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Discrepancies Between HER2 Assessment from Core Needle Biopsies and Surgical Specimens of Invasive Ductal Breast Carcinoma*

Niezgodność oceny receptora HER2 w materiale z biopsji gruczołowej oraz materiału operacyjnego raka przewodowego gruczołu piersiowego

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article; G – other

Abstract

Background. The assessment of HER2 status is particularly important for qualifying patients for trastuzumab treatment of invasive ductal breast carcinoma (IDC). HER2 assessment in core needle biopsies (CNBs) of IDC could contribute to a better therapy schedule.

Objectives. The study aimed at examining the relationship between HER2 immunohistochemistry assessment scores in paired CNBs and whole tissue sections of IDC.

Material and Methods. The study was performed on paired samples of CNBs and whole tissue sections from 49 IDC patients operated on at the Lower Silesian Oncology Center in Wrocław, Poland.

Results. Discrepancies in HER2 scores were noted in eleven (22.45%) of the paired samples analyzed. Three cases (6.12%) were underscored in the CNB specimens as compared to the surgical HER2 specimens, whereas eight cases (16.33%) were overscored in the CNB specimens.

Conclusions. Based on the high level of discrepancy between the tested pairs of IDC tissues, the authors recommend caution in assessing HER2 in CNB tissue specimens as a standard procedure. Wherever possible whole tissue sections should be utilized for HER2 assessment (*Adv Clin Exp Med* 2013, 22, 1, 27–31).

Key words: breast cancer, HER2, needle core biopsy.

Streszczenie

Wprowadzenie. Ocena ekspresji receptora HER2 jest ważnym elementem kwalifikacji pacjentek chorych na raka przewodowego gruczołu piersiowego (IDC) do terapii trastuzumabem. Ocena receptora HER2 w materiale z biopsji gruczołowej (CNB) guzów IDC mogłaby przyczynić się do lepszego planowania terapii przeciwnowotworowej.

Cel pracy. Zbadanie zależności między oceną ekspresji receptora HER2 w materiale IDC z biopsji gruczołowej oraz tkankach pobranych operacyjnie.

Materiał i metody. Badanie przeprowadzono na parach tkanek IDC pobranych metodą biopsji gruczołowej oraz operacyjnie od 49 pacjentek operowanych w Dolnośląskim Centrum Onkologii.

Wyniki. Znotowano rozbieżność w ocenie ekspresji receptora HER2 w jedenastu (22,25%) spośród analizowanych par przypadków. Trzy (6,12%) przypadki miały niedoszacowaną ocenę, a osiem (16,33%) było przeszacowanych w materiale CNB w porównaniu z klasycznymi próbkami HER2.

Wnioski. Na podstawie uzyskanych wyników oraz dużej rozbieżności między badanymi parami tkanek autorzy zalecają ostrożność w ocenie receptora HER2 w materiale CNB w standardowym postępowaniu w przypadku możliwości użycia do oceny preparatów HER2 przygotowanych z materiału operacyjnego (*Adv Clin Exp Med* 2013, 22, 1, 27–31).

Słowa kluczowe: rak gruczołu piersiowego, HER2, biopsja gruczołowa.

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Breast cancer poses a serious health problem worldwide. In 2008, approximately 450,000 new cases of this malignancy were diagnosed in Europe and more than 140,000 patients died of the disease [1]. Therefore, an early diagnosis and effective treatment of the disease are immensely important. In breast cancer diagnosis, core needle biopsy (CNB) is regarded as a reliable method for tissue sampling of palpable as well as non-palpable breast lesions [2, 3]. CNB has been found to be a fast and accurate diagnostic tool allowing for fast preoperative diagnosis and preliminary selection of breast lesion treatment [3, 4]. In comparison to fine-needle aspiration biopsies (FNABs) of breast lesions, CNBs are characterized by a greater sensitivity and allow additional immunohistochemical markers to be determined, due to the amount of tumor material in the biopsied core [5, 6]. Moreover, CNBs have also been shown to yield predictive information, since assessment of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) are possible in tissue samples obtained this way [4, 7–14].

The assessment of HER2 status is particularly important for selecting patients for trastuzumab treatment in patients showing HER2 gene amplification [15, 16]. HER2 has been found to be amplified in up to 30% of breast cancers, and its overexpression is associated with a more aggressive disease course [17, 18]. HER2 testing is performed on formalin-fixed, paraffin embedded tumor tissue. Two complementary methods used for HER2 testing are immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH), which allow for examination of protein overexpression or gene amplification, respectively. In the diagnostic algorithm, the IHC is performed first, in accordance with a well-established worldwide four-grade scale based on estimating the continuity and intensity of membrane reaction. If the result of the IHC are equivocal and do not allow the HER2 expression status to be established, additional FISH examinations are undertaken to determine HER2 amplification [19–21].

