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ORIGINAL ARTICLE

Report of a national external quality assessment program for HER2-low expression in breast cancer—options for increased accuracy with standardized methodology

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Abstract

HER2-low breast cancer is an important new diagnostic tumor category with promising therapeutic options. The aim of this study was to evaluate the quality of HER2-low determination in a national prospective external quality assessment (EQA) and to identify pitfalls and options for improvement. Slides were distributed to 66 pathology institutions for diagnostic assessment according to pre-defined HER2-low categories. After local staining and evaluation, slides were collected for central re-evaluation for quality parameters. Endpoints included the percentage of correct HER2-assessments and subgroup analyses for selected technical parameters. Within this prospective proficiency test, a total of 659 individual HER2 evaluations were performed by 66 participating institutions with 86% correct results. Of the incorrect results, 82% (73 of 89) reported a HER2 category that was too high, and only 18% reported a category that was too low. A successful participation was achieved by 71% of 66 pathology institutions. In central re-evaluation, technical limitations were identified as the most important parameters for suboptimal results. In defined institutional subgroups with optimal technical parameters (antibodies, automation platforms and detection systems) a success rate of up to 86–91% was achieved. In this prospective EQA assessment, the concordance rate of pathology institutions is now much higher compared to previous retrospective studies. However, the quality assurance results are still lower compared to classical HER2 proficiency tests focusing on HER2 3 + tumors, and further optimization is necessary. The results will contribute to further improve the reliability of HER2 testing, which could also be applied to HER2-ultralow in the future.

Keywords Breast cancer · HER2-low · Quality assurance

Introduction

Breast cancer with low HER2 expression [1, 2] is of high clinical relevance and opens new therapeutic options with antibody–drug conjugates. In contrast to the classical anti-HER2 agents, new substances such as trastuzumab-deruxtecan have shown clinical efficacy also in tumors with low HER2 expression, defined as a score of IHC 1 + (or IHC 2 + with negative in-situ hybridization (ISH)) [3, 4]. It has been shown that 60% of hormone receptor-positive and 30% of hormone receptor-negative breast carcinomas show a low expression of HER2 [1, 5]. Therefore, the reliable identification of HER2-low positive breast cancer might open new therapeutic options for a considerable number of patients with breast cancer. The current HER2 consensus statements [6] have been adapted to emphasize the importance of the HER2-low category [2, 7], however, the general assessment of HER2 has not been changed [8], because this category was already present in the original ASCO/CAP guidelines [9].

A comparably high variability for HER2-low between pathologists has been reported in retrospective studies [5, 10–12]. These reports have been important to raise awareness for potential challenges in diagnostic approaches. However, some of these previous evaluations were performed with blinded pathologists [10, 11], who were not aware that the HER2-low status would be clinically relevant and who were not told about the aims of the interobserver study.

Extended author information available on the last page of the article

This obviously resulted in a relevant bias, and these retrospective investigations do not represent the current clinical situation of trained pathologists who are aware of the importance of the HER2-low category.

The new category of HER2-low has led to changes in diagnostic assessment and to increased awareness that the new HER2-low category is clinically important in breast cancer.

Furthermore, national and international training initiatives have been conducted to educate pathologists about the importance of the new diagnostic category [13]. The focus has been on training and education as well as evaluation of existing slides, but typically the institutions have not used their own existing staining protocols. Further adjustment of protocols might be relevant, because some reports using standardized cell lines have shown that in particular the selection of the antibody is central for the determination of HER2. Different antibodies have different ranges for HER2 assessment, which is particularly relevant in low expressing tumors [14]. Therefore, a further adjustment of laboratory procedures might be an essential step for further improvement beyond training and education. To generate a basis for this, it is essential to perform external quality assessment (EQA) programs that evaluate the actual performance of existing laboratory procedures in a large number of pathology institutions.

