Review Article

HER2-low breast cancer: insights on pathological testing

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Abstract: Human epidermal growth factor receptor 2 (HER2) is an important biomarker for predicting prognosis and effectiveness of HER2-targeted therapy in breast cancer. The emergence of novel HER2 antibody-drug conjugate has led to a shift from the binary categorization of HER2 status (i.e., negative and positive) to a ternary categorization gradually (i.e., HER2 0, HER2 low expression and HER2 positive). The heterogeneity of HER2 low expression in breast cancer has also recently aroused widespread concern, and the heterogeneity of tumors has led to differences in the efficacy of HER2-targeted therapy. Therefore, it is crucial to accurately identify the HER2 expression status of breast cancer, which can provide a basis for patients to formulate personalized treatment strategies. In recent years, artificial intelligence (AI) has developed rapidly and been widely used in the pathological accurate diagnosis of breast cancer. The research results show that AI can significantly improve the consistency and accuracy of HER2 interpreted by pathologists in breast cancer. This has provoked many discussions on HER2-low breast cancer, such as quality control prior to HER2-low expression detection, the latest progress of tumor heterogeneity, and the application of AI. In this paper, we discuss the latest testing guidelines and advances on HER2-low breast cancer, aiming to standardize and improve the pathological testing of HER2-low breast cancer.

Keywords: Human epidermal growth factor receptor 2 low breast cancer (HER2-low breast cancer); pathological testing; quality control; heterogeneity; artificial intelligence (AI)

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Introduction

The incidence of breast cancer is increasing year by year and has become the most common malignancy in women (1). Human epidermal growth factor receptor 2 (HER2) is a proto-oncogene located on chromosome 17q21 that encodes a transmembrane glycoprotein with tyrosine kinase activity. Its gene amplification and protein overexpression are associated with proliferation, metastasis, invasion and poor prognosis of tumor cells (2,3). Therefore, accurate assessment of *HER2* gene amplification and protein expression is crucial for the clinical treatment and prognosis of breast cancer. With the widespread application of HER2-targeted drugs, anti-HER2 therapy has achieved remarkable effectiveness, and the prognosis of patients with HER2-positive breast cancer has improved significantly, while patients with HER2 negative breast cancer, on the contrary, are generally considered that they will not benefit from anti HER2 treatment (4).

However, in recent years, the concept of HER2-low breast cancer has been proposed and the results of Phase III clinical trial (Destiny-Breast 04) (5) demonstrated that novel HER2 antibody-drug conjugate significantly prolonged the survival time of patients with HER2-low metastatic breast cancer. Therefore, it also redefines the categorization of HER2 expression in breast cancer and officially opens up a change in the categorization and treatment of breast cancer. The 2021 edition of the French HER2 testing guidelines and the 2023 edition of the UK HER2 testing guidelines also address the pathological detection of HER2-low breast cancer. In this review, we discussed the latest international guidelines and advances on HER2-low breast cancer, with the aim of standardizing and improving the pathological detection of HER2-low breast cancer.

Quality control prior to HER2 low expression testing in breast cancer

The 2021 edition of the French HER2 guidelines emphasizes the importance of quality control prior to HER2 testing. Specifically, the cold ischemia time of the sample must be as minimal as possible, with biopsy samples fixed within minutes and surgical specimens fixed within an hour. The recommended fixation solution is 10% neutral buffered formalin, with a fixation period of 6 to 72 hours (6). Furthermore, in addition to cold ischemia time and fixation time, the detection of biomarkers can be impacted by various factors during tissue processing (7). For instance, HER2 immunoreactivity in paraffin sections deteriorates over time. Mirlacher *et al.* (8) analyzed aged and freshly cut sections stored at 4 ℃ for 6 months under identical experimental conditions. The HER2 positivity rate in aged sections decreased from 16.3% to 9.6% compared to results in fresh sections (P=0.0047). This study confirmed the significant influence of paraffin section storage time on immunohistochemical (IHC) analysis results. A study by Forse *et al.* (9) demonstrated that long-term storage of unstained paraffin sections at –80 ℃ had no significant effect on HER2 assessment in breast cancer. Different paraffin section storage conditions and periods can affect the results of IHC testing of breast cancer specimens.