Assessing the HER2 status in CNBs may result in early treatment planning. Earlier studies concerning the assessment of HER2 status in CNBs and surgical tissue specimens showed some discrepancies, ranging up to 40% [14]. Therefore, the goal of this study was to assess and compare the discrepancies in HER2 testing in pairs of breast cancer specimens obtained by CNB and by standard surgical resection of the tumor.

Material and Methods

The Specimens

The breast cancer tissues utilized in the study originated from 49 female patients diagnosed with IDC and treated at the Lower Silesian Oncology Center in Wrocław, Poland. The CNBs were performed under ultrasound guidance using a true cut needle coupled to an automated biopsy device. The number of cores taken per tumor ranged from three to five. After the CNB, 24 patients underwent quadrantectomy followed by lymphadenectomy; 25 had radical mastectomies. During both procedures surgical tissue specimens were collected before the initiation of systemic treatment.

Pairs of CNBs and surgical tissue specimens were fixed in 10% buffered formalin, embedded in paraffin, cut into 4- μ m thick sections and mounted on SuperfrostPlus slides (Mänzel Gläser, Braunschweig, Germany). The slides were stained with hematoxylin and eosin (H&E) and HER2 using the Pathway HER-2/neu (4B5) Kit (Ventana, Tuscon, USA) in an automated immunostainer (Benchmark System, Ventana) using the protocol recommended by the manufacturer.

HER2 Assessment

The CNB and surgical specimen slides were evaluated by two independent pathologists (AW and PD) under a BX-41 microscope (Olympus, Tokyo, Japan). A four-grade scoring system developed by the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) was used to evaluate HER2 expression, which was encoded as follows: 0 (no staining), 1+ (incomplete, weak membrane staining regardless of the proportion of tumor cells stained), 2+ (non-uniform complete membrane staining or staining with obvious circumferential distribution in at least 10% of the tumor cells, or intense, complete membrane staining $\leq$ 30% of the invasive tumor cells), 3+ (intense membrane staining in $>$ 30% of the invasive tumor cells) [20]. In cases where the two pathologists differed with regard to the HER2 score, the slides were carefully reviewed under a double-headed microscope until a consensus was achieved.

Results

Among the CNB specimens 26 cases (53.1%) were scored 0 and 23 (46.9%) were scored 1+. None of the analyzed CNB specimens had a score of 2+ or 3+. Among the surgical specimens 32 cases

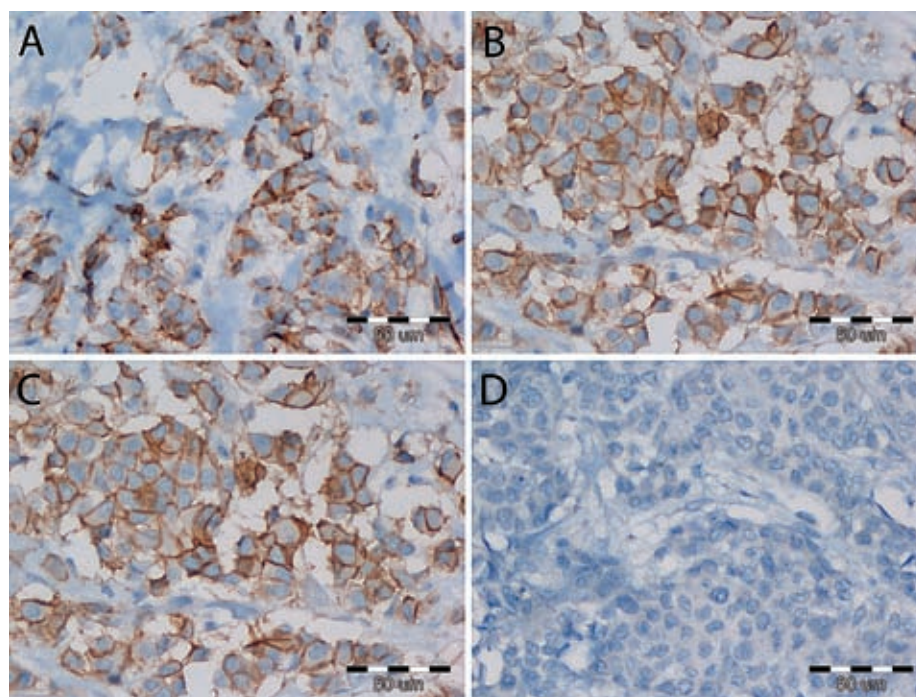


Fig. 1. HER2 IHC staining in CNB (A, C) and surgical specimens (B, D) as examples of under-scoring (A, B) and over-scoring (C, D) of HER2 assessment

