A threefold evaluation of HER2low status of advanced breast carcinomas in core biopsies, matching surgical specimens and their distant metastases. A retrospective study of 47 patients using conventional microscopy, digital pathology and artificial intelligence (AI).

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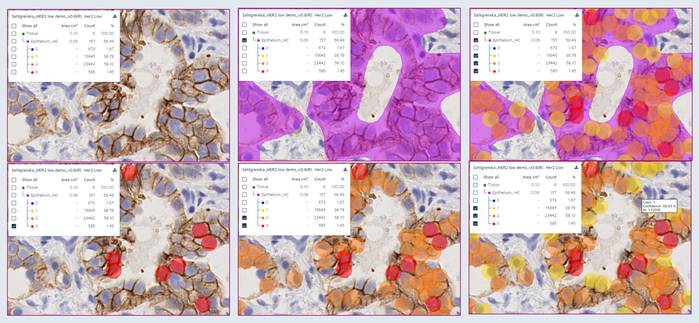
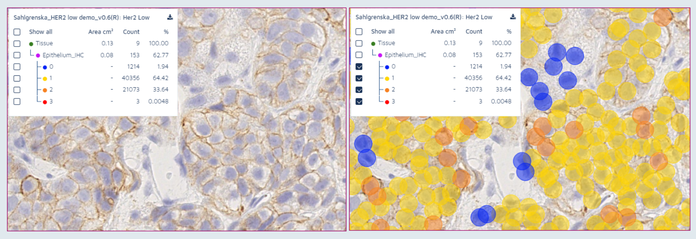
Her2 classification: 1+, 2+ and 3+



Her2 Low classification: 2+, 1+ and 0



Background & objectives

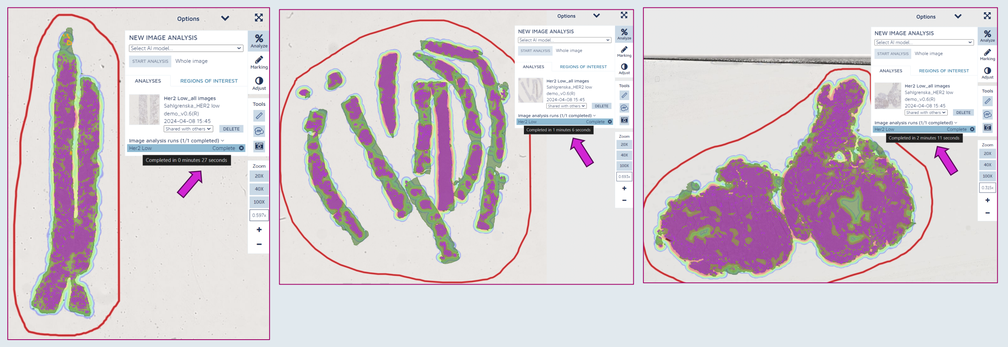
Patients with **advanced breast carcinoma with HER2-low status** (score 1+ or 2+/non-amplified) may benefit from the antibody drug conjugate (ADC) Enhertu® (**Modi**). However, evaluation of immunohistochemistry (IHC) is challenging and further confirmation with molecular assays is currently not available (**Tarantino**). **The aim** was to
(1) compare the outcome of HER2 scoring using conventional microscopy, digital pathology, and artificial intelligence (AI);
(2) assess changes in HER2low status in core biopsies, matching operation specimens, and their distant metastases.

Methods

For patients with invasive breast carcinomas showing HER2-low status (n = 47), immunohistochemistry (IHC) images for HER2 were reevaluated in the core needle biopsies, subsequent matching operation specimens, and distant metastases using **three modalities**:
(1) **Conventional microscopy** (eye balling in the microscope),
(2) **Visual estimation of scanned digital image on the screen** (eye balling on the screen),
(3) **Artificial intelligence** (AI, Aiforia®) evaluation providing an exact percentage of each HER2 score. AI analysis was preceded by deep learning using the pathologist's annotations of the IHC images.
The HercepTest slides were stained between 2013 and 2023 and retrieved from the archives. Four micrometer sections were made from formalin-fixed paraffin-embedded blocks, and pretreated using the Dako PTLink system (Dako, Carpinteria, CA) and processed further on an automated DAKO Autostainer platform with HercepTest (Dako, Cat. SK001). This study was approved by the Regional Ethical Committee (registration no. 287-15, updated by diary number 2023-03030-02 for AI usage) in Gothenburg, Sweden. A.K. received research grants from Sahlgrenska Comprehensive Cancer Centre in 2023 to perform the study.

