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Original article

Statistical modelling of HER2-positivity in breast cancer: Final analyses from two large, multicentre, non-interventional studies in Germany

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ABSTRACT

Background: The German NIU HER2 model was developed based on five variables found to have statistically significant influences on HER2-positivity, to allow exploration of deviations between model-predicted and actual HER2-positivity rates as a measure of testing quality. The prospective, non-interventional EPI HER2 BC study (NCT02666261) compared NIU and EPI data, aiming to validate the NIU model.

Methods: HER2 status and patient-/tumour-related information were collected from eligible patients with invasive breast cancer. The influence of variables on HER2-positivity was compared between studies and the NIU model validated using EPI data with cut-off and variable coefficients from the NIU study. The influences of additional variables, centre effects and laboratory-specific parameters were also explored.

Results: The study included 14,729 EPI and 15,281 NIU samples; HER2-positivity rates were comparable (13.5% versus 14.2%). The five covariates from NIU were shown to significantly affect HER2-positivity using EPI data. The Youden Index for the NIU model refitted to EPI data (0.3632) and the NIU model for prediction of HER2-positivity in EPI (0.3552) was close to that for the NIU model fitted to NIU data (0.3888), validating the NIU model. Replacing hormone receptor status with progesterone and oestrogen receptor expression, and adding method of sample extraction as a variable improved the model's predictive strength (ROC AUC 0.7402; Youden Index 0.3935).

Conclusions: Reliable, high-quality HER2-testing methods are essential for selection of patients with HER2-positive breast cancer for HER2-tageted treatment. Integration of our model into a locally used software or website may improve its viability for use in clinical practice.

1. Introduction

Thirteen to 20% of all breast cancer (BC) cases have overexpression or amplification of human epidermal growth factor receptor 2 (HER2) [1–3]. HER2 testing in BC has been routine for almost two decades, and selection of patients eligible for HER2-targeted treatment (e.g. trastuzumab [Herceptin®; F. Hoffmann-La Roche Ltd, Basel, Switzerland] [4–11], trastuzumab emtansine

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[Kadcyla®; F. Hoffmann-La Roche Ltd] [12], or pertuzumab [PERJETA®; F. Hoffmann-La Roche Ltd] [9,11]) relies heavily on accurate identification of HER2-positive tumours. Thus, reliable HER2-testing methods are essential, particularly when considering false-positive assessments [2,13]. Assessment of HER2-positivity rates is recommended as an indicator of HER2-testing quality [1,2,14]; however, identifying patient- or tumour-related factors that independently affect HER2-positivity rates between centres has proven challenging, particularly when rates of false-positive and false-negative results are balanced [2].

The large, multicentre, non-interventional NIU HER2 study was performed to identify variables that influenced HER2-positivity rates at 57 pathology institutes in Germany [15]. The study included 15,332 invasive BC samples and demonstrated that histological grade had the strongest influence on HER2-positivity rate, followed by hormone receptor (HR) status, histological subtype, age and nodal status ($P < 0.0001$ for all). A statistical model based on these variables was developed, allowing exploration of deviations between model-predicted and actual HER2-positivity rates and potentially highlighting HER2-testing quality issues in local practice.

Here, we report the final analyses from the 'Non-Interventional Study on the Epidemiology and Testing of HER2 in Breast Cancer in Germany' (EPI HER2 BC), which aimed to compare the original NIU data (15,281 reanalysed samples) [15] with the present EPI data (14,729 samples) to validate the NIU model and to draw conclusions for practising pathologists.

2. Methods

2.1. Study design

The NIU study design has been reported previously [15]. EPI was a prospective, non-interventional study of HER2 testing in 64 centres across Germany (NCT02666261), each of which were selected based on their ability to collect and document data on the required number of breast cancer diagnoses (200 or 400 depending on the site contract and documentation capability) within the 2-year duration of the study. Eligible adult patients with histologically confirmed invasive BC (any stage) and HER2 diagnostic data collected between March 4, 2016 and February 28, 2018 from routine pathological testing in accordance with the current guidelines at the time of the study (i.e. the American Society of Clinical Oncology/College of American Pathologists 2013 guidelines for HER2 testing in breast cancer [16]) and local standards were included based on consecutive sampling; data from samples obtained before the study start date were not included. Patient- and tumour-related data, including HER2 status, patient age and tumour characteristics (histological grade, HR status, histological subtype, nodal status, sample origin and method of sample extraction) were collected and documented. Patient information and test results were anonymous.

