Original paper

Potential impact of HercepTest™ mAb PharmDx (Dako Omnis) (GE001) in breast cancer diagnosis

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The HER2 gene is a biomarker for breast cancer prognosis and treatment. Overexpression of HER2 protein determined by immunohistochemistry (IHC) or amplification of the HER2 gene determined by fluorescence in situ hybridization (FISH) is a condition for qualifying patients for anti-HER2 therapy. Due to the high toxicity of anti-HER2 treatment, proper patient selection is essential.
In our study we compared 40 cases with IHC staining of HER2 antibody determined by Ventana PATHWAY anti-HER2/neu antibody (4B5) as HER2 2+ with the new antibody (HercepTest™ mAb PharmDx [Dako Omnis] [GE001]). Then using a double-blind study we compared the (IHC) evaluation with FISH results.
In 65% of cases (26/40) the IHC 2+ score remained unchanged, in 32.5% of cases (13/40) expression of HER2 protein after IHC with new antibody was indicated as 3+ score, and in one case we observed a decrease of HER2 protein expression to 1+. In all cases but one, in which we found IHC HER 3+ with new antibody, there was FISH amplification.
We have reason to believe that the new antibody will reduce the diagnostic time and avoid unnecessary costs. Due to the small study group, further investigation is needed.

Key words: breast cancer, HER2, IHC, FISH.

Introduction

According to data provided by the International Agency for Research on Cancer GLOBOCAN, breast cancer was the most commonly diagnosed cancer in the world in 2020 (more than 2.3 million new cases) [1–3]. It is estimated that by 2040 the number of newly diagnosed cases will rise to more than 3 million [3]. Among important biomarkers for breast cancer prognosis and treatment is the HER2 gene located on chromosome 17 [4]. Other molecular markers used in the prognosis and treatment of breast cancer are oestrogen (ER) and progesterone receptors, Ki-67 proliferative marker, and tumour suppressor protein p53 [4, 5]. We can observe overexpression of the HER2 protein detected by immunohistochemistry (IHC) (using scale from 0 to 3+ while 0, 1+ are negative, 2+ so called equivocal and 3+ is positive) or amplification of the HER2 gene detected by in situ hybridization (ISH). HER2 overexpression and/or HER2 gene amplification in breast cancer cells is used as the target for direct therapies [6, 7]. Patients with HER2 receptor overexpression (IHC 3+ or ISH amplification) are eligible for anti-HER2 treatment. The HER2 gene is amplified in about 15–20% of invasive breast cancer cases, which is closely linked to overexpression of HER2 protein, resulting in a worse clinical course of the disease such as an increase tumour progression and metastasis [7, 8].

