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ORIGINAL ARTICLE

Interlaboratory concordance in HER2 testing: Results of a Serbian ring-study

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Summary

Purpose: *The purpose of this study was to assess the immunohistochemistry and chromogenic in situ hybridization (CISH) inter-laboratory consensus between national pathology laboratories in Serbia.*

Methods: *This study was conducted between 2013 and 2016. In 2013, HER2 results were evaluated using two sets of four different breast cancer specimens in five laboratories. A total of 20 immunohistochemistry and 20 CISH cases were tested. In 2014, there were 6 testing rounds, and a total of 24 specimens were analyzed, whereas in 2015 and 2016, seven testing rounds were conducted, with four additional cases (i.e. a total of 28 specimens). In 2014, 2015 and 2016, all institutions performed immunohistochemical analysis only.*

Results: *We found discrepancies in HER2 immunohistochemical (IHC) results in all four surveys. IHC testing resulted in diagnostic discordance between participating centers in two (2/17) cases in 2013, two (2/24) in 2014, four (4/27) cases in 2015 and three cases (3/27) in 2016. The overall agreement among the centers was 79%, 85.5%, 83.5% and 89.4%, respectively. For CISH analyses, the results for 16 (84.2%) of 19 samples were consistent for all participants. Three results were found to be discordant, indicating a misdiagnosis rate of 15.8%. In all the discrepant cases, interinstitutional discordances were related to technical and evaluation issues.*

Conclusions: *Our study highlights the difficulty encountered during HER2 testing using immunohistochemistry and CISH. This also emphasizes the need for rigorous quality control procedures for specimen preparation and analysis.*

Key words: *HER2 testing, breast cancer, inter-laboratory consensus, quality control*

Introduction

Amplification of the HER2 gene and/or overexpression of its protein product has been shown in 15-20% of breast cancers [1,2]. Studies show that HER2 overexpression represents a target for individualized therapy. Prior to the availability of HER2-directed therapies, HER2-positive breast cancer was associated with worse prognosis compared to HER2-negative cancer, including higher mortality rate in early-stage disease, reduced time until relapse, and shorter survival rate [1]. Trastuzumab (Herceptin) is nowadays offered to breast cancer patients in advanced, adjuvant, and neoadjuvant settings in association with chemotherapy [3]. Moreover, in cases of metastatic HER2 breast cancer a tyrosine kinase inhibitor, Lapatinib [4], as well as an antibody targeting HER2-HER3 dimer, Pertuzumab [5], have been approved for treatment by the U.S. Food and Drug Administration (FDA).

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Guidelines for breast cancer management require all breast cancer patients to be tested for HER2 at initial diagnosis and at the time of recurrence [6]. Methodologies include assessment of protein levels by immunohistochemistry (IHC), or gene copy count by in situ hybridization (ISH). Although they can be used indiscriminately, the first step is usually represented by the assessment of HER2 protein overexpression using IHC which contemplates a well-known four-tier scoring system [7]. HER2 gene status is determined by ISH, which identifies the number of HER2 gene copies, normally in conjunction with the number of chromosome 17 centromere copies, and is generally seen as being more quantitative than IHC [8].

The substantial benefits achieved with anti-HER2 targeted therapy in patients with HER2-positive breast cancer and the lack of benefit in HER2-negative tumors, in addition to the toxic effects of these costly drugs, have emphasized the need for accurate determination of HER2 status in all patients [9,10]. To achieve a high degree of test accuracy, reproducibility, and precision, improvement of technical aspects such as test performance, validation, and accreditation are needed [11]. Differences in HER2 testing methods, interpretation, and reporting criteria exist, which can create uncertainty for oncologists when determining patient eligibility for treatment.

The objective of this study was to assess immunohistochemical and ISH inter-laboratory consensus between national pathology laboratories in Serbia, using a range of breast cancer specimens, in order to identify factors that may contribute to discordant results. The study also aimed to evaluate the use of slide-exchange programs as a quality assessment method.

Methods

Participants

The study was conducted at a national level during 2013 and 2016, to compare and assess immunohistochemical and CISH analyses of HER2 expression by different laboratories. In 2013, 5 institutions in Serbia (Institute for Oncology and Radiology of Serbia, Oncology Institute of Vojvodina, Clinical Center Nis, Medical Center Bezanijska Kosa, University School of Medicine Belgrade) participated in the first survey. Clinical Center Kragujevac and Medical Center Valjevo joined the study in 2014 and 2015, respectively. The ring study was coordinated by the Institute for Oncology and Radiology of Serbia and the Oncology Institute of Vojvodina.