The number of estimations & assessments in this study was **400**:
- 40 core biopsies with 3 modalities = **120** examinations (7 cases were diagnosed by cytology)
- 47 operation specimens with 3 modalities = **141** examinations
- 47 distant metastases with 3 modalities = **139** examinations (one metastasis had only scanned image, here only eye balling on the screen was possible).

Time taken for an analysis run

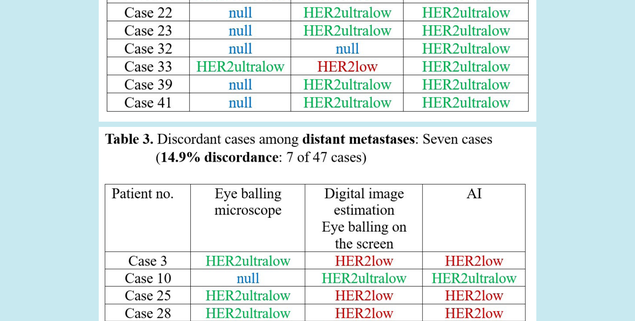


CRITERIA USED FOR HER2 STATUS:
HER2low: >10% score 1+ or 2+/non-amplified
HER2ultralow: 1-10% score 1+
HER2null: score 0 or <1% score 1+

Discussion

The HER2low concept causes diagnostic challenges for pathologists. Score 1+ and 2+ are mostly heterogeneously distributed within both the invasive breast carcinomas and their distant metastases (**Marchió, Sode**). It poses difficulty especially when the number of tumor cells was near to the 10% cut-off point (for HER2low versus HER2ultralow status) or 1% cut-off point (for HER2ultralow versus HER2null status). This differentiation is crucial, because according to the results from the DESTINY-Breast06 trial, even HER2ultralow metastatic breast carcinomas may also benefit from the ADC therapy (**Mehta**).
AI evaluation helped us to determine a more accurate HER2 status, providing an exact percentage of each HER2 score within seconds with very high accuracy (**Palm, Wu**).
The Aiforia® solution provided pinpointed individual findings with visual feedback for pixel-level validation of the outcome prediction.

Her2 Ultra Low classification: 1+ and 0



Results

RESULTS COMPARING HER2 STATUS IN THE THREE PATIENT SAMPLES
(CORE BIOPSY, MATCHING OPERATION SPECIMEN, AND MATCHING DISTANT METASTASIS).

1. IN THE MICROSCOPE (FIGURE 1):

1a. Number of patients with HER2low status in the core biopsies and distant metastases was identical (both 72%).

1b. HER2 ultralow status was very similar in the three patient samples (13% - 19% - 17%).

1c. No HER2null status observed in the core biopsies.

Conclusion: Similarities in HER2low status between core biopsies and distant metastases can be explained that both represent only a small part of the tumor (a random biopsy), not the entire tumor.

2. DIGITAL IMAGES BY EYE BALLING (FIGURE 2):

2a. Same trend observed as it was by the microscopic estimation, that HER2low status was similar in the core biopsies and the distant metastases (83% versus 81%).

2b. More samples with HER2ultralow status both in the operation specimens and matching distant metastases (23%, and 11% respectively) compared to core biopsies (2%).

2c. No HER2null status observed in the core biopsies (similarly to the microscopic evaluation).

2d. More HER2low cases found digitally, in comparison with the microscopic estimation:

(a) in core biopsies 72% versus 83%;

(b) in operation specimens 47% versus 53%;

(c) in distant metastases 72% versus 81%.

CONCLUSION: More HER2low cases had been diagnosed on the digital image by eye balling, in comparison with the microscope. These results support the theory that the scanned images show higher membranous staining intensity. Moreover, more tumor cells can be identified with membranous staining digitally, which results in a higher % number of positively stained tumor cells. The digital estimation may identify more patients eligible for Enhertu® therapy.

3. ASSESSMENT BY THE AI (FIGURE 3):

3a. AI identified only a slightly lower number of patients with HER2low status, than those diagnosed using the digital images by eye balling:

(a) in core biopsies it was identically 83%;

(b) in operation specimens 53% digitally versus 51% by AI;

(c) in distant metastases 81% digitally versus 77% by AI.

