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INTERLABORATORY CONCORDANCE IN HER-2 POSITIVE BREAST CANCER

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SUMMARY – Accurate assessment of HER-2 status is essential for identifying patients who will benefit from HER-2 targeted therapy. The aim of the present study was to show results on the concordance between local and central laboratory testing results in HER-2 positive breast cancer patients. In cases with discordant findings, the immunohistochemical (IHC) and/or in situ hybridization (FISH/SISH) analysis was performed in central laboratories. A total of 104 out of 143 (72.72%) breast carcinoma cases were HER-2 positive (score 3+), while nearly 14% of tumors (20/43) showed weak (score 2+) and 12% (19/143) negative IHC staining (score 0 and 1+). After repeated IHC and ISH, 88% (126/143) were classified as HER-2 positive and 12% (17/143) as HER-2 negative cases. The results obtained are in agreement with many studies that confirmed similar discordance in HER-2 testing by IHC and/or FISH between local and central laboratory. Thus, our findings as well as those from other studies support the importance of regular quality assessment of the staining procedures performed and consistency of interpretation of HER-2 test results.

Key words: *Breast cancer; HER-2; Immunohistochemistry; In situ hybridization, fluorescence; Quality control*

Introduction

Breast cancer is the most common malignancy in women, affecting approximately 2500 women *per* year in Croatia[1]. Although substantial progress has been made, one-third of patients with breast carcinoma will have poor prognosis. The disease shows variable biological and clinical behavior. Besides the classic prognostic factors used in clinical practice, Human Epidermal Growth Factor Receptor 2 (HER-2/neu, ERBB2), a proto-oncogene located on chromosome 17, has become an important prognostic indicator[2]. HER-2 protein, as member of the epidermal growth factor receptors, located on the cell membrane, is involved in signal transduction for proliferation, differentiation, adhesion and motility of epithelial cells[3]. Thus, overexpression of these proteins in patients with breast cancer and positive lymph nodes is associated with poor prognosis with a reduced disease-free inter-

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val and shortened survival time, and similar linkage may exist in node-negative cases[4-6].

Trastuzumab (Herceptin), a humanized monoclonal anti-HER-2 antibody, has been approved by the US Food and Drug Administration (FDA) as an adjuvant therapeutic agent for breast cancer patients with positive HER-2 status[7,8]. Amplification of the HER-2 gene is the underlying molecular abnormality that causes overexpression of the HER-2 protein on the cell surface in approximately 15% to 20% of primary breast cancers[9-11]. Determination of HER-2 status prior to therapeutic interventions has become a standard practice for the management of breast cancer. The evaluation of HER-2 status has become pivotal to determining patient eligibility for HER-2 targeted therapy, since clinical benefit from this therapy can only be expected in patients with HER-2 positive tumor[12,13].

In clinical laboratory, HER-2 status usually is assessed on formalin-fixed and paraffin-embedded tissue using either immunohistochemistry (IHC) or in situ hybridization (ISH) assay to measure HER-2 protein on the cell membrane and HER-2 gene copies, respectively. Considering the important predictive value of this assessment, quality control of the laboratories performing HER-2 testing is necessary. In Croatia, there are more than 10 laboratories performing IHC and 7 laboratories performing ISH in testing for HER-2 status. In the last ten years, several national ring studies and inter-laboratory quality controls of HER-2 staining and scoring have been performed.

The aim is to present results of the last study analyzing inter-laboratory concordance between local and central laboratories analyzing HER-2 positive breast cancer samples and to discuss them according to literature data.

Patients and Methods

The study included ten laboratories from Croatia where IHC testing for HER-2 status was performed during 2011 for a total of 143 patients. Local laboratories performed IHC staining for determination of HER-2 status using the HercepTest (Dako, Carpinteria, CA, USA), according to the manufacturer's instructions. Paraffin-embedded breast tumor blocks and IH stained sections, evaluated as 3+ in local laboratories, were submitted to central laboratory.