Tumor specimens and distribution

Each of the testing centers was designated in turn to select and dispatch invasive breast cancer specimens to other centers. In 2013, the study included five testing

Table 1. Ring 2013 results

Sample/Center	*A*	*B*	*C*	*D*	*E*	*Consensus*
A1	N	E	N	E	N	60%
A2	P	P	P	P	P	100%
A3	*E*	*E*	*N*	*P*	*N*	*Additional scoring*
A4	*P*	*N*	*E*	*P*	*E*	*Additional scoring*
A5	N	N	N	E	N	80%
A6	N	N	N	N	N	100%
A7	P	E	P	P	P	80%
A8	E,N	E	N	E	E	60-80%
A9	N	E	N	E	N	60%
A10	P	P	P	P	P	100%
A11	N	N	N	N	N	100%
A12	E	E	P	E	E	80%
A13	P	E,P	E	P	P	60-80%
A14	N	N	N	N	E	80%
A15	N	N	N	E	N	80%
A16	N	N	N	N	N	100%
A17	X	X	P	N	N	
A18	X	X	X	X	X	
A19	LCIS	LCIS	E	E	E	
A20	N	N	N	N	N	100%
Overall consensus						79%

P-Positive, N-Negative, E-Equivocal. Highlighted samples resulted in diagnostic discordance.

rounds. In each round, immunohistochemical and CISH testing were performed on two sets (A and B) of four different invasive breast cancer specimens (i.e. a total of eight different specimens). Thus, a total of 20 immunohistochemistry and 20 CISH breast cancer specimens were assessed by the testing centers over the first course of the study. All specimens had been fixed with neutral buffered formalin (12-48 h), dehydrated and embedded in paraffin blocks. Tissue sections, 5 μm thick, were mounted on silane-coated slides. Of the 5 participating institutions, 3 performed HER2 testing by immunohistochemical analysis and CISH analyses; 2 participants performed immunohistochemical analysis only.

In 2014, there were 6 testing rounds and total of 24 breast cancer specimens were analyzed, whereas in 2015 and 2016, 7 testing rounds were conducted, and 4 more cases were tested (i.e. a total of 28 breast cancer specimens). In 2014, 2015 and 2016, all institutions performed immunohistochemical analysis only, because CISH reagents were not available.

Study design and data analysis

This study was designed to examine interinstitutional consistency. Sample selection and distribution of sections was described in the preceding section, with the evaluated results analyzed by the study coordinator.

Each testing center, including the sending center, analyzed the HER2 status of set A specimens by immunohistochemistry using in-house assays. Appropriate control specimens were also stained. The ASCO-CAP guidelines were used for the interpretation of staining and HER2 protein expression [12]. HER2 IHC was interpreted using light microscopy without digital imaging.

CISH analysis of set B specimens was carried out by 3 testing centers, including the sending center, using SPOT-Light® HER2 CISH Kit (Invitrogen, California, USA) according to the manufacturer's instructions. Any tumor with <6 HER2 copies per cell in >50% cancer cells was classified as not amplified. Amplification was defined as ≥6 signals per nucleus in >50% of cancer cells or when a mixture of dots or small or large clusters of signals in >50% cancer cells was found. CISH signals were counted using a light microscope with 400x magnification. The scoring system was adapted from the protocol information provided by Invitrogen.

Results from each testing round were sent to the coordinator. A final analysis of the results was conducted by the coordinator. Consensus among the testing centers for each of the HER2-testing techniques was defined as the percentage of centers with the modal score for each immunohistochemistry or CISH specimen tested.