Given that the concept of HER2 low expression has led to a significant distinction between HER2 IHC 0 and 1+, and the difference between IHC 0 and 1+ is extremely small, it is critical to explore the effect of unstained paraffin section storage time on HER2 low expression immunohistochemical results. This is to avoid incorrectly determining HER2 low expression breast cancer due to excessive storage time, which impacts treatment plan development and patient prognosis. The Chinese guidelines for HER2 detection in breast cancer (2019 edition) (10) recommend that unstained sections should not be left at room temperature for over 6 weeks to prevent antigen loss. Our research team also conducted an in-depth discussion on the effect of paraffin section storage time on HER2 expression in invasive breast cancer and presented at the 112th Annual Meeting of the USCAP in 2023. Our results showed that long-term storage of unstained paraffin sections resulted in decreased HER2 expression levels, especially in patients with low HER2 expression. For unstained paraffin sections of breast cancer, HER2 staining is recommended within 4 weeks to prevent antigen loss (11). If sections undergoing HER2 testing have been stored for a prolonged period and show negative expression, retesting with fresh sections is recommended. However, these results need to be further validated by numerous clinical studies. More studies are also expected to jointly explore the effect of paraffin section storage conditions and time on immunohistochemical test results.

Specificity and sensitivity vary among different HER2 detection antibodies

Several HER2 IHC detection antibodies are currently used to detect HER2 protein expression in breast cancer, and they differ in specificity and sensitivity. A study in VIRCHOWS ARCHIV in November 2022 (12) showed that HercepTest and PATHWAY 4B5 were highly concordant (98.2%) in detecting HER2 negative (IHC 0, 1+, 2+ without ISH gene amplification) and positive (IHC 3+, 2+ with ISH gene amplification) cases. Of the 41 tumors identified as completely negative by PATHWAY 4B5, only 19 cases (46.3%) did not stain when using HercepTest (mAb), corresponding to the more strictly defined IHC 0 category per the Ventana scoring algorithm. The remaining 8 cases were assessed as HER2-Low using HercepTest staining (7 IHC 1+ and 1 IHC 2+ with FISH negative). Fourteen cases belonged to HER2 ultra-low (percentage of tumor cells positive <10%). Therefore, HercepTest has higher sensitivity in HER2-low detection. In contrast to HercepTest, Pathway 4B5 showed cytoplasmic non-specific staining; unlike Pathway 4B5, HercepTest showed weak to moderate intensity staining in some normal ducts. This indicates differences in the specificity of staining by different HER2 antibodies. More scholars are expected to study in depth the effect of different HER2 antibodies on detecting HER2 low expression rates, enabling more effective screening of populations that could benefit from HER2 low expression targeted therapy.

Detection of heterogeneity of HER2 low expression

Gene heterogeneity and protein heterogeneity are two important parts of Intratumoral heterogeneity (ITH) of HER2 expression. ASCO/CAP (2013 version) (13) suggested that *HER2* gene heterogeneity be defined as the presence of individual cell populations with different HER2 copy numbers and/or HER2/CEP17 ratios, accounting for at least 10% of the entire tumor cell population. The 2023 UK guidelines (14) for HER2 detection stated that at the

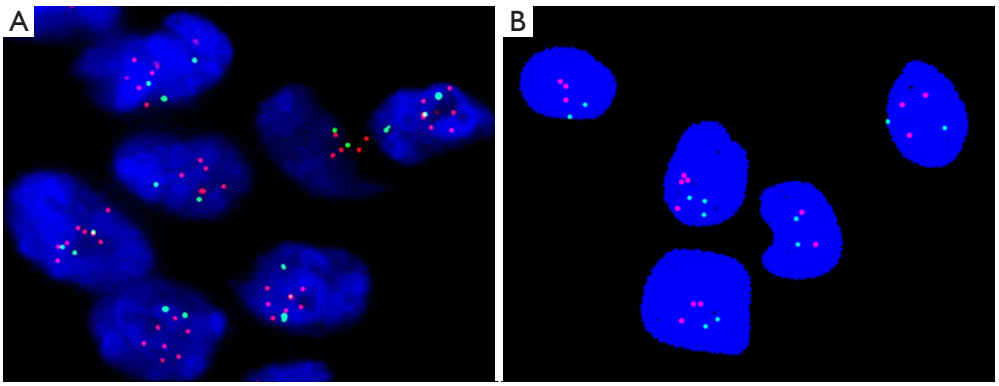

Figure 1 Results of HER2 double probe fluorescence *in situ* hybridization in invasive breast cancer. (A) Positive (HER2 amplification, ×100); (B) negative (HER2 non-amplification, ×100). HER2, human epidermal growth factor receptor 2.

level of HER2 protein expression, heterogeneity is defined as any cluster of cells (subclones) with strong or moderate expression of intact cell membranes in <10% of tumors; then the IHC score was 2+ and should be tested by *in situ* hybridization (ISH) (*Figure 1*). If subclonal cells show weak staining in <10% of tumors that are otherwise regionally negative, the tumor is scored as 1+. Therefore, core needle aspiration biopsy (CNB) specimens need to be carefully examined to identify differentially tumor cell populations with different HER2 staining patterns.