In central laboratory, reevaluation of HER-2 score was performed. In those cases in which IHC score 3+ was not confirmed, two protocols were used, according to the scheme shown in Figure 1.

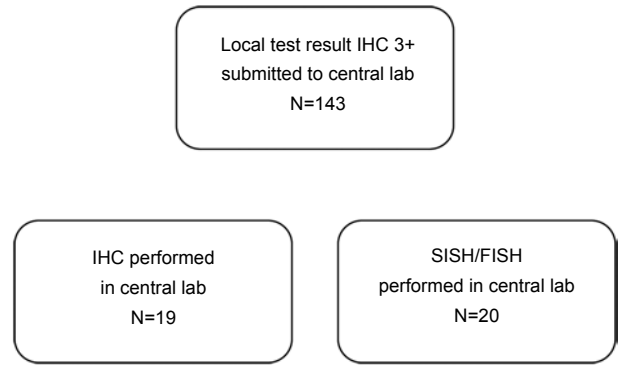

Fig. 1. Schematic diagram summarizes the numbers of breast cancer specimens received and analyzed by immunohistochemistry (IHC), silver in situ or fluorescent in situ hybridization (SISH/FISH) in central laboratories (Split and Rijeka).

Immunohistochemical analysis at central laboratory

Paraffin blocks from the slides that showed negative (0) or weak, discontinuous membrane staining (1+) were chosen for IHC analysis. Serial 4- to 5-μm thick sections were used for IHC using DAKO HercepTest kit and the results were interpreted according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines from 2007[14].

In situ hybridization at central laboratory

In situ hybridization with SISH or FISH method was performed in the cases with IHC score evaluated as 2+. The Path Vysion HER-2 probe kit was used for FISH analysis. In brief, the sections were baked overnight at 56 °C and the invasive carcinoma components were marked based on the corresponding HE stained sections. Unstained sections were deparaffinized in CitriSolv (Vysis), dehydrated in 100% ethanol, and air dried. Then the slides were subjected to protease digestion for 45 to 60 min, denatured, and hybridized with prewarmed probe for HER-2 gene and chromosome 17 centromere (HER2/neu/CEP17 SG probe, Vysis) overnight at 37 °C. Then they were washed