To generate data on the performance of pathologist on a national level as well as pitfalls and technical limitations, we have therefore performed a prospective external quality assessment focused on the determination of the HER2-low status in breast cancer. This national diagnostic prospective proficiency test was performed after the publication of the clinical trial results regarding antibody–drug conjugates (ADCs) in HER2-low breast cancer [3] and the recognition of the clinical importance of the HER2-low category. Therefore, the main limitation of previous retrospective assessments, that pathologists simply did not know about the relevance of the HER2-low category, was excluded from our study. This allowed us to generate data reflecting the real situation in current diagnostic pathology and also to identify those pitfalls and limitations that still contribute to sub-optimal performance.

Methods

Diagnostic proficiency test

The diagnostic proficiency test was part of the QuIP (Quality in Pathology) national quality assurance program. QuIP is a quality management program in Germany aimed at improving the reliability and reproducibility of pathological diagnostics. Established in 2004, QuIP is a joint venture of the German Society of Pathology (DGP) and the German Pathologists' Association (BDP). The program is organized by Quality in Pathology (QuIP) GmbH, Berlin, Germany, which is accredited according to DIN-EN-ISO/IEC17043:2010.

Previous established EQA programs were typically focused on identification of HER2 overexpressing (IHC 3+) tumors as well as on identification of the IHC 2+ category to perform additional in-situ hybridization testing. The new category of HER2-low was not adequately tested in previous ring trials. Therefore, a selection of new cases was performed by experienced pathology centers (= panel institutes). The 7 QuIP panel institutes (Marburg, Lübeck, Kassel, Erlangen, Dresden, Hannover, Hildesheim) selected 10 breast cancer cases. These 10 breast cancer cases selected for the proficiency test were concordantly categorized as HER2-0 ($n = 4$), HER2-low ($n = 5$) or HER2-positive ($n = 1$) by all 7 panel institutes. Anonymous tissue samples were selected in accordance with the ethical conditions for materials for external quality assurance. Samples were not required for diagnostic purposes and were completely anonymized so that no patient personal data was transferred.

To address for potential heterogenous staining, it was decided to use large diagnostic slides instead of tissue microarrays for this EQA scheme. To control for potential heterogeneity within the paraffin block, selected sections from each tissue block were centrally stained to ensure that the HER2 status was identical to the first section. Slides containing formalin-fixed paraffin-embedded tissue of the 10 cases were shipped to participating institutions for evaluation by a combination of HER2 immunohistochemistry and ISH.

The laboratory protocols including primary antibodies and staining platforms could be freely chosen corresponding to the locally established methods; the final HER2-status had to be reported within 10 working days.

Predefined endpoints

The main predefined endpoint of the analysis was the report of the categories HER2-0, HER2-low, or HER2-positive, based on the inclusion criteria of the DestinyBreast-04 study [3]. The potentially upcoming new category of HER2-ultralow, defined as a weak membranous staining of less than 10% of tumor cells, was not evaluated in this proficiency test, because it was not recommended by the international guidelines as a diagnostic category [8, 9].

A maximum of two points was awarded for each of the 10 cases (2 points: fully correct classification; 1 point: invalid due to technical reasons; 0 points: incorrect classification) and a minimal score of 18 was required for successful participation.