Table 2. Ring 2014 results

Sample/Center	*A*	*B*	*C*	*D*	*E*	*F*	*Consensus*
A1	E	E	E	E	E	E	100%
A2	*E*	*E*	*E*	*N*	*E*	*N*	*67%*
A3	N	N	N	N	N	N	100%
A4	P	P	P	P	E	P	83%
A5	P	P	P	P	P	P	100%
A6	*E*	*N*	*E*	*E*	*N*	*E*	*67%*
A7	N	N	N	N	N	N	100%
A8	N	N	N	N	N	N	100%
A9	E	E	E	E	E	E	100%
A10	*P*	*P*	*P*	*P*	*E*	*E*	*67%*
A11	N	N	N	N	N	N	100%
A12	N	N	N	N	N	N	100%
A13	N	N	N	N	N	N	100%
A14	E	P	P	P	E	E	50%
A15	P	E	E	P	P	P	67%
A16	N	N	N	N	N	N	100%
A17	E	E	E	E	E	E	100%
A18	N	N	N	N	N	N	100%
A19	N	N	N	E	N	P	67%
A20	E	E	E	P	E	N	67%
A21	P	P	P	P	P	P	100%
A22	N	N	N	E	N	N	83%
A23	E	N	E	N	N	N	67%
A24	E	N	E	E	N	E	67%
Overall consensus							85.5%

P-Positive, N-Negative, E-Equivocal. Highlighted samples resulted in diagnostic discordance.

Results

Analysis of immunohistochemistry concordance

In the 2013 HER2 survey, 17 of 20 samples were analyzed; 3 cases were excluded from the subsequent analysis because specimens were inappropriate. Complete consensus between the five testing centers was achieved for 6 of 17 immunohistochemistry specimens (35.3%); four cases were scored as negative (0/1+) and two as positive (3+) in all centers. Six cases were negative or equivocal (2+), and further three specimens positive or equivocal. For two specimens, there was diagnostically relevant discordance, that is, two specimens were categorized as positive in one or more centers but negative or equivocal in other centers. The overall agreement among the five centers was 79% (Table 1).

In the 2014 survey, complete concordance between all six testing centers was found in 13 of 24 specimens analyzed by IHC (54.2%); 8 cases were scored negative, 2 cases positive, and 3 cases equivocal in all centers. Five cases were negative or equivocal, and further four specimens positive or equivocal. Disagreement between the six testing laboratories had clinical importance in two cases, since they were categorized as positive in one or more centers but negative in others. The overall agreement among all centers was 85.5% (Table 2).

In the 2015 HER2 survey, complete agreement of the scoring results among the seven testing centers was achieved in 9 (33.3%) of 27 cases: 8 cases were scored negative, and 1 case positive in all centers. Seven specimens were positive or equivocal, and seven other cases negative or equivocal. IHC testing resulted in diagnostic discordance between participating centers in four cases (Table 3). The overall agreement among all centers was 83.5%.

Table 3. Ring 2015 results

Sample/Center	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*Consensus*
A1	N	N	N	N	N	N	N	100%
A2	P	P	P	P	P	P	P	100%
A3	N	N	N	N	N	N	N	100%
A4	E	E	E	P	E	E	E	85.7%
A5	N	N	N	N	N	E	N	85.7%
A6	N	N	E	N	N	N	N	85.7%
A7	E	E	E	E	N	N	E	71.4%
A8	*E*	*N*	*P*	*P*	*E*	*P*	*P*	*57.1%*
A9	E	E	P	P	E	E	E	71.4%
A10	*E*	*E*	*E*	*P*	*E*	*N*	*E*	*71.4%*
A11	N	N	N	N	N	N	N	100%
A12	P	P	P	P	E	P	P	85.7%
A13	N	N	N	N	N	N	N	100%
A14	N	N	E	N	N	E	N	71.4%
A15	*P*	*P*	*P*	*P*	*N*	*E*	*P*	*71.4%*
A16	N	N	N	E	N	N	N	85.7%
A16	*E*	*P*	*P*	*E*	*P*	*E*	*N*	*42.9%*
A18	N	N	N	N	N	N	N	100%
A19	P	P	P	P	P	P	E	85.7%
A20	D	D	E	D	D	N	D	N/A
A21	N	N	N	N/A	N	N	N	100%
A22	E	P	P	N/A	E	E	E	66.6%
A23	N	N	N	N/A	N	N	N	100%
A24	E	E	E	N/A	E	P	E	83.3%
A25	P	P	P	P	E	P	P	85.7%
A26	P	P	P	P	P	E	E	71.4%
A27	N	N	N	N	N	N	N	100%
A28	N	N	N	E	N	N	N	85.7%
Overall consensus								83.5%

P-Positive, N-Negative, E-Equivocal, D-Discarded (DCIS). Highlighted samples resulted in diagnostic discordance.

