Inter-laboratory variability of HER2-low analysis using Artificial Intelligence

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Introduction

Pathologists look for HER2 immunohistochemical expression in breast carcinoma representing HER2 amplification. The used tests are validated for HER2-high, e.g. score 2+ or 3+. Treatment of HER2-low breast carcinoma is emerging for HER2 expressing tumors with a score of 1+ or 2+ without amplification. In this study, immunohistochemical detection of HER2-low is tested for inter- and intralaboratory reproducibility in The Netherlands. Can the HER2-high test be used to detect eligibility for HER2-low treatment as well?

Methods

To define reproducibility of HER2-low, three blanc slides with a dynamic range cell line (HistoCyte, Newcastle,UK) were send to 42 labs. Participants were asked to stain slides in three consecutive days. After staining, slides were scanned and analyzed using AI-software (Qualitopix, Visiopharm, Hørsholm, DK). Membrane staining intensity and completeness per cell was measured by the algorithm to output a percentage of biomarker expression.

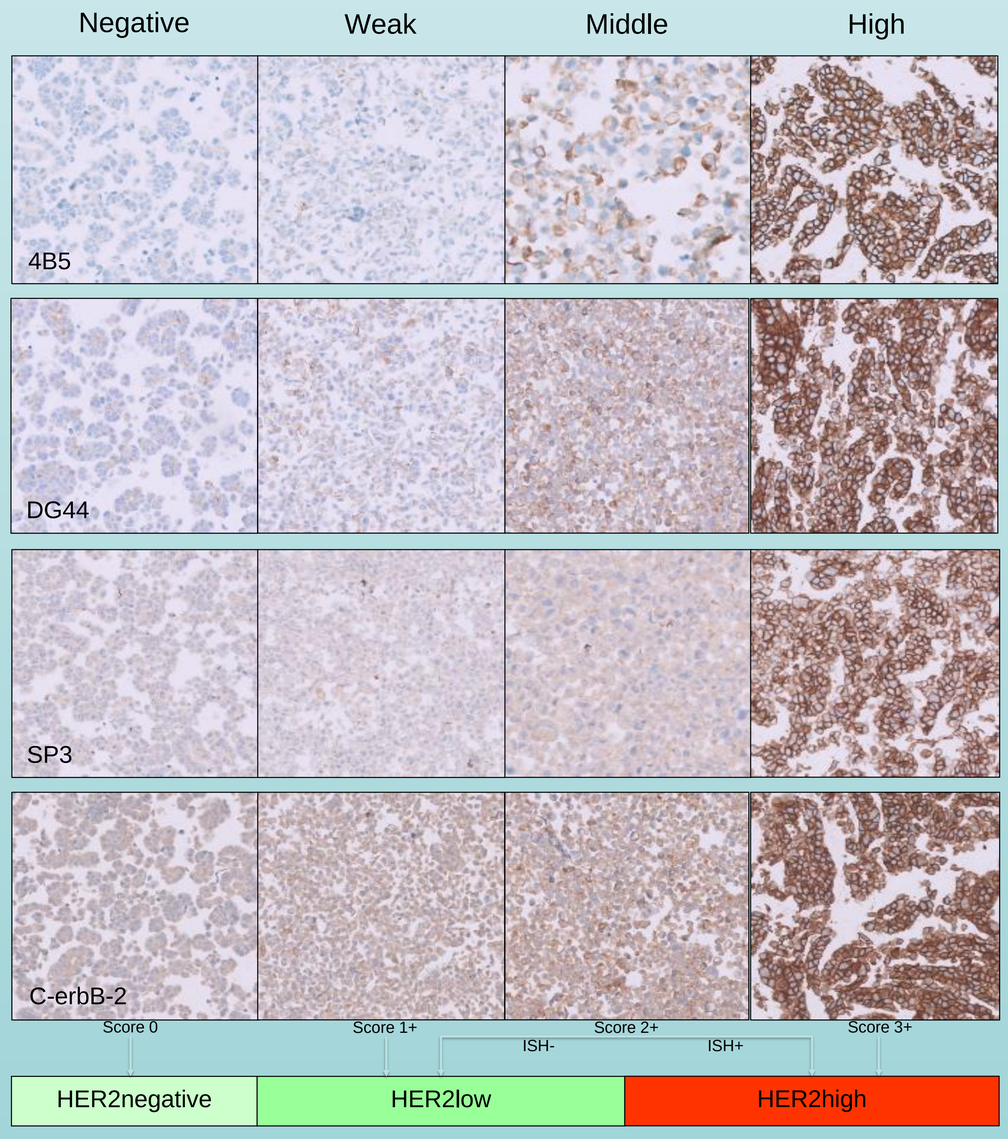

Figure 1, example of stained dynamic range cell line using four HER2 antibodies. Cell line with four cores: negative representing score 0, weak score 1+, equivocal score 2+, positive score 3+

Results

Four antibodies were used by 35 laboratories: 54% clone 4B5, 14% Herceptest (DG44), 14% SP3, 17% C-erbB-2.