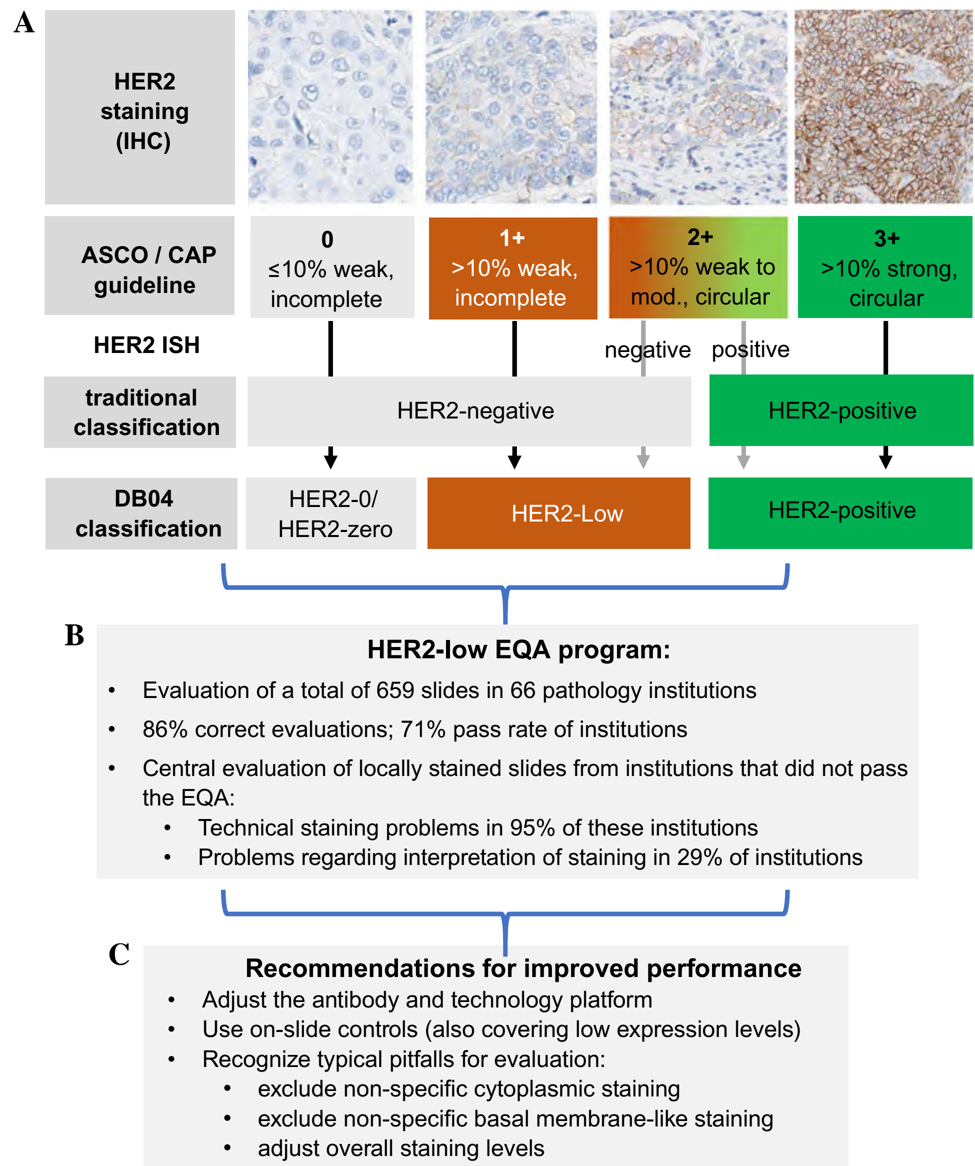

Fig. 1 Overview on setting and workflow of the EQA scheme. **A** Different options for HER2 classification, modified based on Denkert et al. [2]. **B** Organization of the EQA program. **C** Summary of recommendations for improved assessment of HER2-low, based on central re-evaluation of slides

Educational activities and central review of slides

Before the start of the ring trial, an educational online seminar was offered to all participants to explain the relevance of HER2-low from a clinical perspective as well as potential pitfalls for staining and evaluation of this marker. After the proficiency test, slides were centrally reviewed by one of two pathologists (AL or CD) to identify potential pitfalls as a basis for recommendations for improvement of the assessment. The main categories for assessment were differences related to technical staining aspect as well as differences related to the interpretation of staining.

Results

Evaluation of 659 slides in 66 institutions

A general overview on the setting or the HER2-low EQA scheme as well as the main results is given in Fig. 1. In this multi-institutional diagnostic proficiency test, a total of 659 individual HER2 evaluations by IHC and ISH were performed by 66 institutions (one single evaluation was missing due to technical limitations). Of these 659 HER2 assessments, 570 (86%) showed the correct result, while 89 (14%) were incorrect (Fig. 2A). Interestingly, 73 (82%) of the incorrect results were due to a HER2 category that was reported as too high, and only 16 (18%) of the incorrect evaluations were too low (Fig. 2A).