A study by Zhang *et al.* (15) published in Modern Pathology in 2022 showed that 31% of 281 breast cancer patients had low HER2 expression, with greater heterogeneity in HER2 IHC 1+. Therefore, accurate identification of heterogeneity of HER2 low expression is crucial for developing appropriate treatment regimens and predicting prognosis in breast cancer patients. The 2021 edition of the French guidelines for HER2 detection (2021 version) (6) recommends that HER2 immunohistochemical results be clearly reported on three components used to assess HER2 scores, including intact or incomplete HER2-stained cell membranes, staining intensity (which requires careful observation at high magnification), and percentage of positive infiltrating tumor cells.

In recent years, a new concept of HER2 heterogeneity called HER2 micro-heterogeneity has been proposed. It currently relies on gene-protein assay (GPA) method for detection, first developed by Downs-Kelly *et al.* (16). It combines HER2 IHC and HER2 ISH assays, which can be performed simultaneously on the same tissue slice, allowing pathologists to score both HER2 gene and HER2 protein expression levels at the single-cell level. The results of Nitta *et al.* (17) showed that this method can detect breast tumors cell populations with amplified HER2 gene without overexpression of HER2 protein. This mismatch between HER2 gene and protein at individual cell level is called "HER2 micro-heterogeneity". The results of Osamura *et al.* (18) suggest that HER2 micro-heterogeneity may be the biological mechanism of resistance to HER2-targeted therapy. A HER2 micro-heterogeneity xenograft model study showed that the efficacy of anti-HER2 antibody therapy depended on the ratio of HER2 gene amplified tumor cells overexpressing HER2 protein to HER2 gene amplified tumor cells not expressing HER2 protein (19). Therefore, to further explore of HER2 micro-heterogeneity may be one of the directions of HER2 heterogeneity research.

Advances in artificial intelligence (AI) assisted interpretation of HER2-low breast cancer

In recent years, AI has developed rapidly in assisting in IHC diagnosis. The AI algorithms proposed in the current researches also performed excellently in the accurate interpretation of HER2-low expression. Marra *et al.* (20) used the combination of IHC and HER2 mRNA expression as the gold standard and attempted to predict HER2 status of breast cancer by using whole slide image (WSI) of hematoxylin-eosin (H&E) stained sections via a deep learning approach. When distinguishing cases with HER2-low expression, HER2 amplification, and HER2 true negative, the receiver operating characteristic curve area of the model reached 0.78, sensitivity was 76%, specificity was 73%, positive predictive value was 97%, negative predictive value was 21%, and F1 score was 0.85, indicating that the AI model can distinguish tumors completely lacking