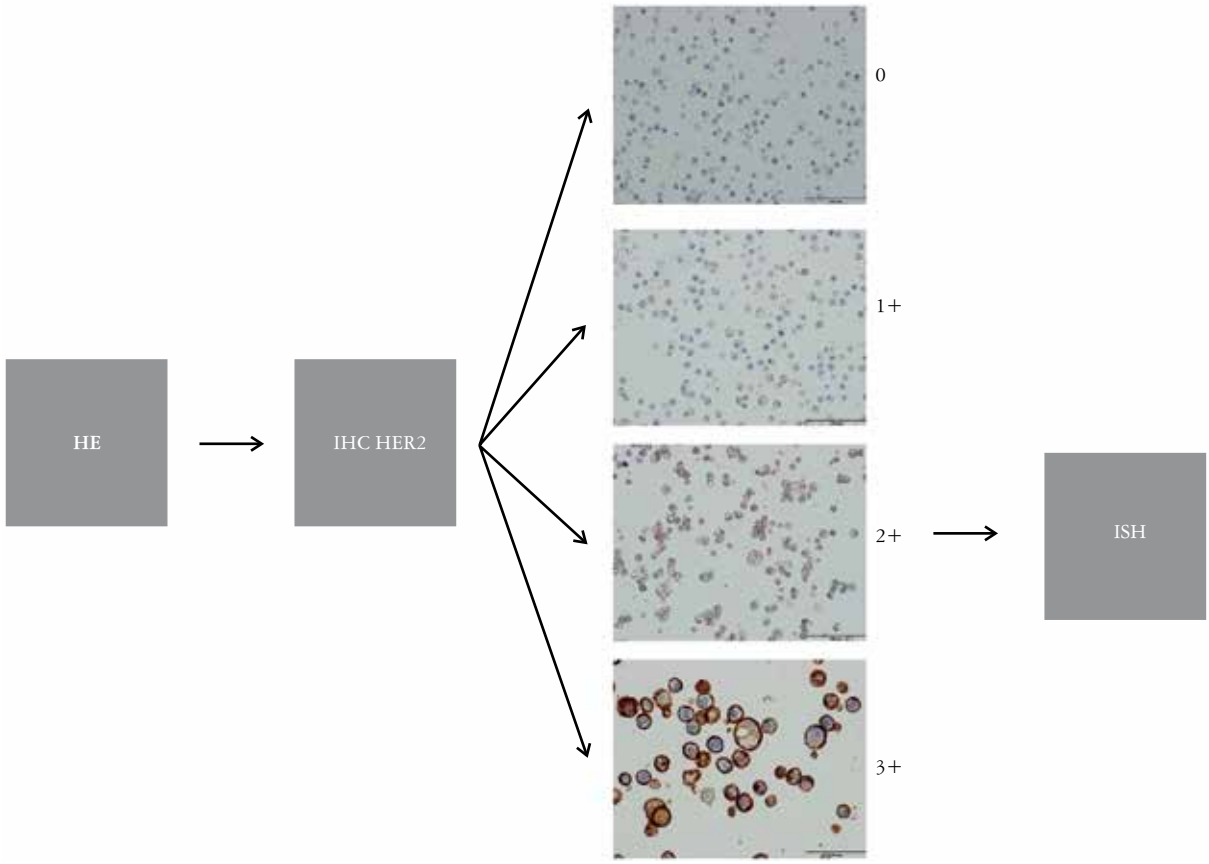

Fig. 1. Diagnostic procedure scheme in breast cancer according to 2018 American Society of Clinical Oncology/College of American Pathologists guidelines; immunohistochemistry HER2 control performed by Ventana PATHWAY anti-HER2/neu antibody (4B5), control panels, magnification 10×

IHC – immunohistochemistry, ISH – hybridization

Table I. Five groups of hybridization results according to 2018 American Society of Clinical Oncology/College of American Pathologists guidelines

FISH categories	HER2/CEP17 ratio	Average HER2 gene copy number	HER2 status
Group 1	≥ 2	< 4	Positive
Group 2	≥ 2	< 4	Negative
Group 3	< 2	≥ 6	Positive
Group 4	< 2	≥ 4 to < 6	Negative
Group 5	< 2	< 4	Negative

FISH – fluorescence in situ hybridization

Due to the high cost and the cardiotoxic effects of anti-HER2 treatment, it is extremely important to appropriately select patients who are included in a given drug programme [4]. According to the Polish Ministry of Health announcement of 20 February 2023, for early HER2-positive breast cancer (ICD10: C50) the HER2 status should be determined by IHC or in situ ISH if HER2 receptor overexpression needs to be confirmed (HER2 equivocal cases) (Fig. 1) [9]. According to the 2018 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines update, an equivocal (2+) IHC result was defined as the presence of a weak to moderate continuous membrane reaction in 10% of invasive cancer cells. Another important update was that HER2 status could be re-determined in post-operative specimens in a patient whose HER2 status determined by a thick-needle biopsy was negative. The hybridization results were divided into 5 groups, and in only 2 cases the ISH result was assessed as positive, i.e. where amplification has occurred with an HER2/CEP17 ratio ≥ 2.0; mean HER2 gene copy number ≥ 4 and HER2/CEP17 ratio < 2; and mean HER2 gene copy number ≥ 6 (Table I) [8, 10, 11]. The new guidelines aim to eliminate equivocal results, which are a problem in therapeutic decisions.

The purpose of this study was to compare IHC staining by using 2 different clones of anti-HER2 antibody and compare the IHC evaluation with ISH results using a double-blind study.