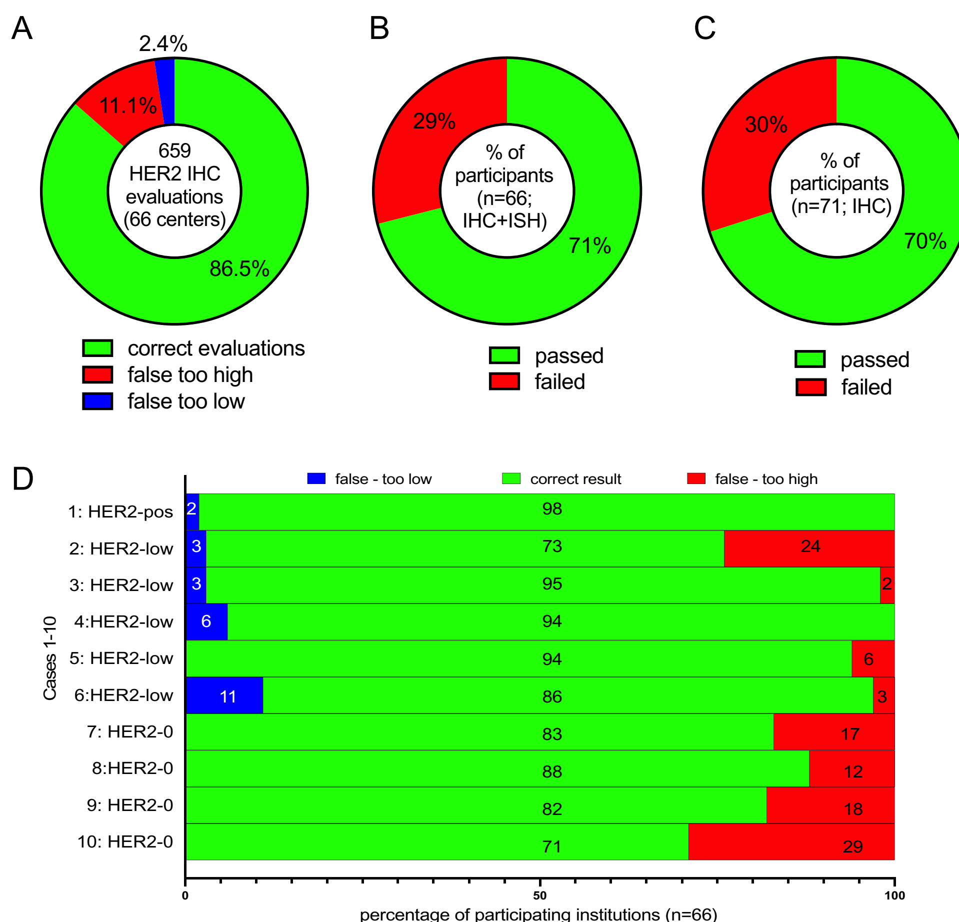

Fig. 2 Summary of the results of the HER2-low quality assurance ring trial. **A** Percentage of correct HER2 evaluations (total 659 evaluations) as well as incorrect evaluation leading to a result too high or too low. **B** Successful participation of 71% of all 66 institutions participating in the complete IHC/ISH ring trial. **C** Successful participation of 70% of all 71 institutions participating in the IHC part of the ring trial. **D** Case by case overview sorted by HER2 level displaying percentage of correct evaluations (green), incorrect high evaluations (red) and incorrect low evaluations (blue), based on the results of 66 participants with complete IHC/ISH evaluation

A total of 66 institutions completed the HER2 assessment with immunohistochemistry and in-situ hybridization, of these, 47 institutions fulfilled the passing criteria (score points of at least 18 of 20), resulting in a success rate of 71% (Fig. 2B). Five additional institutions performed just the immunohistochemistry, without in-situ hybridization, which was formally not sufficient for completion of the proficiency test. If these institutions were included, the success rate was very similar (70%) for the total of 71 institutions (Fig. 2C).