In the 2016 survey, complete concordance between all seven testing centers was found in 15 of 27 specimens analyzed by IHC (55,55%); 11 cases were scored negative, 3 cases positive, and 1 case equivocal in all centers. Five cases were negative or equivocal, and further four specimens positive or equivocal. Disagreement between the seven testing laboratories had clinical importance in three cases; they were categorized as positive in one or more centers but negative in others (Table 4). The overall agreement among the six centers was 89.41%.

Analysis of CISH concordance

CISH analysis was only performed in 2013. Complete concordance between all three testing centers was found in 16 of 19 specimens analyzed by CISH (84.2%; 9 negative, 7 positive). One case (B14) was excluded from the analysis because there was insufficient tumor tissue for analysis. Cases B6 and B7 were reported as amplified by two centers but not amplified by center C and center A respectively. Case B7 was reported as not amplified by two centers but amplified by center B. There were no cases in this series where noticeable heterogeneity in the number of HER2 signals per tumor cell was observed.

Discussion

For CISH analyses, results for 16 (84.2%) of 19 samples were consistent for all participants. Three results were found to be discordant, indicating a misdiagnosis rate of 15.8%. This is more than what has been reported in the study by Di Palma et al. where concordance was 98.5% [13], and by van de Vijver 92-95% [14].

The first discrepant case, case B7, was reported as non-amplified by two centers but amplified by

Table 4. Ring 2016 results

Sample/Center	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*Consensus*
A1	N	N	N	N	N	N	N	100%
A2	P	P	P	P	P	P	P	100%
A3	E	N	E	E	E	N	N	57%
A4	N	N	N	N	N	N	N	100%
A5	P	P	P	P	P	E	E	71.4%
A6	P	E	P	P	P	P	P	85.7%
A7	N	N	N	N	N	N	N	100%
A8	N	N	N	N	N	N	N	100%
A9	P	E	P	P	P	E	E	57%
A10	P	P	P	P	P	P	P	100%
A11	N	N	N	N	N	N	N	100%
A12	N	N	E	N	N	N	N	85.7%
A13	P	P	P	P	P	E	P	85.7%
A14	N	N	N	N	N	N	N	100%
A15	N	N	N	N	N	N	N	100%
A16	*E*	*E*	*E*	*P*	*N*	*E*	*E*	*71.4%*
A17	N	N	N	N	N	E	N	85.7%
A18	N	N	N	N	N	N	N	100%
A19	N	N	N	N	N	N	N	100%
A20	*P*	*P*	*P*	*P*	*N*	*P*	*P*	*85.7%*
A21	N/A	N/A	P	N/A	P	N/A	P	N/A
A22	E	E	E	E	E	N/A	E	100%
A23	P	P	P	P	P	N/A	P	100%
A24	N	N	N/A	N	N	N/A	N	100%
A25	*E*	*P*	*P*	*E*	*E*	*N*	*P*	*71.4%*
A26	N	N	N	N	N	N	N	100%
A27	N	E	N	E	N	N	N	71.4%
A28	E	N	E	E	E	E	E	85.7%
Overall consensus								89.4%

P-Positive, N-Negative, E-Equivocal. Highlighted samples resulted in diagnostic discordance.

Table 5. Overview of concordance by year

	2013	*2014*	*2015*	*2016*
Complete Concordance	6/17 (35,3%)	13/24 (54,2%)	9/27 (33,3%)	15/27 (55,5%)
Negative	4/6 (66,6%)	8/13 (61,5%)	8/9 (88,9%)	11/15 (73,3%)
Positive	2/6 (33,3%)	2/13 (15,4%)	1/9 (11,1%)	3/15 (20,0%)
Equivocal	0/6 (0,0%)	3/13 (23,1%)	0/9 (0,0%)	1/15 (6,67%)
Negative/Equivocal	6/17 (35,3%)	5/24 (20,8%)	7/27 (25,9%)	5/27 (18,5%)
Positive /Equivocal	3/17 (17,6%)	4/24 (16,7%)	7/27 (25,9%)	4/27 (14,8%)
Clinical Importance	2/17 (11,8%)	2/24 (8,3%)	4/27 (14,8%)	3/27 (11,1%)
Overall consensus	79%	85,5%	83,5%	89,4%