Material and methods

The research was performed at Department of Cancer Pathology, Greater Poland Cancer Centre, Poznań, Poland.

For the purpose of this work, we used archival material belonging to the Department of Tumour Pathology, Greater Poland Cancer Centre, where 1552 cases of breast cancer were diagnosed in 2022, of which IHC HER2 0 comprised 29%, IHC HER2 1+ comprised 43%, IHC 2+ comprised 17%, and IHC 3+ comprised 11%.

In our department we use the following procedure: HER2 status is determined in formalin fixed paraffin-embedded tissue by assessing protein expression on the membrane of tumour cells using IHC. An IHC HER2 test is performed by standard laboratory procedure according to the manufacturer's recommendation with Ventana PATHWAY anti-HER2/neu antibody (4B5). In our department, according to 2018 ASCO/CAP guidelines, all cases with HER2 2+ (equivocal) are verified by fluorescence in situ hybridization (FISH).

Fluorescence in situ hybridization determination is performed with a double-probe IQFISH pharmDX following an established laboratory procedure according to the manufacturer's recommendations. The average number of *HER2* signals and the average number of CEP17 per nucleus is assessed in 30 representative invasive tumour cells to generate the HER2 to CEP17 ratio. Interpretation of HER2 status is performed using the 2018 ASCO/CAP guidelines. Fluorescence in situ hybridization assessment is performed in the same tissue area where a 2+ response was found in invasive cancer cells when HER2 IHC was assessed.

For this study we selected 40 consecutively evaluated breast cancer cases, including thick-needle biopsies, mammotomic biopsies, and postoperative material. All cases selected for the study had a completed pathological diagnosis, and thus in these patients the HER2 status was determined with the Ventana PATHWAY anti-HER2/neu antibody (4B5). All cases were assessed as IHC HER2 2+, but the results of further FISH analysis were hidden for the sake of further experiment.

Formalin fixed paraffin-embedded tissue from all selected cases were again sectioned, and another HER2 IHC test was performed with a new HER2 antibody clone (HercepTest™ mAb PharmDx [Dako Omnis] [GE001]) by standard laboratory protocol according to the manufacturer's recommendation.

A double-blind method was used. HER2 expression determined by HercepTest™ mAb PharmDx (Dako Omnis) (GE001) results were blinded for independent viewers according to standard rules (blinded trial in accordance with the ASCO 2018/CAP guidelines).

After evaluation of IHC results for all cases the FISH results were revealed and finally evaluated and analysed.

Results

Both HER2 IHC results, the earliest determined with the Ventana PATHWAY anti-HER2/neu antibody (4B5) and repeated with a new clone of the antibody (HercepTest™ mAb PharmDx [Dako Omnis] [GE001]), were evaluated by 2 independent assessors. In the case of differences in evaluation, the higher score was accepted for further statistics.

Compared to the previous HER2 status primary determination using Ventana PATHWAY anti-HER2/neu antibody (4B5), 65% of cases (26/40) determined by the new HercepTest™ mAb PharmDx (Dako Omnis) (GE001) remained unchanged and were described as HER2 2+ score.

In 32.5% (13/40) of all cases, expression of HER2 protein after IHC with the new antibody was indicated as 3+ score.

We observed a decrease of HER2 protein expression to the 1+ score in only one case (2.5%) (Table II). In all cases included in this study there were 38% cases in which HER2 amplification was determined by FISH protocol. We found that in all cases but one, in which we found IHC HER 3+ with new antibody, there was FISH amplification. In the case where we observed a decrease of HER2 protein expression to the 1+ score using IHC HercepTest™ mAb PharmDx (Dako Omnis) (GE001), we also obtained amplification confirmed by FISH protocol.

For the present study we used tissue blocks which were archived and anonymised with no interest of changes on the patients' follow-up. According to Polish law, permission from the bioethical commission is not needed in such cases (Fig. 2, 3).

