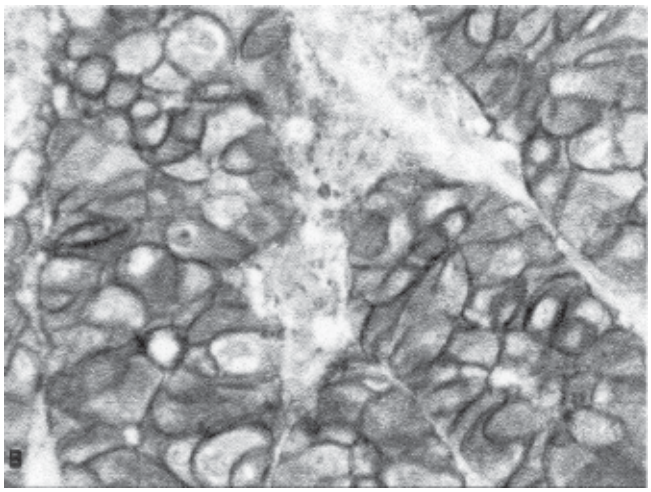

Fig. 1b.

Fig. 1. C-erbB2 immunostaining of a case of invasive ductal breast carcinoma using (a) HercepTest and (b) routine immunohistochemistry (DAKO, A0845). 3+ positive staining (Haematoxylin counterstain, original magnification x310).

cases and 5 positive cases). Minute variations of staining intensities were observed in 0, 1+ and 2+ staining, but 3+ staining showed a marked variation (43.9%, 22%, 19.5% and 14.6% versus 41.5%, 19.5%, 14.6% and 24.4% respectively) between HercepTest and DAKO A0485 antibody. Pairwise kappa value indicated an almost perfect agreement (k = 0.898) for binary variables (positive and negative results). For staining intensity, moderate agreement was attained (k = 0.554).

Discussion

Immunohistochemistry is one of the most widely used tools for diagnosis and research. It is currently the most convenient, readily performed method in routine surgical pathology practice for the detection of c-erbB2 protein overexpression using specific antibodies.[2,15] Many antibodies, such as CB-11 (monoclonal antibody, Ventana), A0485 (polyclonal antibody, DAKO), Mab-1/Pab 1 (cocktail antibody), TAB250 (Zymed) and HercepTest kit (DAKO), are used by many laboratories worldwide.[16] Each antibody has different levels of sensitivity and specificity. The HercepTest (Rabbit anti-human HER2 protein; code # K5204) has high sensitivity and specificity, and has been approved by the FDA for therapeutic applications. Variability in results of c-erbB2 immunostaining is partly related to the use of different anti-HER2/neu antibodies, as these antibodies may differ in binding affinity, epitope specificity and/or cross-reactivity with non-HER2/neu proteins.[17] Standardisation of procedures may assist in obtaining reliable results.

Table 1. Staining Intensity of Herceptest and Dako A0485 Antibody Immunohistochemistry

Intensity	Frequency in HercepTest (%)	Frequency in DAKO A0485 (%)
0	18 (43.9)	17 (41.5)
1+	9 (22)	8 (19.5)
2+	8 (19.5)	6 (14.6)
3+	6 (14.6)	10 (24.4)
Total	41	41

In this study, the primary objective was to compare the FDA-approved assay method (HercepTest, DAKO) with the routine diagnostic immunohistochemistry assay method (A0485, DAKO) to determine if there was a significant variation in the results. There was good concordance between both methods, when the results were analysed as binary variables (positive versus negative results). Immunostaining with HercepTest fully concurred with the DAKO A0485 antibody in 95% of paraffin-embedded breast cancer specimens when the positive versus negative results were analysed (k = 0.898). On the other hand, there was a significant statistical variation in individual staining intensities (k = 0.554; moderate agreement). Of the 2 discordant cases, 1 showed no staining with HercepTest against 3+ result on routine c-erbB2 immunohistochemistry; the second case showed 1+ result for HercepTest and 2+ result on routine c-erbB2 immunohistochemistry. Both cases are clinically significant: based on routine immunohistochemistry, they would have been amenable to Herceptin treatment; according to the HercepTest, they would not have been candidates. Presently, patients with breast carcinomas showing 1+ staining for c-erbB2 protein are usually not offered Herceptin therapy, whereas those with 2+ are.[16] All patients with 3+ staining are candidates for Herceptin therapy.

We have previously shown that using routine c-erbB2 immunohistochemistry, all cases with 3+ and approximately 50% of cases with 2+ immunostaining intensities revealed c-erbB2 amplification on FISH.[18] None of the cases with 0 or 1+ immunostaining were FISH-amplified. This lends weight to the reliability of routine c-erbB2 immunohistochemistry in our pathology laboratory. The cost of using the HercepTest versus routine c-erbB2 immunohistochemistry is also significant. The HercepTest costs significantly more than a vial of c-erbB2 antibody in routine c-erbB2 immunohistochemistry. With 95% concordance established in this study between the FDA-approved HercepTest and c-erbB2 immunohistochemistry, it is reasonable to conclude that routine c-erbB2 immunohistochemistry is a reliable, cheaper and cost-effective alternative to the HercepTest.

Most studies have shown inter-laboratory or inter-observer agreement in different assays or methods. This study is an inter-test agreement study that needs further evaluation. A recent study had shown a high level (97%) of inter-laboratory agreement in assessing c-erbB2 status based on immunohistochemistry assays using the same primary antibody, but with different detection systems.[19] In this study, a single immunostaining detection system (DAKO autostainer) was used; the differences identified in the staining intensity may be due to differences in the primary antibodies, and may not be due to detection systems. This observation concurred with that of Press et al.[20]

In conclusion, factors important in improving the quality of immunohistochemistry results are standardisation of tissue fixation and processing, use of appropriate positive and negative controls, and participation in external quality assurance programmes. In many ways, immunohistochemistry can probably be considered the most appropriate assay for routine c-erbB2 testing if laboratories follow standardised procedures and adopt appropriate quality controls. Further prospective studies to assess the predictive values, sensitivity and specificity of different antibody assays are warranted to choose the most suitable method for assessing the management of breast cancers.

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