center B (with remarks of low level amplification). This center miscounted the HER2 signals in the tumor cells as >6 and interpreted this as low-level amplification. However, this case, with a signal number close to 6, falls within the equivocal gene copy number. The IHC result in this case was 2+, which favors the view that this patient was unlikely to benefit from Trastuzumab-based therapy. Cases B6 and B9 were reported as amplified by two centers but non-amplified by center C and center A, respectively. In both cases, the discordance was related to technical issues. Center C and center A misinterpreted the cases as non-amplified, but the HER2 signal was not detectable in >50% tumor cells. The absence of signals probably resulted from under-digestion or over-digestion. The pretreatment of tissue sections, especially the pepsin digestion time is a critical step in achieving a good CISH result. The optimal pepsin digestion time differs between tumors. It is possible that pepsin digestion time used in the preparation of received slides was not the optimal value for all tumor samples, thus no HER2 signal could be detected.

The percentage of technically not interpretable or equivocal cases showed a decrease over the last years. In a large cohort, Middleton et al. reported a drop from 10 to 3.4% [15], which is quite similar to Varga et al.'s observations (3.6% to 1.6%) [16].

We found discrepancies in HER2 immunohistochemistry results in all four surveys. IHC testing resulted in diagnostic discordance between participating centers in two to four cases during this study (Table 5). In all discrepant cases, interinstitutional discordances were related to technical and evaluation methods.

In the first survey, two cases were misinterpreted as HER2 over-expressed. In both samples, staining was present in the benign epithelium, but the pathologist did not recognize the failure of the test. Membrane staining of the tumor cells in these cases was not intense and linear, and there was also positive intensive staining in the cytoplasm. Case A5 and A15 were interpreted equivocal by center D, whereas these cases were reported as clearly negative by other centers involved in the study. Normal ductal epithelium was included in case A5, and it was positively stained, but normal epithelium was not included in A15 specimen. In 2013, center D categorized more specimens as positive and equivocal than other centers. It should be noted that centers A and D were low volume local laboratories without extensive experience in HER2 testing.

In the 2014 survey, two cases, A19 and A20, were most discordant. In case A19, lack of concordance was due to center F who misinterpreted the specimen as positive, and center D who reported it equivocal. In both cases, moderate or weak and incomplete membrane staining of invasive tumor cells was observed. Case A20 was found positive by center D, even though complete membrane staining was detected in less than 30% of the invasive tumor cells. This result highlights the difficulty in interpreting some equivocal cases and assessing cases with intratumor heterogeneity. We also assume that the clearly negative result observed in case A20 by center F represents a false negative result due to laboratory errors and fixation issues.

In 2015, three cases were reported negative by centers B, E and G, respectively, but we assume that these cases more likely represent false negatives due to laboratory errors. Case A10 was interpreted as HER2 negative by center F (Table 3), but there was weak heterogeneous staining in 15-20% of the tumor cells over the peripheral area. Estimating the percentage of positive staining areas in cases with heterogeneous patterns was difficult.

The 2016 survey showed less discrepancies (11%). Case A25 was reported negative by center F, while all other centers described it positive or equivocal. Case A20 was agreed to be positive in all centers except center E, who considered it negative. Case A16 was found equivocal by 5 laboratories, positive in center D and negative in center E (Ta-

ble 4). Laboratories E and F have reported lower HER2 status during the entire survey compared to other laboratories, so we assume they provided false negative results related to technical issues.

The present ring study demonstrates poor agreement for immunohistochemical detection of HER2, although a mild increase in concordance can be observed. Dowsett et al. conducted an international ring study with 5 participants from different countries—the Netherlands, Canada, France, Belgium, and Germany—using 20 sets for immunohistochemical analysis and FISH. The concordance rate for immunohistochemical analysis was 45% (9/20) in categories of negative, equivocal and positive; and for FISH, the rate was 80% (16/20) [17]. In another, Japanese ring study [18], the concordance rate was similar to that in the study by Dowsett et al.