Discussion

Patients whose tumours show amplification of the human epidermal growth factor 2 (HER2) gene and overexpression of the HER2 protein have a disease manifested with aggressive clinical behaviour and poor prognosis, and with shorter disease-free and overall survival [11–14]. In these cases, the use of HER2-targeted therapies such as trastuzumab, lapatinib, and pertuzumab helped to increase progression-free survival [11, 12]. A response to treatment was observed only in patients with

Table II. Comparison of immunohistochemistry and fluorescence in situ hybridization results

Ventana PATHWAY anti-HER2/neu antibody (485)	HercepTest™ mAb PhannDx (Dako Omnis) (GE001)	Ratio	
		ISH	Amplification
2+	2+	1	N
2+	2+	1.4	N
2+	2+	1.7	N
2+	2+	1.4	N
2+	2+	1	N
2+	2+	1	N
2+	2+	1	N
2+	2+	1.1	N
2+	2+	1.5	N
2+	2+	1.3	N
2+	2+	1.3	N
2+	2+	1	N
2+	2+	1	N
2+	2+	1	N
2+	2+	1.8	N
2+	2+	1.4	N
2+	2+	1.2	N
2+	2+	1.3	N
2+	2+	1	N
2+	2+	1	N
2+	2+	1.3	N
2+	2+	1.8	A
2+	2+	2.2	A
2+	2+ (3+)	1.2	N
2+	2+ (1+)	1	N
2+	1+ (2+)	1.2	N
2+	2+ (1+)	1.6	N
2+	2+ (3+)	2.2	A
2+	3+ (2+)	2.3	A
2+	2+ (3+)	2.3	A
2+	2+ (3+)	2.1	A
2+	1+	3.5	A
2+	3+	3.5	A
2+	3+	2.2	A
2+	3+	2.2	A
2+	3+	4.5	A
2+	3+	3.3	A
2+	3+	3.7	A
2+	3+	3.4	A
2+	3+	2.2	A

ISH – hybridization
In cases with 2 values, where one is in parentheses, there were differences in evaluations by 2 independent viewers, and the higher value was adopted.

Ventana PATHWAY anti-HER2/neu antibody (4B5) 2+ HercepTest™ mAb PharmDx (Dako Omnis) (GE001) 2+

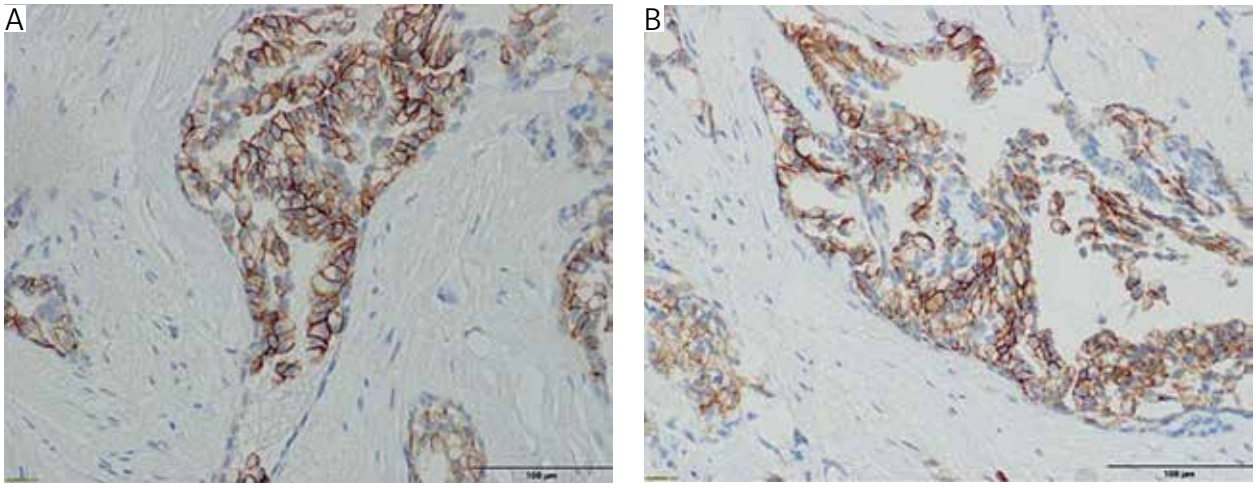

Fig. 2. Comparison of immunohistochemistry staining with 2 different antibodies, both evaluated as HER2 2+
Magnification 10×