As shown in Fig. 2D, in most of the cases, more than 80–90% of the institutions scored correctly, and only two cases had more than 20% incorrect scores. A total of 19 (29%) participants had difficulties in the analysis of case 10 (Fig. 1D) due to a too high classification based on HER2-IHC. For case 2 (Fig. 1D) 18 (27%) participants deviated from the target value. This case also showed an increased number of false-positive ISH analyses. Although this tumor had a slight increase in *HER2* and *CEN17* signals, these were not sufficient for an ISH-positive classification according to the ASCO/CAP criteria [8].

Comparison of different technical approaches

There were no specific requirements regarding the used HER2 assay; therefore, the different institutions used variable antibodies, staining platforms, and detection kits. For all 71 institutions performing immunohistochemistry, Fig. 3 shows the results for selected methodological subgroups using the identical technique in more than 10 institutions. All methodological subgroups with less than 10 institutions were combined to one mixed group. Highest success rates were observed in those centers using the antibody 4B5 (86% success rate, total $n = 28$), a Ventana staining platform (80% success rates, total $n = 35$), and the Ultraview detection kit (91% success rate, total $n = 22$). Due to the diversity of the staining methods, the success rates could not be assessed for all elements of the staining protocol.

Independent review of slides

All stained slides were submitted for central review to identify possible problems of the staining and interpretation. A total of 21 of 71 institutions were not successful in immunohistochemical HER2 evaluation. We performed a systematic

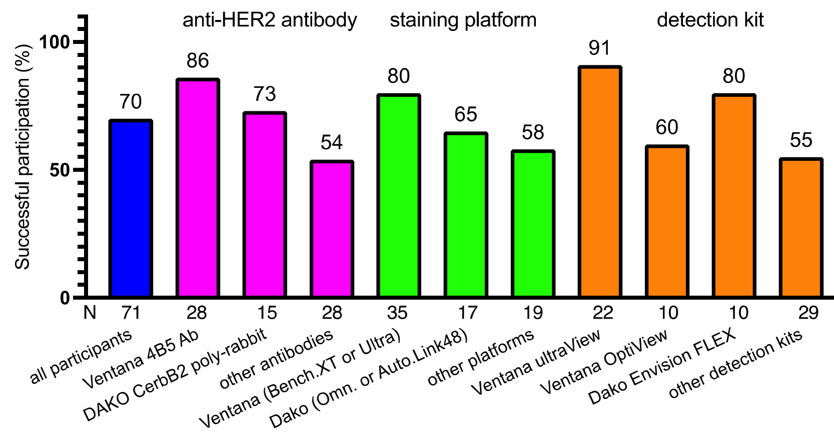

Fig. 3 Technical parameters and results of the HER2-low proficiency test. Percentage of institutions with successful participation in the proficiency test stratified by anti-HER2 antibody (magenta columns), staining platform (green columns) as well as detection kit (orange columns). In some technical subgroups, success rates of 86–91% could be achieved. The figure shows only those methodological subgroups using the identical technique in at least 10 of 71 participating institutions; the remaining technical subgroups are shown as a combined group

analysis and categorization of the individual locally stained slides of those institutions that did not pass the EQA test.

For 20 (95%) of these institutions, technical staining problems were identified during the central review, typically resulting in a generally too intense staining as well as an increased cytoplasmic staining. In addition, problems regarding the interpretation independent of the technique were identified in 6 (29%) of these 21 institutions, these were typically related to the interpretation of the cytoplasmic staining.

Examples of selected slides from the central review are shown in Fig. 4. These examples illustrate the tendency to produce false-too high scores; this was due to cytoplasmic staining (Fig. 4D, G), due to too high membrane staining (Fig. 4E, H) as well as due to a generally to high level of the immunohistochemistry (Fig. 4F, I). The tumor in Fig. 4C was scored as IHC 2+ with a negative ISH by the panel institutes, but was assessed as IHC3 + by several participating institutions (Fig. 4F, I).