2.2. Statistical methods

The main objectives were: (1) to review the distributions of the main variables and their consistency in an independent data set and compare findings with the NIU study; (2) to explore the predictive power of the previous NIU model and validate it based on the newly collected EPI data; (3) to improve the model based on the new EPI data, if possible; (4) to analyse data by centre to identify those with documented HER2-positivity rates that deviated significantly from the model-predicted HER2-positivity rates, after adjustment for the centres' patient and sample characteristics.

Data were analysed descriptively with standard summary statistics, Wilson score-based confidence intervals (CIs) and graphical methods, as appropriate. Multiple logistic regression (MLR) was used to evaluate the combined impact of multiple parameters on HER2-positivity in routine pathological testing.

The NIU model was applied to predict the probability of HER2-positivity for individual patients and centres of the EPI study with cut-off and variable coefficients from the NIU study (Supplementary material S1). The model was validated by comparing the predictive performance for the new EPI data with that for the original NIU data. Bivariate analyses were used to assess the association of additional variables with HER2-positivity (Supplementary material S2).

The most influential variables from the MLR and best-fitting models (based on the smallest corrected Akaike information criterion) were determined using stepwise mixed forward inclusion and backward elimination of candidates. The relative influence of individual covariates on HER2-positivity rate was assessed using their *P* value from the model as a measure of statistical significance and by estimating their level of contribution to the variation of the predicted probability of positivity. Sensitivity and specificity of the model, the related Youden Index and the area under the receiver

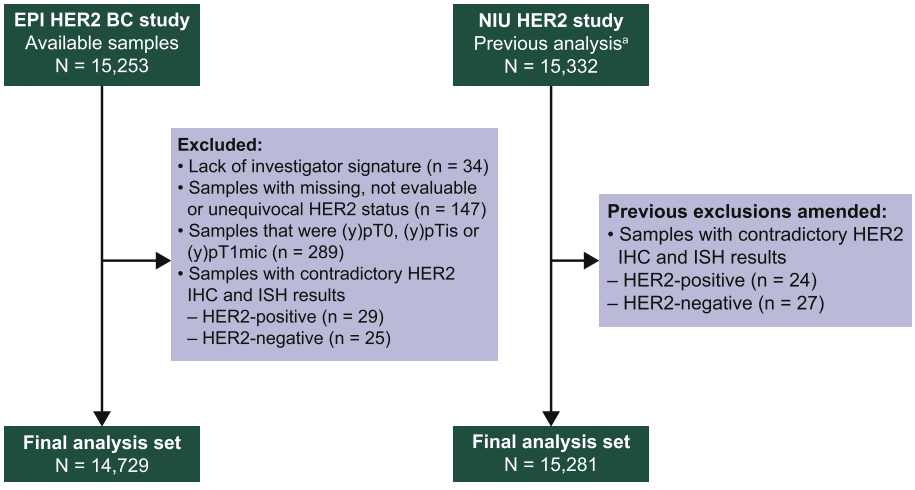


Fig. 1. Flow diagram of the main analyses for the EPI HER2 BC and NIU HER2 studies. BC, breast cancer; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridisation. [a] Statistical analysis of the NIU HER2 study samples set has been published previously [15]. Previous exclusions of samples were amended to achieve full consistency with the EPI HER2 BC study.

operating characteristic curve (ROC AUC) were used to assess the predictive strength of the model and to compare models within and between the NIU and EPI studies (higher values mean improved predictive strength). Prediction profiles were applied to visualise the relationship between model-predicted probability of HER2-positivity and the adjusted influence of covariates. The covariates of the best-fitting models were further reviewed for clinical and scientific appropriateness.