Ventana PATHWAY anti-HER2/neu antibody (4B5) 2+ HercepTest™ mAb PharmDx (Dako Omnis) (GE001) 3+

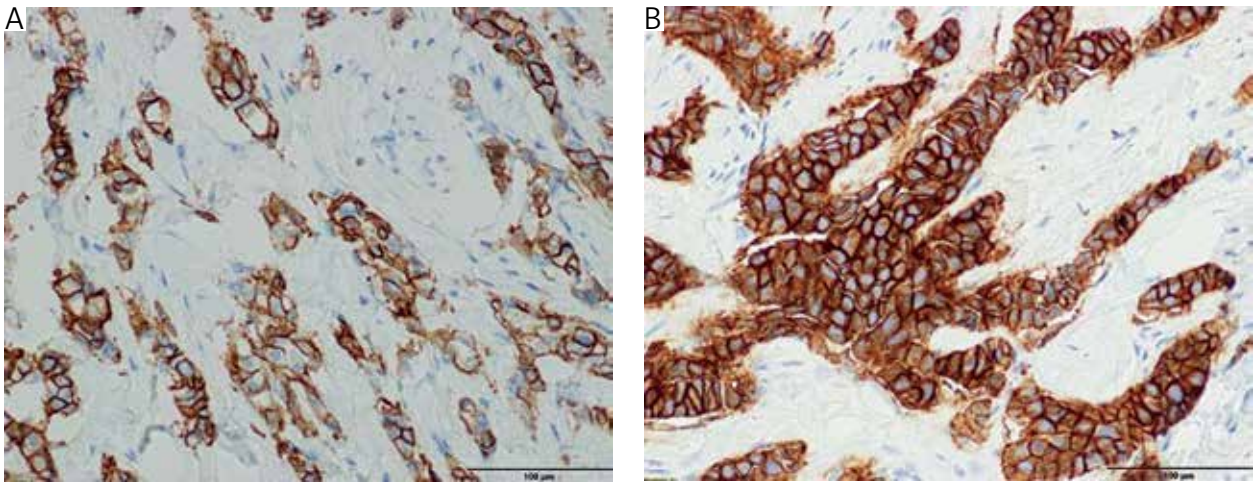

Fig. 3. Comparison of immunohistochemistry staining with 2 different antibodies Ventana PATHWAY anti-HER2/neu antibody (4B5) evaluated as HER2 2+ and HercepTest™ mAb PharmDx (Dako Omnis) (GE001) evaluated as HER2 3+
Magnification 10×

tumour cells presenting overexpression of HER2 at 3+ or amplification confirmed by FISH [12]. There is no clinical benefit of targeted therapy for HER2-negative patients [11]. Considering high drug toxicities and the risk of cardiac complications, it is important to avoid unnecessary treatment with associated risk [12, 15, 16]. Accordingly, indications for treatment are regulated locally by national health institutions (e.g. the Ministry of Health). In the case of our department, according to the Ministry of Health of the Republic of Poland announcement of 20 February 2023, early HER2-positive breast cancer (ICD10: C50) HER2 status is determined by IMH or ISH if HER2 receptor overexpression needs to be confirmed, and overexpression is a qualification for anti-HER2 treatment. Using the IHC method to determine the HER2 status is standard laboratory practice because of its short time of implementation and low cost. However, the IHC method has its limitation due to its high incidence of equivocal scores occurring with varying frequency. Overall, an HER2 2+ score represent around 15% of invasive breast cancer, and HER2 amplification is detected in 20–40% of them [17, 18]. In several studies conducted by Aznab *et al.* [4] 81.8% showed equivocal HER2 results, 73% in research conducted by Wang *et al.* [19], and over 90% of equivocal HER2 results in research conducted by Lim *et al.* [11]. For comparison, in research conducted by Pauzi *et al.* 52.7% cases were evaluated as equivocal HER2 2+ score [20]. Such a large divergence in results between laboratories may be the result of different kinds of patients selected for the study groups. According to 2018 ASCO/CAP guidelines, all cases of HER2 2+ score should be confirmed by

ISH procedure [11]. An equivocal (2+) IHC result was defined as the presence of a weak to moderate continuous membrane reaction in 10% of invasive cancer cells [10, 21]. A recent study showed that in HER2 equivocal (IHC 2+) tumours, FISH is positive in around 10–20% of cases [22].