Discussion

Evaluation of HER2 is central for therapeutic strategies in breast cancer, and the new category of HER2-low offers improved therapeutic options. Therefore, high-quality reporting of HER2 expression in pathology reports is essential for optimal patient care.

HER2-low breast cancer is currently regarded not as a defined subtype, but mainly as a clinically relevant diagnostic category [15]. It has been shown that HER2-low tumors have a reduced response to neoadjuvant chemotherapy, in particular in the hormone-receptor positive subgroup [1]. In the hormone-receptor negative subgroup, an improved survival was observed for HER2-low tumors. Similarly, an improved overall survival has been shown in a large study of 41,610 patients from the Danish Breast Cancer Group database [16]. These significant differences suggest that standard immunohistochemistry approaches are able to identify this subset of tumors and that certain biological differences exist, which might be related to crosstalk between HER2 signaling and other cellular pathways. The main clinical relevance of the HER2-low subset of tumors was emerging based on the results of clinical trials that showed an improved response to anti-HER2 directed antibody–drug conjugates in tumors with HER2 overexpression (IHC 3+) [17], but also in tumors with low HER2 expression (IHC 1+ or IHC 2+ with negative ISH) [3]. The immunohistochemical category IHC 1+ is defined as at least 10% of tumor cells with weak incomplete membrane staining for HER2. Obviously, this 10% cutpoint is an arbitrary cutoff which is not driven by biology, and it has been shown in the DESTINY-Breast06 trial that even patient with tumors with a lower expression of HER2, designated as HER2-ultralow tumors, have a similar benefit from an anti-HER2 directed antibody–drug conjugate (ADC) treatment [18]. The experience gained in this diagnostic proficiency test focused on HER2-low, especially regarding the technical parameters, will be useful for further improvement of HER2 testing including HER2-ultralow tumors as a potential additional category.

The emergence of HER2-low has been recognized as a challenge for diagnostic pathology, and different strategies have been developed to address this challenge. As a first step, retrospective evaluations have shown that traditional reporting performed by pathologists that were not aware of the relevance of HER2 low had a comparably poor concordance.