A primary model was established based on statistical performance measures and clinical viewpoints and validated using ten-fold cross-validation (Supplementary material S3).

Centre effects were assessed using a descriptive and a modelling approach (Supplementary material S4). Centres that deviated based on either approach were compared and investigated further.

HER2-positivity rates were consistent over the course of the study (eight quarters in 2 years), indicating that there was no bias over the study periods (Fig. S1).

Statistical analyses were performed using SAS JMP V13.2.1 (SAS Institute, Inc., Cary, NC, USA).

Results

3.1. Sample inclusion and exclusion criteria

EPI data were collected from 15,253 samples; samples with (y)pT0, (y)pTis and (y)pT1mic stage (n = 289) were excluded as ductal carcinoma *in situ* was ineligible for inclusion. Following sample exclusions, the final EPI data comprised 14,729 samples (Fig. 1).

To account for slight differences between the EPI and NIU study protocols, and to allow comparison between the studies, the NIU data were reanalysed to exclude samples with contradictory immunohistochemistry or *in situ* hybridisation measurements and fit the original histological subtype data to one of two categories ('lobular' and 'ductal or other'). The final NIU analysis set, with sample exclusions consistent with the EPI study, included 15,281 samples (Fig. 1).

3.2. Distribution of the main variables for the EPI and NIU studies

Overall, the distributions of relevant variables were comparable between studies; HER2-positivity rates were 13.5% and 14.2% in the EPI and NIU studies, respectively (Table 1).

3.3. The adjusted NIU model fitted to NIU data for comparison to the EPI models

Adjustment of the NIU model slightly improved the predictive strength of the model (Supplementary material S5; Table 2 [Row 2 versus Row 1]); however, the prediction profiles for each variable remained unchanged (Fig. 2a) and the order of influence of the variables on HER2-positivity was per the original NIU model (Fig. 2b).

3.4. Validation of the NIU model

The NIU model was validated using the independent EPI data (Supplementary material S6; Table 2). The best fit of the NIU model was to the NIU data, as expected. When all five covariates of the NIU model were refitted to the EPI data, the ROC AUC and the Youden Index for this model were close to those for the NIU data (Table 2 [Row 4]). In addition, when the adjusted NIU model was used to predict HER2-positivity in the EPI data without any refitting based on variable coefficients and cut-off values determined with the NIU data (Table 2 [Row 6]), the resulting Youden Index was still reasonably close to that of the adjusted NIU model fitted to the NIU data. Therefore, the original NIU model was considered successfully validated using EPI data.

3.5. Novel variables for further improvement of the model

Where percentage of progesterone receptor (PgR%; n = 364) and percentage of oestrogen receptor (ER%; n = 361) were missing, PgR% and ER% values were imputed (Supplementary material S7). PgR% and ER% fitted to HER2 status showed that both variables statistically significantly influenced HER2 status ($P < 0.0001$) and supported inclusion of PgR% and ER%, rather than HR status, as continuous variables in the model. Method of sample extraction (operative resection, non-operative biopsy or unknown) was also identified for inclusion in the model, with bivariate analysis demonstrating a statistically significant influence on HER2-positivity ($P < 0.0001$). HER2-positivity was detected in 10.8% of samples extracted by operative resection and 14.7% of samples by non-operative biopsy.

Table 1
Distribution of relevant variables for the EPI HER2 BC and NIU HER2 study data.