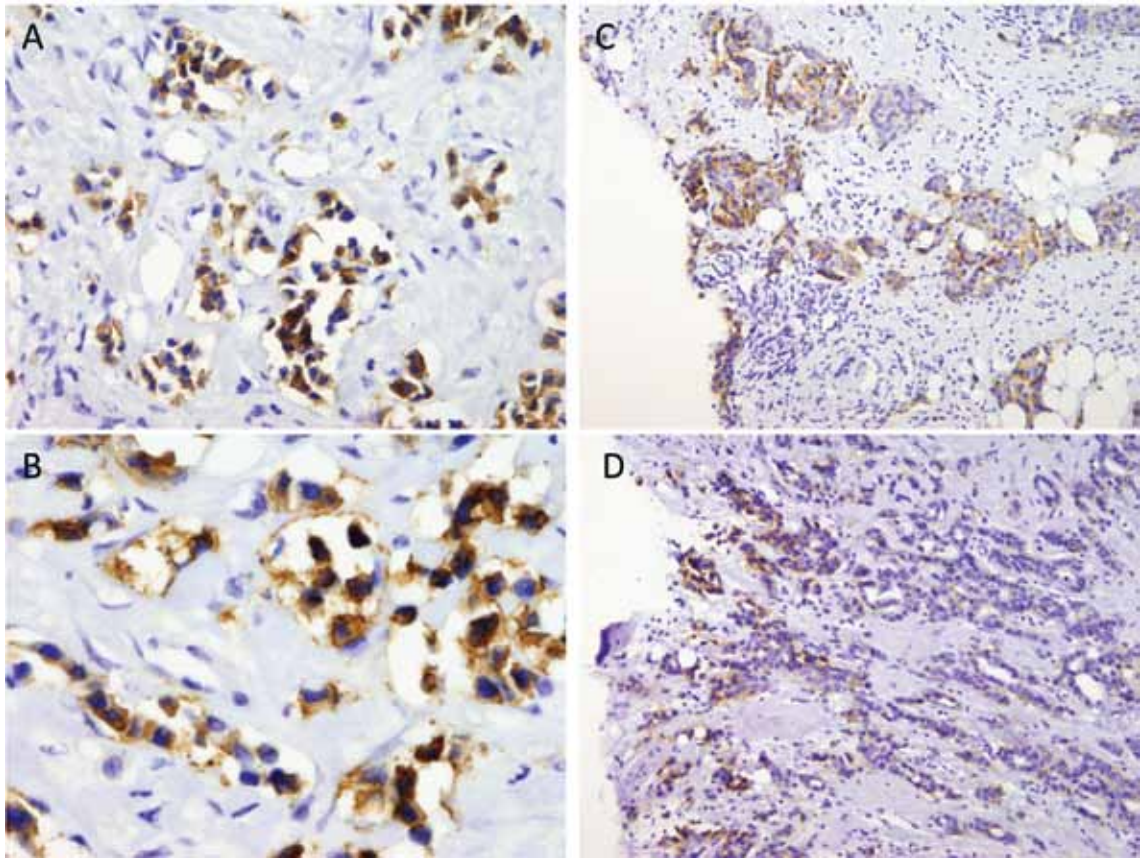

Fig. 2. Cases with insufficient and false-positive immunohistochemical staining for HER-2 protein in ductal breast carcinomas (A, B); insufficient staining due to retraction of stroma and developed clefts in which the chromogen precipitated. False-positive staining at tumor margins that would not be evaluated (C, D).

with posthybridization wash buffer at 72 °C and counterstained with 4.6-diamidino-2-phenylindole (DAPI) in antifade solution, mounted and scored. Slides were first scanned at low magnification (x100) using DAPI filter to identify areas of optimal tissue digestion and no overlapping nuclei within the area of invasive carcinoma. The signal enumeration was performed under high magnification (x1000). The number of chromosome 17 signals, HER-2 signals, and tumor nuclei score were recorded for each tumor. Tumors were interpreted as amplified, positive when the ratio of HER-2 signals divided by chromosome 17 centromere was equal to or greater than 2.0 and negative when it was less than 2.0 according to the manufacturer's protocol.

The Ventana INFORM HER2 Dual SISH probe was used for Dual SISH analysis, using fully automated protocol developed on the Ventana BenchMark XT. In the invasive area, at least 20 cells were counted, recording the HER2 and CHR17 results from each nucleus. At the end, the HER2/CHR17 ratio was calculated. Staining results were scored according to the ASCO/CAP guidelines from 2007[14].

Results

Clinicopathologic characteristics of patients

The clinical and pathological characteristics of patients with HER-2 positive invasive breast cancer involved in this study are summarized in Table 1. Most of the patients (nearly 30%) were between 50 and 60 years old, with pathological tumor size between 2 and 5 cm (pT2) (around 44%), and with high grade carcinoma in most cases (55%). In addition, most of the tumors (43% of cases) showed high proliferative activity (>30%) measured by Ki67 antibody (43%) and lym-

Table 1. Patient and tumor characteristics in HER-2 positive invasive breast cancers enrolled in the study

Characteristic	n (%)
Age at diagnosis (yrs)	
20-30	3 (1.97)
31-40	6 (3.95)
41-50	17 (11.18)
51-60	50 (32.9)
61-70	39 (25.66)
71-80	33 (21.71)
>80	4 (2.63)
Tumor size	
T1a	1 (0.65)
T1b	12 (7.74)
T1c	49 (31.61)
T2	68 (43.87)
T3	25 (16.13)
Histologic grade	
I	3 (1.96)
II	66 (43.13)
III	84 (54.91)
Ki67 index	
1%-14%	23 (15.54)
15%-30%	62 (41.89)
>30%	63 (42.57)
LVI	
No	30 (44.12)
Yes	38 (55.88)
ER	
Negative	61 (38.85)
Positive	96 (61.15)
PR	
Negative	88 (56.05)
Positive	69 (43.95)

LVI = lymphovascular invasion; ER = estrogen receptor; PR = progesterone receptor

phovascular invasion (56%). According to the steroid receptor status, most tumors were estrogen receptor (ER) positive (61%) and progesterone receptor (PR) negative (56%).

