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Research Article

HER2-Low Breast Cancer: Incidence, Clinicopathologic Features, and Survival Outcomes From Real-World Data of a Large Nationwide Cohort

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ABSTRACT

Patients with breast cancer (BC) with low levels of human epidermal growth factor receptor 2 (HER2) expression (HER2-low) could benefit from novel antibody–drug conjugates. However, there is conflicting information regarding the characteristics of HER2-low BC and its outcome. We assessed the clinicopathologic characteristics and outcomes of HER2-low BC using real-world data from the Dutch National Pathology Registry. This retrospective study incorporated all patients with primary invasive BC, without neoadjuvant therapy, reported in the Dutch National Pathology Registry synoptic reporting module between 2014 and 2022. HER2 status was categorized as HER2-0 (defined as an immunohistochemistry score of 0 according to the current American Society of Clinical Oncology/College of American Pathologists guidelines) or HER2-low (immunohistochemistry score 1+ or 2+ without amplification). Clinicopathologic characteristics and overall survival of HER2-low BC were compared with HER2-0, adjusted for estrogen receptor (ER) status. We included 65,035 patients with BC, resulting in 69,424 tumors. The proportion of HER2-low BC was 62% in the ER+ cohort and 38% in the ER− cohort. A substantial number of patients had a different HER2 category between the needle biopsy and the corresponding surgical resection (28%) or among multiple tumors (28%). After multivariable logistic analysis, HER2-low tumors were significantly associated with histologic subtype, a higher ER, and lower progesterone receptor expression in the ER+ cohort, whereas within the ER−cohort, HER2-low tumors were associated with a lower tumor grade. However, the absolute differences were limited, and there was no significant difference in overall survival between HER2-low and HER2-0 tumors within the ER+ or ER− cohort. The classification of HER2 expression (HER2-0 vs HER2-low) varies between biopsies and corresponding resection specimens and within multiple tumors in the same patient, which could affect clinical decision making in case only HER2-low cases are eligible for novel HER2-targeting agents. The limited follow-up time and the lack of substantial clinicopathologic differences between HER2-low and HER2-0-cases could explain the lack of differences in overall survival.

Introduction

The human epidermal growth factor receptor 2 (HER2) is a well-known negative prognostic biomarker in breast cancer (BC). For >2 decades, it has been used to select patients who could benefit from HER2-targeted therapy.[1,2] Currently, the latest version of the American Society of Clinical Oncology/College of American Pathologists guidelines recommends HER2 testing by immunohistochemistry (IHC) assay or in situ hybridization.[3] Tumors with an IHC score of 3+ or 2+ and the presence of gene amplification in a reflex test are considered HER2 positive,

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whereas tumors with an IHC score of 0, 1+, or 2+ without amplification are considered HER2 negative.[3]

For a couple of years now, this dichotomous division in either HER2 negative or HER2 positive has been evolving because of the development of novel HER2-targeting agents, including antibody–drug conjugates (ADCs). These drugs offer therapeutic opportunities not only for patients with HER2-amplified BC but also for tumors with a lower expression level of HER2 (HER2-low) on the cell membrane, ie, tumors with an IHC score of 1+ or 2+ with negative in situ hybridization.[4-6] The mechanisms of action of trastuzumab–deruxtecan (T-DXd) differ from other ADCs (eg, trastuzumab–emtansine), because there is delivery of a topoisomerase I inhibitor linked to a humanized anti-HER2 antibody by a unique cleavable peptide-based linker.[4] After cleavage, the released cytotoxic deruxtecan molecule, which is cell membrane permeable, can diffuse into antigen-negative surrounding cells, causing the so-called "bystander effect."[7] Currently, several ongoing clinical trials are testing the effectivity of T-DXd in HER2-positive and HER2-low BC. The DESTINY-Breast04 trial compared T-DXd with the physician's choice of chemotherapy and showed substantial improvement in progression-free and overall survival in patients with unresectable or metastatic HER2-low BC.[8]