	EPI HER2 BC study N = 14,729	NIU HER2 study N = 15,281
HER2 status, n (%)		
Positive	1984 (13.5)	2176 (14.2)
IHC3+	1511 (76.2)	1740 (80.0)
IHC2+/ISH+	399 (20.1)[a]	321 (14.7)
ISH+	33 (1.7)	115 (5.3)
Negative	12,745 (86.5)	13,105 (85.8)
Median age, years (range)	64.0 (20.0–100.0)	64.0 (20.0–104.0)
Sample source, n (%)		
Primary tumour	13,029 (88.5)	13,590 (88.9)
Local recurrence	512 (3.5)	728 (4.8)
Metastasis	805 (5.5)	805 (5.3)
Unknown	383 (2.6)	158 (1.0)
HR status, n (%)[b]		
ER-negative/PgR-negative	2172 (14.7)	2241 (14.7)
ER-negative/PgR-positive	203 (1.4)	350 (2.3)
ER-positive/PgR-negative	1729 (11.7)	1816 (11.9)
ER-positive/PgR-positive	10,170 (69.0)	10,696 (70.0)
Unknown	455 (3.1)	178 (1.2)
Histological grade, n (%)		
Grade 1	2184 (14.8)	2302 (15.1)
Grade 2	7752 (52.6)	8049 (52.7)
Grade 3	3927 (26.7)	3739 (24.5)
Unknown	866 (5.9)	1191 (7.8)
Nodal status, n (%)		
(y)pN0	4412 (30.0)	3419 (22.4)
(y)pN1	1625 (11.0)	1256 (8.2)
(y)pN2	442 (3.0)	375 (2.5)
(y)pN3	280 (1.9)	251 (1.6)
Unknown	7970 (54.1)	9980 (65.3)
Histological subtype, n (%)[c]		
Lobular	2073 (14.1)	2173 (14.2)
Ductal or other	12,656 (85.9)	13,108 (85.8)
Method of sample extraction, n (%)		
Non-operative biopsy	9808 (66.6)	10,601 (69.4)
Operative resection	4811 (32.7)	4468 (29.2)
Unknown	110 (0.7)	212 (1.4)

BC, breast cancer; ER, oestrogen receptor; HER2, human epidermal growth factor receptor; HR, hormone receptor; IHC, immunohistochemistry; ISH, *in situ* hybridisation; PgR, progesterone receptor.

[a] An additional 41 samples were classified by the participating centres as HER2-positive with an IHC2+ status but with a missing confirmatory ISH result.

[b] A sample was defined as ER- and/or PgR-positive if the ER and/or PgR status was ≥1%.

[c] Histological subtypes from the NIU HER2 study were originally categorised as ductal, lobular, other and unknown [15]. To improve comparability between the NIU and EPI studies, and to validate the NIU model using EPI data, the original NIU-defined levels of histological subtype were adapted to those of the EPI study.

Table 2
ROC AUC, sensitivity and specificity for all relevant models fitted.

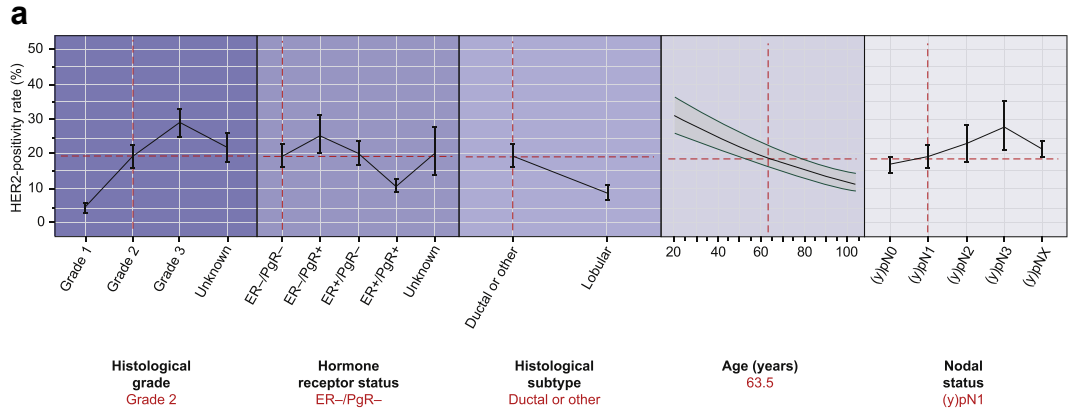
Model(s)/data	ROC AUC	Sensitivity	Specificity	Youden Index[a]
NIU model as published [15]	0.7355	0.7209	0.6654	0.3863
NIU model adapted: NIU data	0.7366	0.7298	0.6590	0.3888
NIU models: EPI data				
All five covariates refitted	**0.7244**	**0.7112**	**0.6520**	**0.3632**
One-parameter logistic regression refitted	0.7206	0.6855	0.6750	0.3605
NIU model for prediction of EPI data	0.7206	0.6996	0.6556	0.3552
EPI models: EPI data[b]				
Improved EPI model	0.7402	0.7621	0.6314	0.3935
Cross-validation model	0.7377	0.7933	0.5056	0.3890