SP3 is used at dilutions ranging from 1:200 to 1:1000, polyclonal C-erbB-2 dilutions varied between 1:200 and 1:500. The negative core showed a positive result for both SP3 and C-erbB-2.

Most variation was found in the middle core:

- Monoclonal 4B5: 43.0+/-8.7% (mean+/-SD)
- Monoclonal DG44: 70.9+/-4.4%
- Monoclonal SP3: 52.4+/-21.5%
- Polyclonal C-erbB-2 73.6+/-18.9%

Discussion

Four HER2 antibodies were used in Dutch laboratories. 4B5 and DG44 showed consistent HER2 results.

Surprisingly, C-erbB-2 and SP3 showed a HER2-positive result in negative controls. Furthermore, different antibody concentrations were used for C-erbB-2 and SP3, showing inconsistent HER2-expression.

HER2 antibodies validated to detect HER2-high need to be re-validated for HER2-low. The use of standardized reference material in combination with arteficial intelligence is a promising method to determine HER2-low for clinical use.

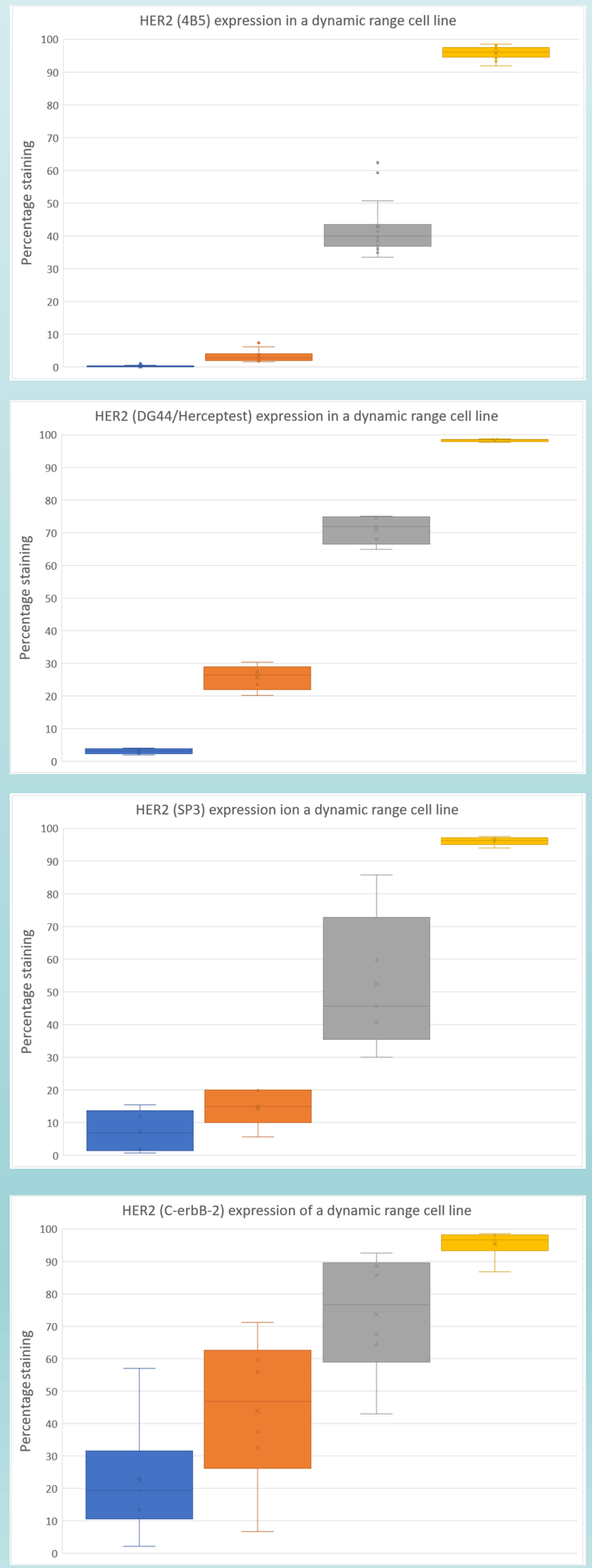

Figure 2, boxplots of HER2 stained cell line results for 4B5 (n=19), DG44/Herceptest (n=5), SP3 (n=5) and C-erb-B2 (n=6). Blue plots = negative, orange plots = weak, grey plots = middle and yellow plots = high cell line controls.

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