Reassessment of HER2 Status and Testing in the Context of HER2-Low in Breast Cancer: Is RNAscope a better determinant?

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Introduction

HER2 overexpression has traditionally been associated with aggressive forms of tumour and poor prognosis in breast cancer. The development of HER2 targeted therapies has significantly improved outcomes for HER2 positive patients. The DESTINY-Breast04 trial reshaped our understanding of HER2 by introducing a new subset called “HER2-low”, characterised by IHC scores of 1+ or 2+ with negative FISH. Trastuzumab deruxtecan, an antibody-drug conjugate that combines the specificity of a monoclonal antibody with the cytotoxic effects of chemotherapy, demonstrated clinical benefit in patients with HER2-low breast cancer.

Aim

- Determine the prevalence of HER2-low by rescoring previous HER2 IHC slides in the local laboratory service.
- Determine inter-observer agreement between assessors.
- Determine whether RNAscope testing is a potentially superior predictor of HER2-low status compared to IHC.
- Investigate the suitability of RNAscope to provide a more reproducible semi-quantitative measure of HER2-low status.

Methods

This study analysed a cohort of 432 invasive breast cancers. A retrospective clinical audit assessed the concordance of reported HER2 status, with the intention of identifying the true incidence of HER2-low cases. RNA *in situ* hybridisation using RNAscope and manual counting was used to evaluate ErbB2 mRNA in relation to HER2 IHC in 80 cases.

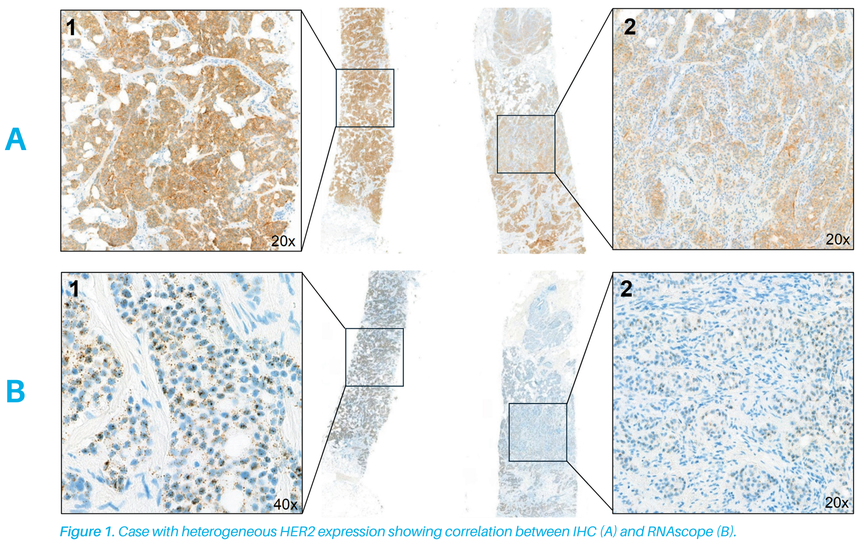

Figure 1. Case with heterogeneous HER2 expression showing correlation between IHC (A) and RNAscope (B).

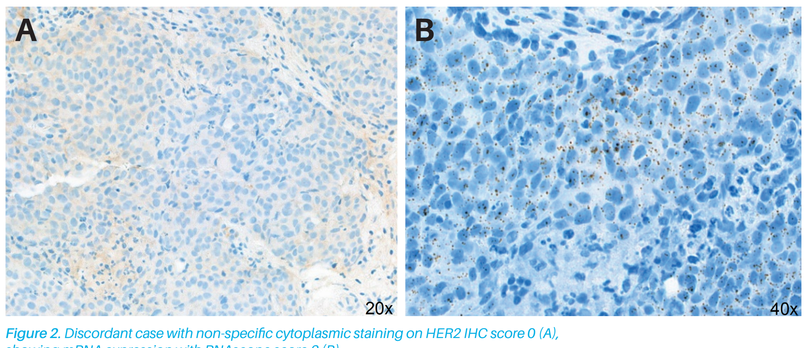

Figure 2. Discordant case with non-specific cytoplasmic staining on HER2 IHC score 0 (A), showing mRNA expression with RNAscope score 2 (B).