ER%, percentage of oestrogen receptor; HR, hormone receptor; PgR%, percentage of progesterone receptor; ROC AUC, area under the receiver operating characteristic curve.
[a] Youden Index = sensitivity + specificity − 1.
[b] HR status was included and was highly statistically significant in all models listed in this table except the EPI models for the EPI data (the last two models), where PgR% and ER% were included as new related variables. When PgR% and ER% were included, HR status no longer provided any additional significant contribution and, therefore, it was removed from the model. The replacement of HR status with PgR% and ER% resulted in the improved model in the EPI study compared to NIU, where PgR% and ER% data were not available. As such, PgR% and ER% was deemed more informative to clinicians then the categorical HR status.

3.6. The improved EPI model

An EPI model was developed with PgR% and ER% as substitutes for HR status, and method of extraction as an additional variable. The predictive strength of the EPI model was improved compared with the NIU model; the Youden Index increased (Table 2 [Row 6 versus Row 2]) and the corrected Akaike information criterion favourably decreased (11,268.3 [adapted NIU model] versus 10,432.1 [improved EPI model]). Prediction profiles of each variable for the probability of HER2-positivity are shown in Fig. 3a.

The variable with the highest influence on HER2-positivity in the improved EPI model was PgR% followed by histological grade, histological subtype, nodal status, ER%, age and method of sample extraction (Fig. 3b). The ROC AUC and Youden Index of the ten-fold cross-validation model were only slightly lower than the corresponding parameters for the overall EPI model (Table 2 [Row 8 versus Row 7]) and were slightly better when compared with the originally published NIU model and the NIU model fitted to the EPI data. Therefore, the EPI model was considered validated by ten-fold cross-validation.



b

Column	Main effect	Total effect[b]	*P* value
Histological grade	0.407	0.480	<0.0001
Hormone receptor status	0.278	0.334	<0.0001
Histological subtype	0.111	0.153	<0.0001
Age (years)	0.063	0.090	<0.0001
Nodal status	0.028	0.043	<0.0001

Fig. 2. (a) Prediction profiles for probability of HER2-postivity[a] and (b) importance of variables on HER2-positivity based on the adapted NIU HER2 study model. +, positive; −, negative; ER, oestrogen receptor; HER2, human epidermal growth factor receptor; PgR, progesterone receptor; (y)pNX, unknown nodal status. [a] The order of the panels in part a corresponds with the order of influence of each parameter on HER2-positivity shown in part b. Each panel indicates the dependence of the model-estimated probability of positivity from the levels of the corresponding parameter adjusted for the other parameters at the vertical dotted red lines. Across all panels, the horizontal dotted red line indicates the predicted probability of HER2-positivity of 19.1% for the combination of all levels of covariates as indicated by the vertical dotted red lines in their corresponding panels. [b] Total effect, which denotes the importance of a variable, includes the main effect plus its combination effect with other variables. Adjacent bars provide a visualisation of this number; the distance between each bar = 0.2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.7. Investigation of centre effects: Descriptive analysis

Centre-specific analysis showed large variability between reported percentages of HER2-positive samples (range 0–50.0%) (Fig. 4). Differences in HER2-positivity between centres and deviations from the overall observed HER2-positivity in EPI could be partly explained by high inter-centre variability of influential variables, e.g., histological grade (Fig. S2). The mean predicted probabilities of positivity were outside the 99% CIs of the reported positivity rate for six centres, with three centres below and three above the 99% CIs.