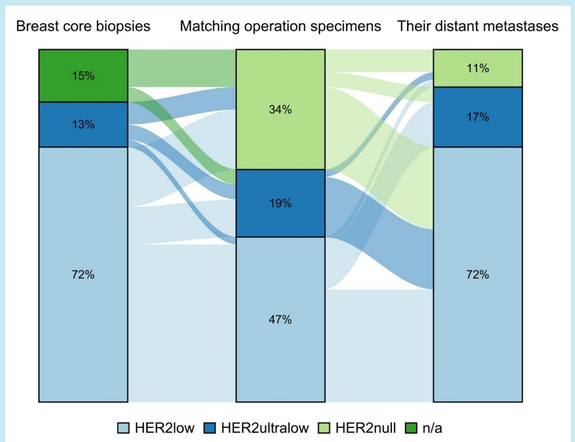


Figure 1. HER2 estimation in three patient samples in the microscope.

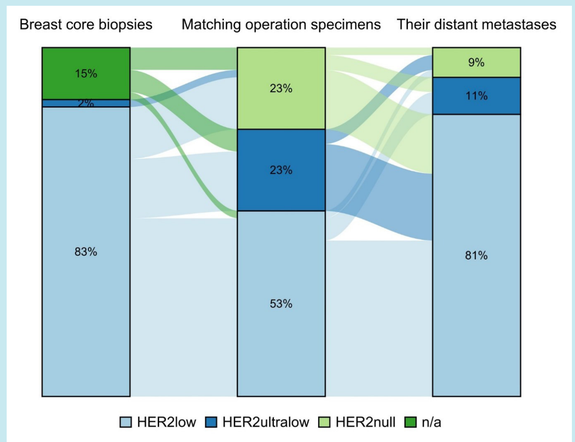


Figure 2. HER2 estimation by eye balling of the digital images.

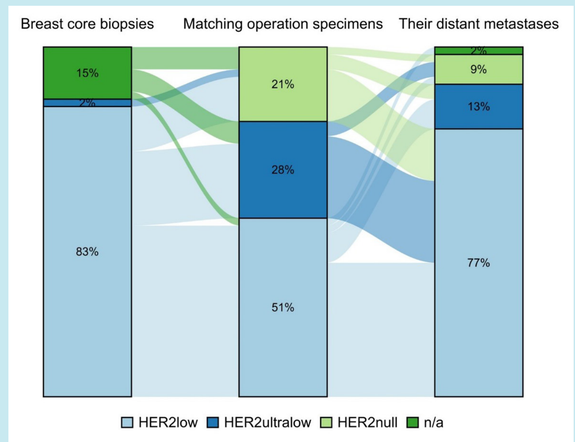


Figure 3. HER2 assessment in the three patient samples by AI.

RESULTS COMPARING HER2 STATUS IN THE THREE MODALITIES
(CONVENTIONAL MICROSCOPY, VISUAL ESTIMATION OF THE SCANNED IMAGE ON THE SCREEN, AND AI).

1. COMPARISON OF RESULTS BY THE THREE MODALITIES IN THE CORE BIOPSIES (FIGURES 4-7):

1a. Digital estimation on the screen and AI scored the same number of cases with HER2low status. It was 11% higher compared to the conventional microscopy, meaning that both the AI and digital estimation identifies 11% more patients eligible for Enhertu® therapy. Interestingly, visual digital estimation and AI showed identical results (both 83%).**1b.** HER2 ultralow status was very similar in the three patient samples (13% - 19% - 17%).

1b. There was no discrepancy regarding HER2null status among the three modalities (15% for each modality).

2. COMPARISON OF RESULTS BY THE THREE MODALITIES IN THE OPERATION SPECIMENS (FIGURES 8-11):

2a. Both the digital visual estimation and AI identified a slightly higher number of patients with HER2low status, and even higher number with HER2ultralow status compared to the microscopic estimation. Consequently, more patients with HER2null status were identified using the microscopic evaluation, further highlighting that the digital image shows stronger membranous staining and even more cells can be identified showing membranous staining.

2b. AI scored more patients with HER2ultralow status compared to microscopy and digital evaluation. This result can be explained by the difficulty in estimating the number of tumor cells with membranous staining manually near the cut-off point 10% (for HER2low versus HER2ultralow status) or 1% cut-off point (for HER2ultralow versus HER2null status).

3. COMPARISON OF RESULTS BY THE THREE MODALITIES IN IN THE MATCHING METASTASES (FIGURES 12-15):

3a. These results are similar to the scores seen in the core biopsies. However, there were more HER2ultralow cases in all three modalities in the distant metastases compared to the core biopsies, but fewer HER2ultralow cases compared to the operation specimens. HER2low status was exactly the same as it was in the core biopsy group (72%).

3b. HER2low status was 20-30% higher in the distant metastases compared to the operation specimens. This fact needs further explanation.

3c. There were fewer distant metastases with HER2null status compared to the core biopsies and operation specimens.