Ryc. 1. Reakcje IHC HER2 w materiale z biopsji gruczołowej (A, C) i operacyjnym (B, D) oraz przykłady niedoszacowania (A, B) oraz przeszacowania (C, D) oceny HER2

(65.3%) were scored 0, 15 (30.6%) were rated 1+ and two (4.1%) were scored as 2+. None of the cases received a score of 3+. The two cases that were scored 2+ in the surgical specimens of IDC underwent subsequent FISH testing, but the final results were negative. In the CNB specimens as compared to the surgical HER2 specimens, three cases (6.12%) were underscoring, whereas eight cases (16.33%) were overscoring (Fig. 1). Overall, discrepancies between the HER2 scores were observed in 11 cases (22.45%), which are listed in Table 1.

Discussion

Because HER2 expression status is of great importance for selecting therapy for breast cancer patients, early information concerning its overexpression could result in a better therapy schedule for trastuzumab treatment [15, 16]. Nonetheless, concerns may arise, as some earlier studies dealing with HER2 expression in CNB specimens and whole tissue sections reported poor concordance (60% and 80%) between the two types of breast cancer specimens [13, 14]. Such vast discrepancies, similar to those observed in the current study, are not acceptable from the clinical point of view. Interestingly, studies performed on larger cohorts of patients reported higher concordance rates, where the discrepancies in HER2 testing reached only 1.2% and 2% [7, 9].

Recent studies have shown that many factors may contribute to discrepancies in HER2 IHC assessment. The pathologist's experience seems to

Table 1. List of cases where discrepancies were noted between the HER2 scores from CNBs and surgical specimens

Tabela 1. Lista przypadków, w których zanotowano rozbieżność w ocenie materiału CNB oraz chirurgicznego

Case (Przypadek)	HER2 score (Punktacja HER2)		FISH
	CNB specimen	surgical specimen	
1	0	2+	–
2	0	1+	
3	1+	2+	–
4	1+	0	
5	1+	0	
6	1+	0	
7	1+	0	
8	1+	0	
9	1+	0	
10	1+	0	
11	1+	0	

account for up to one third of the discrepancies in the final HER2 scores, as shown by the large multicenter study by Umemura et al. [22]. In addition to this, in one fourth of the instances of discrepancies, the difference was attributed to the staining procedures only [22]. A combination of the two factors was found in 41.7% of the cases of discrepancies [22]. Other studies also identify these factors to be key in overall HER2 staining assessment [23–25]. In the current study, the slides were evaluated by two experienced pathologists (>10 years of experience in HER2 assessment) at a large pathology center (more than 800 HER2 assessments

annually), and all the staining was performed using the same automated staining devices and the Ventanas Pathway HER-2/neu protocol, which in the authors' opinion should have enhanced the study's reproducibility.

Another key factor that may be responsible for the discrepancies noted in breast cancer specimens is the morphological heterogeneity of the tumor itself, as the authors reported earlier regarding ER and PR expression [26]. To some extent the use of tissue micro-arrays (TMAs) may reproduce the conditions (limited amounts and random parts of the tumor) obtained in the CNB specimens [27, 28]. In numerous studies, the use of TMAs for HER2 assessment showed great concordance with HER2 IHC scores noted in whole tissue specimens, but some studies reported a discordance in HER2 scores and lower specificity and sensitivity in TMAs when compared to the results obtained in whole tissue sections [29]. Similar findings were noted in the study by Lin et al., who found that HER2 and PR expression are underestimated in TMAs [30].

A study by Tamaki et al. compared the outcomes of ER, PR and HER2 status in CNBs de-

pending on the number of cores obtained from the tumor [31]. The rate of HER2 assessment concordance between CNBs and whole tissue sections strongly depended on the number of cores utilized. For one core the concordance rate reached 85.6%; for two cores it was 91.4%; and for three and four cores it reached 100% concordance. Using three or four cores was initially recommended for breast cancer biopsies in a pioneer study comparing different needle calibers and excursions [31, 32]. In the current study the number of cores taken during the biopsies ranged from three to five, which according to the earlier studies should be optimal.

In summary, this study showed discrepancy rates reaching 22.45% in HER2 IHC scoring between CNBs and whole tissue specimens, although the slides were evaluated by two experienced pathologists and a reasonable number of cores (3–5) were taken during the initial biopsy of each tested tumor. Based on these findings, the authors recommend caution when HER2 assessment is conducted using CNB tissue specimens in cases where whole tissue sections could be utilized for HER2 assessment.

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