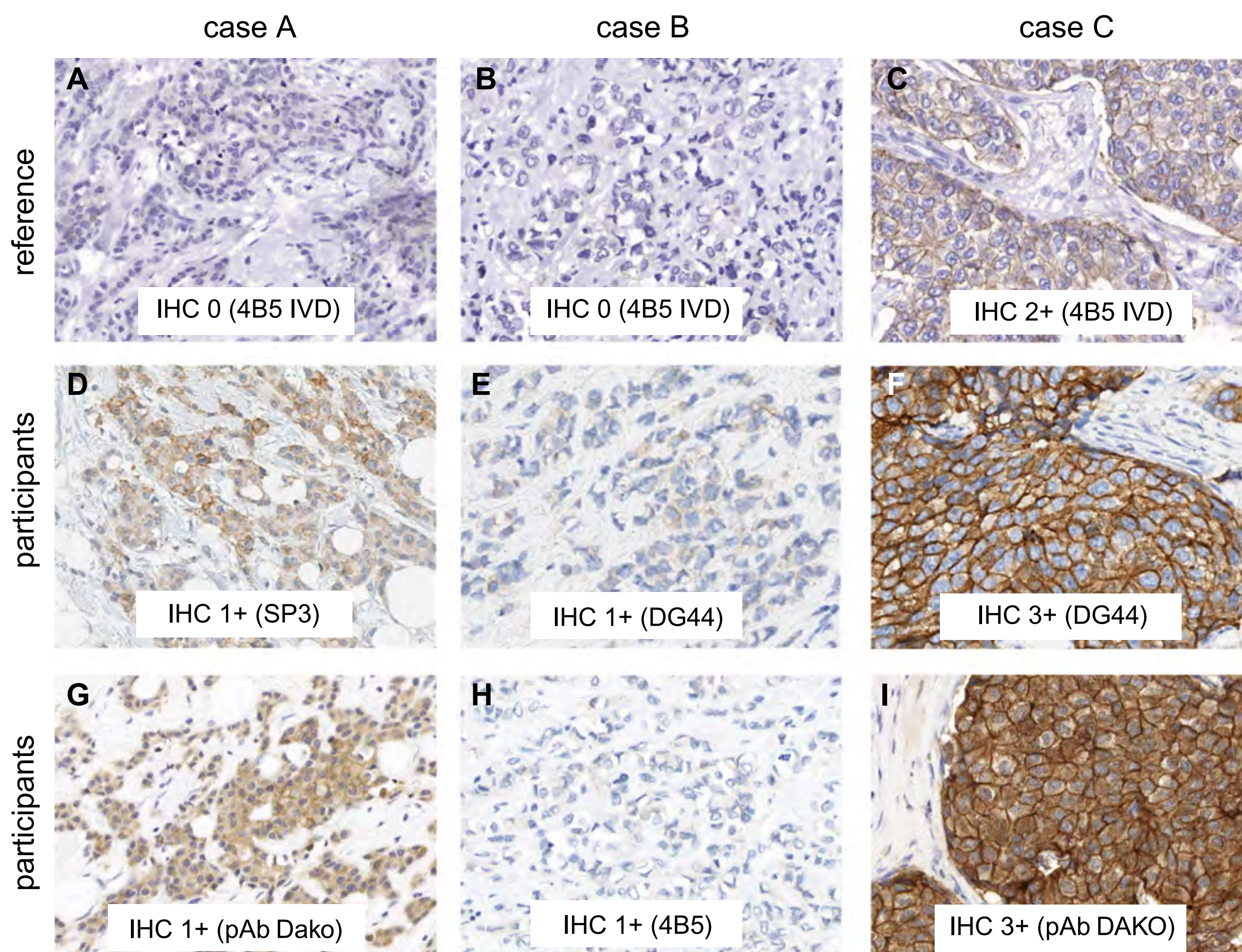

Fig. 4 Central review of slides from participating institutions of the HER2-low EQA scheme. Examples of HER2-low staining of three cases are shown. **A**–**C** The first row shows the reference immunohistochemistry for each of the three cases, that was reproduced in consensus among the 7 panel institutes. **D**–**I** Examples for selected stainings of the three cases in different pathology institutions participating in the ring trial. (columns, Case A: **A**, **D**, **G**; Case B: **B**, **E**, **H**, Case C: **C**, **F**, **I**) Inserts indicate the interpretation result as well as the antibody for each staining. The figures illustrate the tendency to produce false-too high scores, this was due to cytoplasmic staining (**D**, **G**), due to too high membrane staining (**E**, **H**) as well as due to a generally to high level of the immunohistochemistry, that was evident in case C (**F**, **I**). Case C was scored as IHC 2+ with a negative ISH by the panel institutes, but was assessed as IHC3 + by several participating institutions (magnification, ×400)

This is not surprising, because even published international proficiency-testing ring studies have reported HER2 negative (IHC 0/1 +) as a combined category [19], simply because the distinction was not clinically relevant at that time.

With the emergence of HER2-low as an important clinical category and subsequent training of pathologists, the concordance rates were largely improved. In a recent large global accuracy study including 77 pathologists from 14 countries, baseline pretraining agreement rates were > 80%; after training, agreement was improved to around 90% [13].

In a national Australian initiative, standardized slides and evaluation schemes were generated, that resulted in an accuracy of 89% [20]. In contrast to our study, the global accuracy study as well as the Australian were performed using centrally stained digital slides, so that differences related to interlaboratory variance of antibodies or staining procedures were excluded.