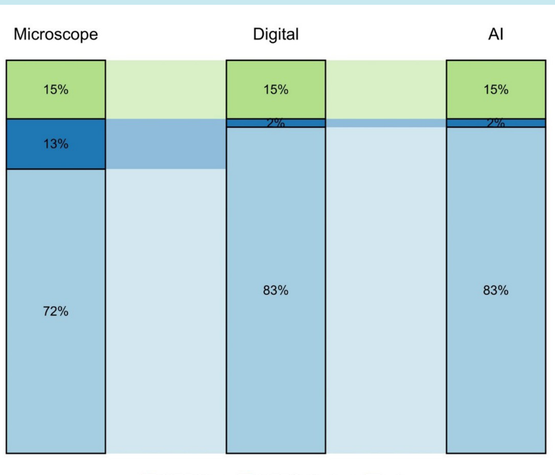


Figure 4. HER2 assessment of core biopsies by the three modalities.

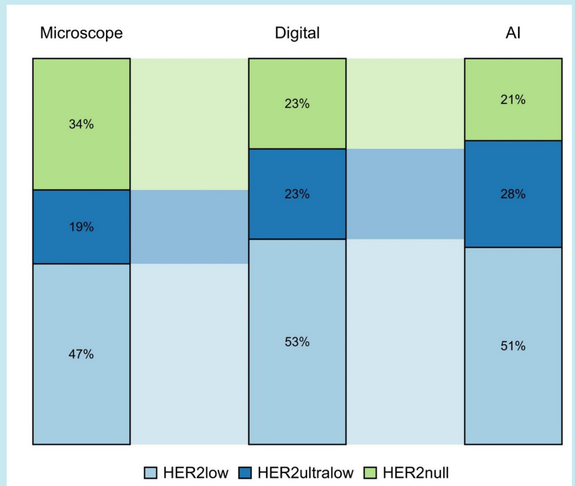


Figure 8. HER2 assessment of operation specimens by all three modalities.

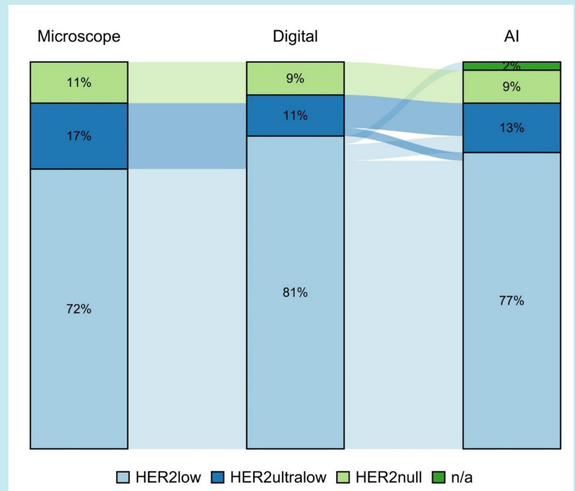


Figure 12. HER2 assessment of distant metastases by all three modalities

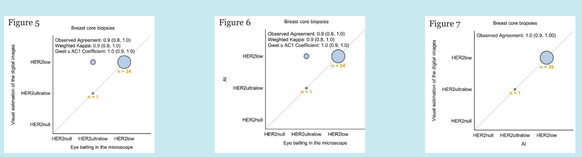


Figure 5. **Breast core biopsies**: Concordance in HER2 status between microscopic estimation and visual estimation of the digital images on the screen.
Figure 6. **Breast core biopsies**: Concordance in HER2 status between microscopic estimation and assessment by AI.
Figure 7. **Breast core biopsies**: Concordance in HER2 status between visual estimation of digital images on the screen and assessment by AI.

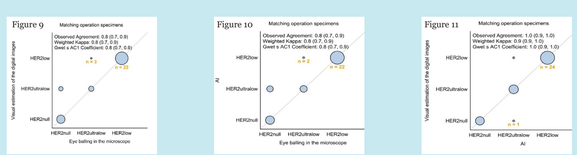


Figure 9. **Operation specimens**: Concordance in HER2 status between microscopic estimation and visual estimation of digital images on the screen.
Figure 10. **Operation specimens**: Concordance in HER2 status between microscopic estimation and assessment by AI.
Figure 11. **Operation specimens**: Concordance in HER2 status between visual estimation of digital images on the screen and assessment by AI.

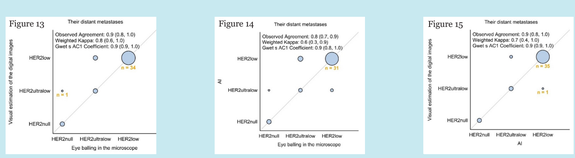


Figure 13. **Distant metastases**: Concordance in HER2 status between microscopic estimation and visual estimation of digital images on the screen.
Figure 14. **Distant metastases**: Concordance in HER2 status between microscopic estimation and assessment by AI.
Figure 15. **Distant metastases**: Concordance in HER2 status between visual estimation of digital images on the screen and assessment by AI.