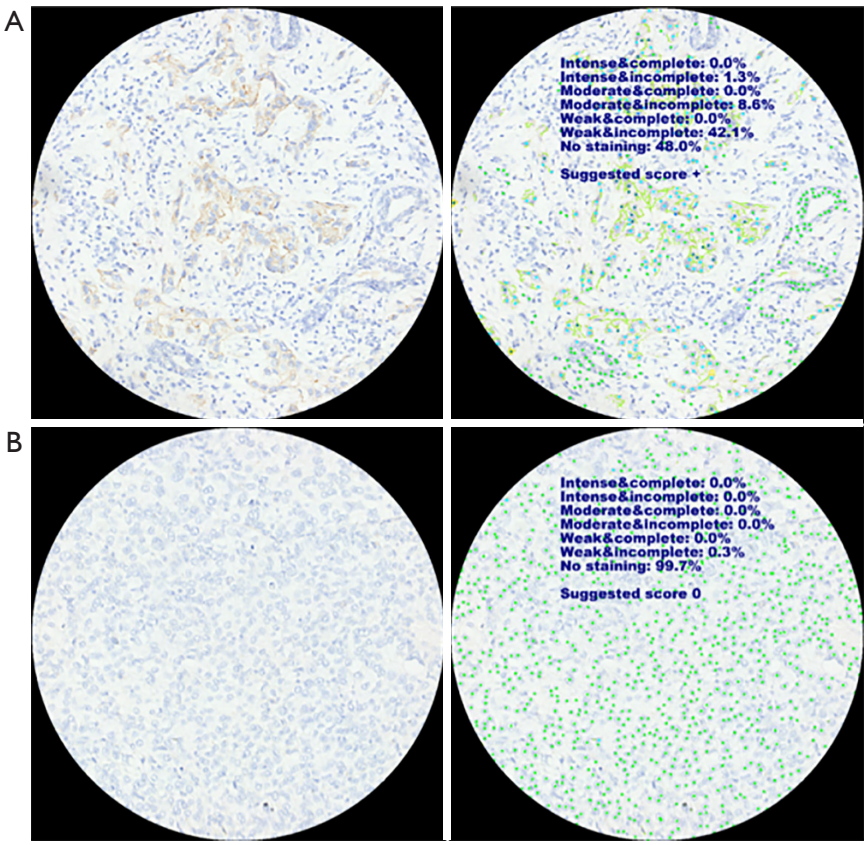

Figure 2 AI assisted microscope assisted accurate interpretation of HER2 low expression in breast cancer. (A) IHC 1+ (40×); (B) IHC 0 (40×). AI, artificial intelligence; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemical.

HER2 protein and mRNA from tumors with HER2-low expression. Atallah *et al.* (21) refined and redefined the definition of thresholds for HER2 IHC 0 and 1+ based on mRNA levels by the AI model, suggesting that the threshold for HER2 >10% weakly incomplete membrane staining be updated to ≥20%, expanding on the original guidelines for different expression pattern scenarios such as ≤10% weakly complete, >10% weakly complete and <10% moderately incomplete membrane staining. The study showed a closer association between the improved scoring and clinicopathologic features.

Our team has previously explored the role of AI in accurately differentiating HER2 IHC 0 and 1+ in breast cancer (22). The results of the study showed that AI-assisted interpretation improved the accuracy and consistency of pathologists' interpretation of HER2 IHC 0 and 1+, especially in HER2 IHC 1+ cases (*Figure 2*). It was also able to improve the accuracy of interpretation in cases with HER2 heterogeneity, especially in the scattered type. We have demonstrated for the first time the role of AI in assisting pathologists in distinguishing HER2 IHC 0 and 1+, as well as in enhancing the interpretation of cases with heterogeneity. These results show that AI can effectively assist in the accurate detection of HER2 in breast cancer, and there are more diversified application prospects. The 2023 edition of the UK HER2 testing guidelines (14) recommends the need for rigorous validation and quality control before the application of AI in clinical practice. The accuracy of automated image analysis techniques in interpreting HER2 in the presence of abnormal staining patterns remains to be considered, and regular review and monitoring will likely reduce the probability of misclassification. At this time, automated image analysis techniques and AI models are not approved for the assessment of HER2 status in routine diagnostic workups, but if new reliable evidence emerges in the future, it will be reviewed and the next evaluation plan will be developed.

Conclusions

In recent years, pathologists have been paying attention to the effect of storage time and storage conditions of unstained paraffin sections on immunohistochemical staining for HER2-low expression, and our results showed that the preferable time to store paraffin sections at room temperature is within 4 weeks, which can effectively avoid the loss of antigenicity. Research has shown that heterogeneity is more common in HER2 low breast cancer, and the concept of HER2 micro-heterogeneity has been proposed, which puts forward higher demands for further subdivision of HER2 heterogeneity. Previous studies have also shown that HER2 micro-heterogeneity may affect the efficacy of HER2-targeted therapy, and its biological mechanisms in HER2-targeted therapy resistance and potential role in predicting the prognosis of breast cancer still need to be further explored in large multi-center studies. AI has developed rapidly and has irreplaceable advantages for quantitative analysis of auxiliary immunohistochemistry. In recent years, the application of AI in the detection of HER2-low breast cancer has achieved encouraging results. It is believed that with further in-depth research, AI will assist pathologists to solve the pain points and difficulties in pathological evaluation of HER2-low breast cancer, so as to help standardized accurate diagnosis and treatment, and benefit patients with breast cancer.

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Footnote

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Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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