Variability in HER2 testing can arise from pre-analytic, analytic, and post-analytic factors. The relevance of these factors varies depending on the testing method but each may affect accuracy, reliability and reproducibility of the results [19]. There are at least three main reasons to explain the poor agreement for IHC detection of HER2 in the present ring study. First, pre-analytic factors relate mainly to tissue sample handling. Based on established literature data it can be assumed that the most critical moments in IHC testing are related to the time and duration of fixation as well as the use of divergent fixatives in laboratories [12]. We believe that the reasons for divergent test results in the study are related to pre-analytical factors, because basic tissue processing was conducted in different laboratories and was not controlled or fully standardized. Every specimen used for HER2 testing in the study was fixed in 10% neutral buffered formalin. However, the duration of fixation and cold ischemia time can vary widely. Recent studies highlighted the impact cold ischemia time may have on HER2 testing [20,21]. Ideally, cold ischemic time should not exceed 1 h, then, upon sampling formalin fixation should be applied within a time frame comprised between 6 and 72 h [6]. However, controlling the time of fixation is a difficult matter, because immersion in formalin of a large surgical specimen does not mean initiation of fixation of a tumor [22,23].

The second reason that can affect the poor agreement of the results in the study are the analytic factors associated with the assay. A wide range of antibodies is available but their ability to detect overexpression is extremely variable. Press et al. conducted a study analyzing the sensitivity and specificity of 7 polyclonal and 21 monoclonal anti-HER2 antibodies on paraffin-embedded tissues of 187 breast cancers with known HER2 protein overexpression and gene amplification [24]. The sensitivity of the antibodies ranged from 6% to 80% and none of the antibodies could detect all the cases with HER2 overexpression. In a recent study with the help of College of American Pathologists (CAP), HER2 proficiency was evaluated with use of HER2 peptide analyte controls. Of the 109 participants, suboptimal staining was identified in 20 (18.3%) cases, due to antigen retrieval errors (35%), antibody or staining protocol problems (20%), or a combination of both (45%) [25].

There is evidence that HER2 test performance in routine practice has improved. Recent data indicate a significant reduction in false-positive, and a much lower false-negative rate, and, importantly, a significant reduction in the proportion of inconclusive cases [11].

In all pathology laboratories involved in the study, in-house assays were used. We believe that it affected the concordance of our results, since in-house assays do not provide standard reagents, procedures, or controls. The type of antigen retrieval, as well as factors such as microwave kinetics and the chemical composition of the retrieval solution can also have a large impact on tissue staining patterns. We also assume that the manually conducted HER2 IHC stains with the poly c-erbB2, combined with signal interpretational difficulties, led to this poor performance.

Our ring study reveals that the use of validated controls is mandatory in immunohistochemical analysis. Internal tissue controls are also important in ensuring the assay's success. The only possible negative internal control was normal ductal epithelium, most of which stained as 0 or 1+. However, there were cases in the study where normal ductal epithelium was stained as 2+ or 3+. We believe that the implementation of validated IHC tests or standardization of in-house tests would lead to more precise HER2 testing. The Immunohistochemical Staining Methods Guidebook was written by Serbian technicians, and issued in 2012, offering recommendations for optimal technical performance of IHC.

Post-analytical factors related to the interpretation of assay findings and cutoff values are an important source of variability between laboratories in the study [26]. We believe that our results varied depending on the experience and alertness of the observer. Besides, the scoring method was not followed strictly in the study. To reduce variation in interpreting HER2 assays, we intend to publish a national manual for scoring of HER2 in breast cancer specimens according to the latest ASCO/CAP recommendations.

The need for standardization and quality controlled HER2 testing were a subject already ad-

dressed in the early 2000s, when HER2 status in breast cancer became an essential predictor of response to Herceptin therapy [27-29]. Recent papers on HER2 testing emphasize the need for pathology institutions to participate in external national or international quality assurance and proficiency programs [12,17,25]. Pathologists must also be aware of the pitfalls in HER2 testing, such as polysomy and co-amplification of HER2/CEP17, when reporting HER2 status in breastcancer [30].

In summary, our study highlights the inherent difficulty encountered during HER2 testing using immunohistochemistry and CISH, even for experienced laboratories. These results show how hard it is to ensure a high standard of quality assessment in view of Serbia's health system financial woes. A slide-exchange program such as this used in the study may help not only to resolve technical issues but also to remedy discrepancies in the interpretation of HER2 testing.

Conflict of interests

The authors declare no conflict of interests.

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