1. The digital image on the screen often showed a stronger membranous staining intensity in comparison with the image examined by eye balling in the microscope. It also resulted in a higher score digitally, e.g.
(a) Some HER2null cases in the microscope became HER2ultralow digitally;
(b) Some HER2ultralow cases in the microscope had been scored as HER2low digitally.
(c) Consequently, a microscopic score was never higher than the digital score.
In summary, more HER2low cases were identified digitally and found by the AI, as in the microscope (**Table 1-3**).

Table 1. Discordance among **core biopsies**: Five cases with HER2ultralow status in the microscope, but HER2low status on the screen digitally (**12.5% discordance**: 5 of 40 cases)

Patient no.	Eye balling microscope	Digital image estimation Eye balling on the screen	AI
Case 11	HER2ultralow	HER2low	HER2low
Case 19	HER2ultralow	HER2low	HER2low
Case 31	HER2ultralow	HER2low	HER2low
Case 32	HER2ultralow	HER2low	HER2low
Case 37	HER2ultralow	HER2low	HER2low

Table 2. Discordance among **surgical specimens**: Ten cases (**21.3% discordance**: 10 of 47 cases)

Patient no.	Eye balling microscope	Digital image estimation Eye balling on the screen	AI
Case 3	null	HER2ultralow	HER2ultralow
Case 9	HER2ultralow	HER2low	HER2low
Case 12	null	null	HER2ultralow
Case 21	HER2ultralow	HER2low	HER2low
Case 22	null	HER2ultralow	HER2ultralow
Case 23	null	HER2ultralow	HER2ultralow
Case 32	null	null	HER2ultralow
Case 33	HER2ultralow	HER2low	HER2ultralow
Case 39	null	HER2ultralow	HER2ultralow
Case 41	null	HER2ultralow	HER2ultralow

Table 3. Discordant cases among **distant metastases**: Seven cases (**14.9% discordance**: 7 of 47 cases)

Patient no.	Eye balling microscope	Digital image estimation Eye balling on the screen	AI
Case 3	HER2ultralow	HER2low	HER2low
Case 10	null	HER2ultralow	HER2ultralow
Case 25	HER2ultralow	HER2low	HER2low
Case 28	HER2ultralow	HER2low	HER2low
Case 29	HER2ultralow	HER2ultralow	HER2low
Case 44	HER2ultralow	HER2low	HER2low
Case 47	HER2ultralow	HER2low	HER2low

2. AI often included DCIS in the assessment, which demanded revision by the pathologist to exclude the areas of DCIS before the re-run by AI. Annotations should be performed in order to teach AI to exclude DCIS.

3. We found lower number of carcinomas with HER2low status and higher number with HER2ultralow and HER2null status in the operation specimens compared to core biopsies and distant metastases in all three modalities (Na).

4. HER2low status was 20-30% higher in the distant metastases compared to the surgical specimens. The question arose: which tumor cells are represented in the distant metastases? Their relationship to neoadjuvant chemotherapy (NACT)? In our material only 10 patients received preoperative neoadjuvant therapy (patient 3. 6. 8. 15. 16. 20. 25. 29. 31. 38.).

5. There is a discrepancy in HER2low status with different antibodies (Ventana versus DAKO's two antibodies). Ventana's antibody and DAKO's monoclonal antibody identifies higher HER2 staining score (Rüschoff, Zhang). We used Dakos's polyclonal HercepTest antibody in this study material.

CONCLUSION

How digital and AI assessment could change the indication for Enhertu® treatment compared to the HER2 status given in the microscope? Which modality will be the gold standard? However, recent studies elucidated that AI was proven to be an accurate method for reducing the number of equivocal cases not affecting the sensitivity of the assessment (**Jacobsen, Palm, Wu**). Moreover, the relatively low interobserver concordance in identifying HER2 ultralow status warrants the need for increased precision in HER2 assessment by digital tools using AI (**Mehta**).

Usage of digital pathology varies greatly among the different European countries. Those countries using conventional microscopy may identify fewer advanced breast carcinomas with HER2low status.
Further clinical studies are needed to verify the predictive value of the three different scoring modalities: (a) conventional microscopy with visual semi-quantitative estimation, (b) visual estimation of the scanned HER2 image, and (c) HER2 assessment by AI.

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