Concordance between local and central laboratory

Reevaluation of IHC stained slides in central laboratory showed that 104 (72.72%) out of 143 breast carcinomas were strongly HER-2 positive (score 3+), while nearly 14% of tumors (20/43) showed weak (score 2+) and 12% (19/143) negative (score 1+ and 0) staining (Table 2). Figure 2 presents some representative cases with false-positive score at tumor margins or strong reaction in the clefts.

The results of repeated IHC at central laboratory are summarized in Table 3. Eleven of 19 (59%) cases showed strong membrane staining (3+), while the remaining 8 cases were classified as weak (2+?) or negative (0 and 1+). Among 2+ tumors (20/143) that were selected for ISH, HER-2 gene was amplified in 11 of 20 (55%) cases, while no amplification was found in 9 of 20 (45%) cases.

Final results, after repeated IHC and performed ISH, are summarized in Table 3. Most of the tumors (126/143 or 88%) were HER-2 positive, while 12% (17/143) were negative.

Discussion

HER-2 protein overexpression and/or gene amplifications are prognostic for patients with node-positive and node-negative breast cancers, predictive of some chemotherapeutic and hormonal agents, and an indication for HER-2 targeted therapy[4-6]. However, the risk of cardiac toxic effects and the spectrum of patients with false-positive results accruing into breast cancer clinical trials have drawn attention to the sensitivity and specificity of clinical HER-2 assays. The last guidelines drafted by the ASCO/CAP have high-

Table 2. Comparative evaluation of HER-2 protein expression by immunohistochemistry (IHC) assay 3+ score of invasive component of a breast cancer specimen between central laboratory and local laboratories

Outside lab IHC score 3+	Central lab IHC score				
	0	1	2	3	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
	2 (1.4)	17 (11.9)	20 (13.9)	104 (72.8)	143 (100)

Table 3. Results of HER-2 status confirmed in central laboratories on stained immunohistochemical (IHC) sections and after repeated IHC staining or in situ hybridization (SISH/FISH) analysis

	IHC	SISH/FISH	Repeated IHC	Total
	n (%)	n (%)	n (%)	n (%)
HER-2 positive	104 (72.8)	11 (55)	11 (57.9)	126 (88.1)
HER-2 negative	39 (27.2)	9 (45)	8 (42.1)	17 (11.9)
Total	143 (100)	20 (100)	19 (100)	143 (100)

lighted that up to now 15% to 20% of current HER-2 testing are inaccurate, thus significantly affecting therapeutic decision making[15]. So, how to best assess HER-2 status in breast cancer is an important issue, particularly after the US FDA has approved the use of trastuzumab that targets HER-2 protein[7,8].

Two types of IHC test to determine HER-2 status are approved by the FDA: HercepTest (Dako, Carpinteria, CA, USA) and Pathway (Ventana Medical System Inc., Tucson, AZ, USA). More recently, FISH/SISH, which directly measures HER-2 gene amplification, has been added to the armamentarium of tests that can be used to identify patients with HER-2 positive tumor for trastuzumab therapy. The hallmark of HER-2 abnormality in breast cancer is protein overexpression, which in most cases apparently occurs as the result of the corresponding gene amplification[9-11]. Consequently, either IHC assay or FISH has been explored as a single assay to evaluate respective HER-2 protein or gene status. In many studies, good concordance from 89% to 93% was found between the group scored as IHC 3+ and FISH results[15-18]. Nonetheless, some authors consider the ISH to be a more accurate and consistent scoring system for determining HER-2 amplification than Hercep Test[19]. In addition, according to some authors' experience, ISH studies should be performed on all 3+ and 2+ staining to avoid inappropriate and toxic treatment[20]. Most data support an algorithm in which ISH testing is restricted to IHC 2+ tumors[21]. ISH is more time consuming and expensive than IHC, and is therefore not preferred for primary screening for HER-2 status[22].