Despite this difference in the study setting, the overall concordance reported in our study was in a similar range (86%), suggesting that optimized training is an important first step for improved concordance.

The main advantage of our study in comparison with previous retrospective evaluations is that the pathologists participating in the proficiency test were aware of the importance and the clinical implications of the identification of HER2-low status. The overall percentage of 86% correct evaluations is encouraging. However, the institutional

success rate of 71% (or 70% for the IHC analysis) is still lower compared to previous classical HER2 diagnostic interlaboratory comparisons [21] which were focused on the identification of 3 + cases.

The tendency for overscoring could result in a larger group of patients that would be diagnosed as HER2-low and treated with ADCs, which might result in unnecessary toxicities. On the other hand, the risk of under-therapy would be comparably lower, as only 16 (2%) of the 659 evaluations reported a score that was too low. This suggests that there is still room for improvement in the diagnostic evaluation of low HER2 expressing tumors. To identify areas for improvement, we performed a central re-evaluation of all slides, that also allowed a direct comparison of the locally stained slides with the reference slides. We identified technical staining problems in 95% of the institutions that did not pass the EQA test. This suggests that an adaptation of the antibody and staining technology would be the most promising strategy to improve the assessment of HER2-low tumors.

A recent study by Hempenius et al. [14], has evaluated the performance range of different anti-HER2 antibodies using standardized cell lines as well as quantitative IHC calibrators consisting of HER2 coated glass microbeads. They found that the large interlaboratory variation observed in HER2 testing in the lower expression range could be explained by the antibody and staining protocol used.

This is in line with the results of our proficiency test. Due to the complexity of HER2 staining protocols and the variability of the used protocols, systems, and reagents, we report the specific success rates only for those technical parameters that were used by at least 10 institutions, and it seems not appropriate to evaluate the success rates for those antibodies, staining platforms, or reagents that were used by a few institutions only. It should be noted that the 4B5 antibody and the associated technical platform that had the highest success rates in the proficiency test were also used in the Destiny-Breast-04 trial [3] as well as the DAISY [22] and Destiny-Breast-06 trial. The latter have also included the category of HER2-ultralow tumors. This might open new options for further standardization. The application of on-slide controls, which also cover the low expression levels, could be a helpful tool for continuous quality control and optimization of staining quality and differentiation of HER2 IHC scores.

Independent of the technical issues, problems regarding the interpretation of the staining were identified in 29% of the institutions that did not pass the EQA test. Typical problems in the interpretation included a cytoplasmic staining that was interpreted as a weak membranous staining. These pitfalls have also been identified by other concordance studies of HER2-low breast cancer, such as the program of the UK National Coordinating Committee for Breast Pathology [23]. For further training, QuIP offers a free digital evaluation test (https://www.qualityinpathology.com/en_GB/zerpa/trials?select=individual) for this purpose, which can be used individually by pathologists.

The special focus of our HER2-low interlaboratory comparison on the interpretation and differential diagnosis of HER2-low tumors, which is similar to the situation in routine diagnostics, allowed the identification of existing problems, mainly in technical staining quality, but also in interpretation. Our results suggest that training and education initiatives have increased the concordance for reliable identification of HER2-low tumors, but that challenges remain that are linked mainly to antibodies and technical staining procedures, and to a lesser extent also to interpretation of the staining. Strategies to resolve these problems might include adaptation of the method for HER2 detection, considering the differences in success rates for different antibodies, platforms and detection reagents.

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Author contribution The study was designed by CD, FS, and AL. CD, FS, AP, RE, PJ, H-US, KJ, M G-N, CL, AF, RvW, and AL contributed to data acquisition. Data analysis was performed by FS, AL,and CD. Sample collection as well as data collection was performed by CD, FS, AP, RE, PJ, H-US, KJ, M G-N, CL, AF, RvW, and AL. All authors interpreted the data. The first draft of the report was written by CD. Verification of the underlying data was performed by FS, AL, and CD. The decision to submit the report for publication was made by all the authors. All authors contributed to the review of the manuscript.

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