In our department we aim to minimise the number of equivocal cases as much as possible by reducing the diagnostic time, and to diminish the high costs of the FISH procedure. For this purpose we tested the new clone of antibody (HercepTest™ mAb PharmDx [Dako Omnis] [GE001]).

In total, 30% (12/40) of cases previously evaluated as HER2 equivocal, were evaluated as HER2 3+ with the new antibody, and all of those cases were determined by FISH method as HER2 amplification. This result makes it possible to draw the conclusion that by using the new clone of the antibody (HercepTest™ mAb PharmDx [Dako Omnis] [GE001]) we could partly eliminate the FISH procedure.

In just one case we observed HER2 3+ score determined by IHC method, but there was no HER2 gene amplification determined by FISH protocol. On the other hand, we observed HER2 1+ score determined by IHC method, in which HER2 gene amplification performed by FISH technique was observed.

Amplification of the HER2 gene in most cases leads to overexpression of the protein. Correlation occurs in about 95% of cases. In a small group (about 5%) protein overexpression may occur by another mechanism [23]. HER2-positive breast cancer is a heterogenous disease, and an improved understanding of tumour biology could help us to realise that overexpression of HER2 protein does not mean that we are going to observe HER2 gene amplification [12, 24]. Moreover, overexpression of HER2 protein and amplification of HER2 gene are usually homogeneous within the whole tumour. However, sometimes we can observe intratumoural heterogeneity [19, 25–28]. There are studies suggesting that HER2 gene amplification could be acquired during tumour progression, and we could observe HER2 overexpression/amplification differences between primary tumour and metastasis [25, 26]. In addition, there is a study demonstrating heterogeneity in the primary tumour [25–28].

It is important to remember that according to 2018 ASCO/CAP guidelines HER2 status determinations by IHC and FISH should be performed in the same tumour, and in this case we can expect the same results. When discrepancies occur, the most likely cause is that one of the assays was performed incorrectly, but it is also possible to have protein overexpression without gene amplification or gene amplification without protein overexpression, and there may also be significant heterogeneity within the tumour [23].

On 24 October 2022, the Cancer Pathology Department of the Greater Poland Cancer Centre received an Accreditation Certificate, which confirms the high quality of the procedures we perform, which is extremely important in the patient diagnostic process. Since 2011, our department has also participated in the NordiQC external audit, which in the case of HER2 involves both IHC and ISH staining. The ISH report is zero-based. We get feedback on whether or not there is amplification in a given preparation. With IHC, on the other hand, even in the NordiQC control, there are preparations that leave a certain range of interpretation to the assessors, e.g. 1–2+ and 0–1+, which shows that the assessment of HER2 expression, despite many regulations, is quite subjective. Hence, the FISH protocol became the gold standard to confirm HER2 gene amplification [29]. Both, IHC and ISH techniques are associated with interpretation issues and can be conditioned by a number of variables such as tumour sampling, fixation process, and intratumoural heterogeneity. Results may be different between laboratories; thus, regular quality assessment of the staining procedure is very important [19, 29–31]. We focused on IHC HER2 equivocal cases, so there is no assurance that the antibody being tested (HercepTest™ mAb PharmDx [Dako Omnis] [GE001]) will not cause a change of the HER2 expression from 1+ to 2+. This problem requires further investigation.

Conclusions

The results obtained in this study with the new antibody clone are promising, and HercepTest™ mAb PharmDx (Dako Omnis) (GE001) will help shorten testing times and avoid unnecessary costs. Considering that the studied group was small and consisted of only previous HER2 2+ results, further investigation is required.

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The authors declare no conflict of interest.

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