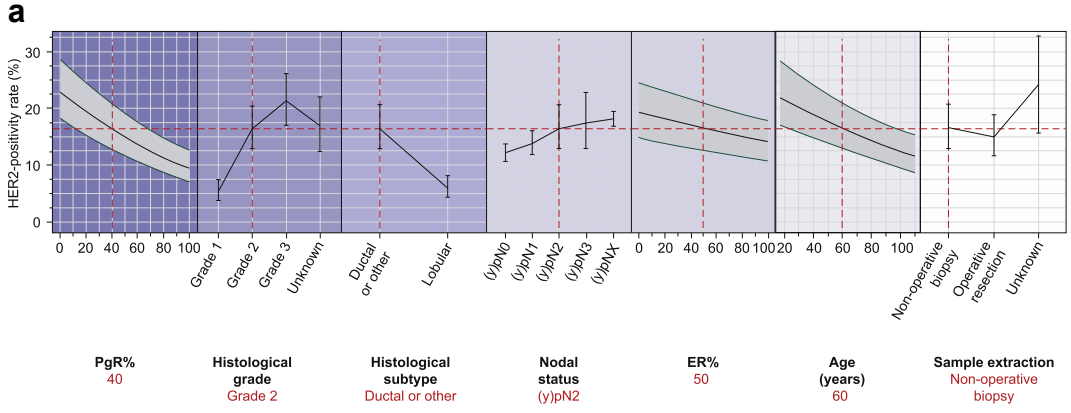
Inter-centre effects could thus not be fully explained by the tumour- and patient-specific characteristics included in the model, suggesting that there may be additional reasons for these increased centre effects, including, but not limited to, quality issues. Details of the investigation of centre effects using the modelling approach can be found in Supplementary material S4.

3.8. Laboratory-specific characteristics

The influence of laboratory-specific characteristics on HER2-positivity was evaluated using models extended by laboratory parameters. Across all centres, the most influential laboratory-specific characteristics were the certification status of the centre, the *in situ* hybridisation cut-off value and the manufacturer of the detection reagents used for immunohistochemistry ($P < 0.05$) (Table S1; Supplementary material S8). Additional laboratory-specific parameters were explored in the extended model but were not considered to be relevant (Supplementary material S9). Due to the observational nature of EPI and the small number of deviating centres, it could not be determined whether the associations between laboratory parameters and HER2-positivity rates were random effects or if they truly contributed to the deviations of the six centres.

4. Discussion

The EPI study aimed to compare the NIU and EPI data and to validate the previous findings from the NIU model [15]. Our results demonstrate that the NIU model could be successfully validated using the EPI data and that the overall strength of the model could be improved by replacing HR status with PgR% and ER% and by adding method of sample extraction as a variable. For the purpose of supporting HER2-testing quality control and considering the retrospective observational nature of the study, the results for the improved EPI model are very promising. We also considered (y)pT as a candidate for inclusion in the model (Supplementary material S10; Fig. S3). While it was not included, (y)pT should still be assessed for individual patients in clinical practice. Significant differences between recorded and model-predicted HER2-positivity rates at some centres, which could not be explained by tumour- or patient-specific variables, indicate potential HER2-testing quality issues and should trigger an assessment of the local centre's testing routine.



b

Column	Main effect	Total effect[b]	*P* value
PgR%	0.313	0.370	<0.0001
Histological grade	0.259	0.318	<0.0001
Histological subtype	0.163	0.199	<0.0001
Nodal status	0.074	0.103	<0.0001
ER%	0.031	0.048	<0.0001
Age (years)	0.024	0.041	<0.0001
Method of sample extraction	0.007	0.011	0.0331

Fig. 3. (a) Prediction profiles for probability of HER2-postivity[a] and (b) importance of variables on HER2-positivity based on the improved EPI HER2 BC study model. BC, breast cancer; ER%, percentage of oestrogen receptor; HER2, human epidermal growth factor receptor; PgR%, percentage of progesterone receptor; (y)pNX, unknown nodal status. [a] The order of the panels in part a corresponds with the order of influence of each parameter on HER2-positivity shown in part b. Each compartment of the plot indicates the dependence of the model estimated probability of positivity from the levels of the corresponding parameter adjusted for the other parameters at the vertical dotted red lines. Across all compartments, the horizontal dotted red line indicates the predicted probability of HER2-positivity of 16.3% for the combination of all levels of covariates as indicated by the vertical dotted red lines in their corresponding compartments. [b] Total effect, which denotes the importance of a variable, includes the main effect plus its combination effect with other variables. Adjacent bars provide a visualisation of this number; the distance between each bar = 0.2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