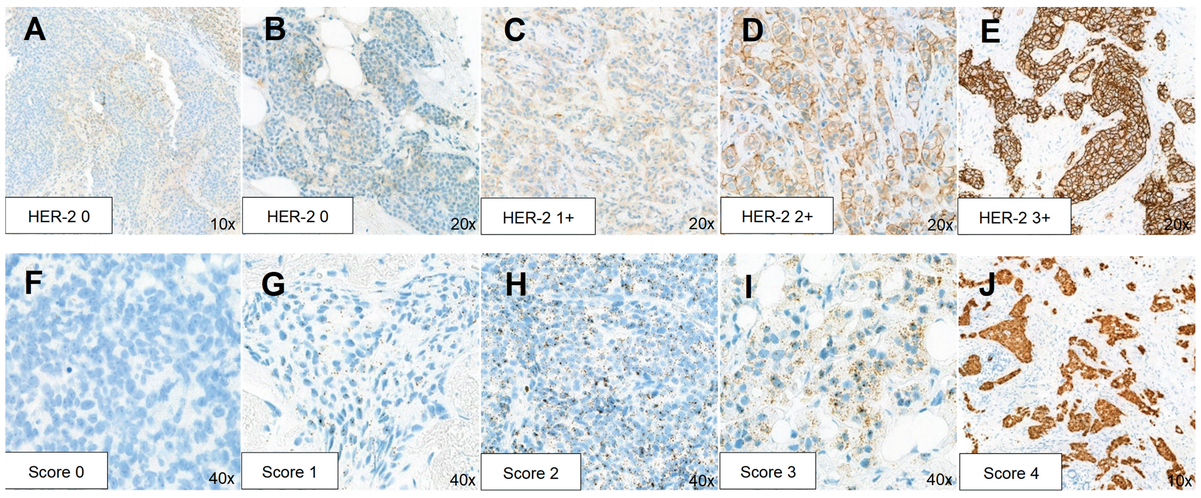

Figure 3. Representative images of HER2 IHC scores of 0 (A-B), 1+ (C), 2+/FISH-positive (D), and 3+ (E), with corresponding RNAscope scores 0 to 4 (F-J).

Results

Prevalence of HER2-low

Based on the original HER2 status, 65.51% (n=283) were HER2-low, with 63.3% (n=179) scored 2+ without gene amplification and 36.8% (n=104) scored 1+. Among the original 2+ cases (n=195), 91.8% lacked amplification, while 8.2% showed amplification.

Level of Agreement

Agreement between raters was moderate (κ=0.564, p<0.001, 95% CI 0.507–0.624). Agreement was substantial for IHC 0 (κ=0.605) and almost perfect for IHC 3+ (κ=0.868), but only moderate for 1+ (κ=0.423) and 2+ (κ=0.475). Rescoring led to a major shift in classification; cases scored as 1+ increased from 24.1% to 51.9%, while 2+ decreased from 45.1% to 22.9% (Figure 4).

Evaluation of ErbB2 mRNA expression in relation to HER2

HER2 IHC scores correlated significantly with ErbB2 mRNA expression (Figure 5; p<0.001, Kruskal–Wallis). Higher IHC scores were associated with higher mRNA expression, except between IHC 0 vs 1+, and 2+ (FISH+) vs 3+. RNAscope score distribution was 0 (n=5), 1 (n=32), 2 (n=30), 3 (n=5), and 4 (n=8). Most cases (77.5%) showed low to moderate expression (scores 1-2), while 10% showed high expression (score 4). Discordance was observed in 5 out of 80 cases, including mRNA positivity in IHC 0 and loss of mRNA in some IHC 1+ (Figure 2).

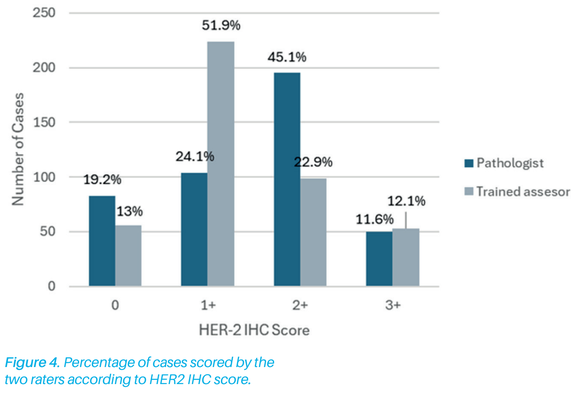

Figure 4. Percentage of cases scored by the two raters according to HER2 IHC score.

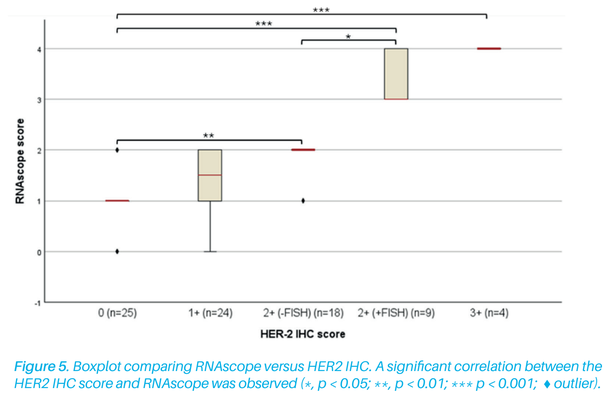

Figure 5. Boxplot comparing RNAscope versus HER2 IHC. A significant correlation between the HER2 IHC score and RNAscope was observed (⁎, p < 0.05; ⁎⁎, p < 0.01; ⁎⁎⁎ p < 0.001; ♦ outlier).

Conclusion

This study showed significant inter-observer variability in routine HER2 IHC interpretation (Figure 4). It also demonstrated the use of RNAscope for assessing ErbB2 mRNA in FFPE tissue, comparable to IHC for protein expression (Figure 5). No significant difference was seen between HER2-low and HER2 zero, and mRNA threshold to define HER2-low was not set. RNAscope offers potential as a complementary tool to IHC by reducing non-specific findings and providing quantitative insights. Further validation in larger cohorts, particularly HER2 IHC 0/ultra-low and 1+, is recommended.

References

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