The quality of the staining procedures performed and consistency of interpretation require regular assessment. Many international ring studies were conducted to investigate the quality of HER-2 testing[23-27]. In our country, in the last ten years, several national ring studies were also performed in order to evaluate HER-2 staining and interpretation reproducibility among participants. The present study was designed with the aim to demonstrate agreement in IHC HER2 3+ scoring between the coordinating institution and 10 participating laboratories. All laboratories used HercepTest for IHC analysis, whereas ISH was performed by FISH and SISH assays in two laboratories. Repeated IHC done at central laboratory proved the strong positive IHC 3+ result by HercepTest in 82 of 104 (79%) cases, while HER2 gene amplification was confirmed with FISH/SISH in 126 of 143 (88.1%) cases. Similarly, the Breast Intergroup report for protocol N9831 describing the first 119 women with 3+ IHC immunostaining at the accruing institution shows that only 74% were 3+ by HercepTest done centrally[23]. When local and central evaluation used the same methodology, concordance was 81.6% for IHC[25]. The sources of error are most likely primarily preanalytical. For example, IHC testing can yield false-negative results when the HER-2 epitope is destroyed by formalin fixation. This process may occur in up to 20% of HER-2 positive samples and can be problematic when testing archive samples that have been stored long term (e.g., when testing primary tumor samples at relapse). Conversely, variability in tissue processing, the reagent used and antigen retrieval can contribute to false-positive IHC results. However, interpretation of results may also be a key factor, especially in low-volume testing laboratories.

Discordant inter-laboratory results found in our study are in line with literature data. External quality assurance studies emphasize the need of rigorous application of standardized procedures with reliable and reproducible diagnostic HER-2 testing for precise identification of breast cancer patients eligible for HER-2 targeted therapy.

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Sažetak

MEĐU-LABORATORIJSKA PODUDARNOST HER-2 POZITIVNIH KARCINOMA DOJKE

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Ispravna procjena HER-2 statusa je osnova za pronalaženje bolesnika kojima će koristiti HER-2 ciljana terapija. Cilj istraživanja je bio prikazati rezultate podudarnosti između testiranja HER-2 pozitivnih bolesnika oboljelih od raka dojke pri mjesnom i centralnom laboratoriju. U slučajevima nepodudarnih rezultata analize imunohistokemije (IH) i/ili in situ hibridizacije su se izvodile u centralnim laboratorijima. Ukupno 104 od 143 (72,72%) slučaja karcinoma dojke su bili HER-2 pozitivni (biljeg 3+), dok je skoro 14% tumora (20/43) prikazalo nisko (biljeg 2+) i 12% (19/143) negativno imunohistokemijsko bojenje (biljeg 0 i 1+). Nakon ponovljene analize IH i ISH 88% (126/143) se klasificiralo kao HER-2 pozitivni i 12% (17/143) kao HER-2 negativni slučajevi. Dobiveni rezultati su sukladni mnogim istraživanjima koja potvrđuju slične nepodudarnosti pri HER-2 testiranju imunohistokemijom i/ili FISH analizom između mjesnog i centralnog laboratorija. Prema tome, naši rezultati kao i rezultati drugih istraživanja podupiru značenje pravilnog procjenjivanja izvođenja protokola bojenja i dosljednosti interpretacija rezultata HER-2 testiranja.

Ključne riječi: *Rak dojke; HER-2; Imunohistokemija; In situ hibridizacija, fluorescentna; Kontrola kvalitete*