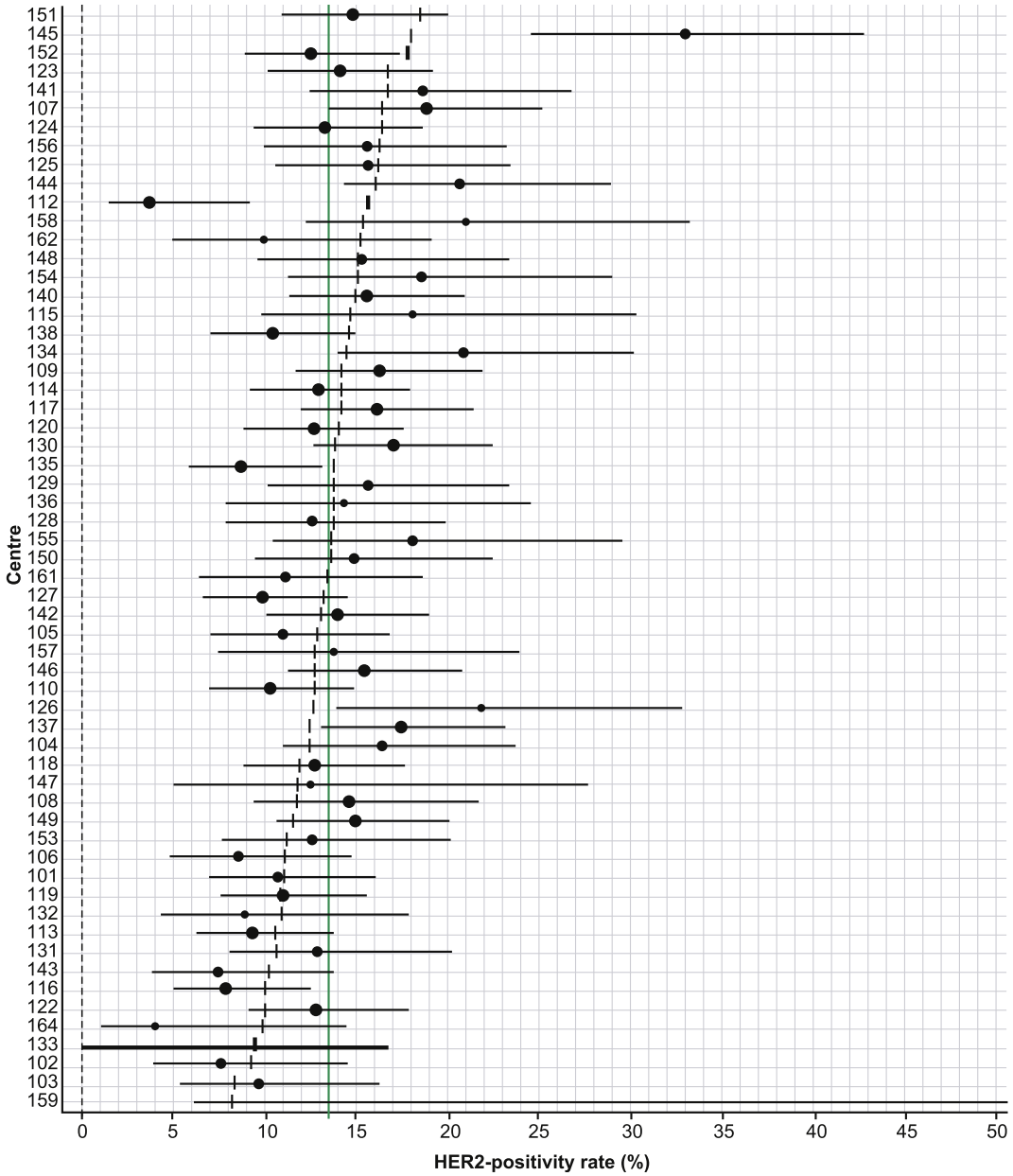


Fig. 4. HER2-positivity rates by centre.[a] CI, confidence interval; HER2, human epidermal growth factor receptor 2. [a] HER2-positivity rates reported by each centre (dots) with 99% CIs and model-predicted probability of HER2-positivity (vertical lines). The size of the dots is proportional to the number of samples provided by each centre. The bold black line indicates the centre (133) with a reported HER2-positivity rate of 0%. Centre 159 (bottom row) provided only two samples, one of which was HER2-positive and the other HER2-negative, resulting in a documented HER2-positivity rate of 50%; the small dot is barely visible.

Validation is a prerequisite for use of the model in routine clinical practice. Several steps are required for routine use of the recommended model for quality control. The first is determination of HER2-testing status and the results of all seven covariates from the proposed model for a group of consecutively collected samples. In the event of missing covariates, imputation of values may be used; however, for the two most influential variables (PgR% and histological grade) no data should be missing, if possible. Second, the predicted probabilities of HER2-positivity for individual samples and centres from the test set should be calculated using the model; documented HER2-positivity rates including 95% CIs should also be calculated. Results from these two steps would allow integration of the data under investigation into Fig. 4, allowing for discussion of deviations as performed in this analysis. The calculation of the model-predicted HER2 probabilities requires adequate implementation of a comprehensive prediction formula (Fig. S4).

The testing approach presented so far uses documented rates and predicted probabilities by centre only and a more detailed approach based on individual samples is under investigation. In the first instance, typical statistical analysis is required to perform a model-based quality control, with a goal of automation of the analysis and making the process available online. Our data support the consideration of patient- and tumour-specific characteristics when assessing HER2-testing quality using HER2-positivity incidence in a specific pathology laboratory.

From a surgical pathologist's point of view, EPI provides important information by highlighting the parameters that are

closely linked to HER2-positivity. For daily practice, the plausibility of these parameters is crucial. For example, for a patient with immunohistochemically HER2-positive, grade 1 BC, HER2 status should be confirmed using *in situ* hybridisation and grade should be rechecked. Further to this, participation in round-robin tests and monitoring of HER2-positivity rates are currently recommended quality assurance measures.

In addition to the parameters investigated here, other factors that may influence HER2-positivity rates, such as mode of detection of BC, should be investigated. Patients with screening-detected BC may have improved outcomes that cannot be fully explained by stage shift at diagnosis (lead-time bias) and favourable prognostic factors (length bias) [17]. Stratification of tumours into different size or nodal status categories to reduce the magnitude of lead-time bias persistently showed better 10-year distant disease-free and overall survival for screening-detected BC [17]. The distribution of St Gallen molecular subtypes in primary tumours may also differ significantly according to the mode of detection, with a shift to more luminal A-like tumours and a decrease in luminal B-like HER2-positive tumours in screening-detected patients [18]. As the rate of HER2-positivity may be lower in screening-detected BCs, detection mode and rate of screening-detected BC should be included in the analysis of HER2-positivity rates for use as a quality indicator in future studies.

Together with the previous NIU data, EPI has allowed the proposed model to be established and validated. Further improvements to the model could potentially be made if more complete data sets for PgR% and ER% were available in future studies. However, this may not be possible in retrospective analyses of real-world data like the study reported here.

5. Conclusion

Reliable, high-quality HER2-testing methods are essential for selection of patients with HER2-positive BC for HER2-tageted treatment. Integration of our model into a locally used software or website may improve its availability for use in clinical practice.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2019.12.005.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the principal investigator. The study protocol was also available for submission to the local committees of the participating centres. As all data were obtained through routine pathological testing, and all patient information and test results remained anonymous, no written informed consent was required from patients for this study.

Data-sharing statement

Qualified researchers may request access to individual patient-level data through the clinical study data request platform: www.clinicalstudydatarequest.com. Further details on Roche's criteria for eligible studies are available here: https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Roche.aspx. For further detail on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here: https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm.

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