This pharmacologic development led to many studies, which attempted to describe the clinicopathologic and molecular characteristics of HER2-low BC using retrospective cohorts. Approximately 15% of BC cases are HER2-positive, whereas 55% to 60% of all BCs are considered HER2-low, of which approximately 80% are luminal-like tumors and 15% to 20% are triple-negative (TNBC).[9-14] Schettini et al,[9] who evaluated 3600 patients affected by BC, reported that the most (67.6%) of the HER2-low tumors have an IHC score of 1+.[9] Zhang et al[15] reported that 34.5% of HER2-low cases had a high risk of disease recurrence by the MammaPrint assay—28.8% had a luminal B molecular subtype and 4.6% of cases had a basal molecular subtype.[15] Regarding the prognostic importance of HER2-low expression, data remain inconsistent. Several studies have reported a longer overall survival in patients with HER2-low BC compared with those affected by BC with an IHC score of 0,[10,13,16,17] whereas others have not found any statistical association between these 2 groups.[9,18-21] A few studies have reported an association between HER2-low expression and a worse outcome.[12,22] These contradictory data illustrate the imperative need for a large-scale population-based study because unicentric cohorts might be prone to bias.

With the introduction of T-DXd and its potential benefits for patients with HER2-low BC, as well as multiple other ADCs under investigation, it is important to thoroughly study the characteristics of HER2-low tumors using real-world data from a nationwide cohort of patients with BC.[23] In this study, we aimed to provide data regarding incidence, clinicopathologic characteristics, and outcome of HER2-low BC by assessing a unique National Pathology Registry, resulting in the largest cohort size on this topic that has ever been published, to our knowledge.

Materials and Methods

Data Acquisition

A retrospective analysis was performed, including all primary invasive BC resection specimens reported via synoptic reporting in the Dutch Pathology Registry (PALGA) between January 1, 2014 and December 31, 2021. The advantage of this synoptic reporting module is that parameters are captured in uniform numeric variables instead of free text fields, which offers the unique opportunity to study these reports simultaneously.[24] We expanded our search by including the history and follow-up data of ipsilateral breast and axilla from a period of ≤3 months before and after resection to obtain additional information from the preoperative needle biopsy and nodal status because not all patients underwent axillary lymph node dissection at the time of their breast surgery.

Patient and Tumor Characteristics

All patients were aged >18 years and did not receive neoadjuvant treatment for BC. We collected clinical characteristics, including sex, age at diagnosis, type of surgery, and overall survival, defined as the interval between needle biopsy diagnosis and death. Histopathologic features of the resection specimen included histologic subtype (according to the World Health Organization classification of breast tumors),[25] diameter (ie, tumor size), tumor grade, angioinvasion, and resection margin status. According to the Dutch guidelines for Breast Cancer Treatment, resection margins are defined as free (no ink on tumor), focally positive (ink on tumor ≤4 mm), or more than focally positive (ink on tumor >4 mm).[26] The presence of macro- or micrometastasis in sentinel node or an axillary nodal dissection was used to determine the nodal status (positive or negative).

Furthermore, we collected detailed information regarding the expression of estrogen receptor (ER), progesterone receptor (PR), and HER2. Hormone receptor (HR) status was defined as positive if ≥10% of the cancer cells showed nuclear staining, independent of intensity, according to the latest "Dutch Clinical Guideline of Breast Cancer."[26] According to the synoptic reporting module of PALGA, the pathologists are only obligated to report the percentage of ER and PR expressions in HR-positive cases, resulting in missing data on the exact percentage of ER and PR expressions in HR-negative (<10%) cases. In this synoptic reporting module, detailed information about the method and results of HER2 testing is mandatory. This comprises the type of tissue on which the test was performed (needle biopsy or resection specimen), the result of IHC (0, 1+, 2+, and 3+), and the type of reflex test (chromogenic in situ hybridization, silver in situ hybridization, fluorescence in situ hybridization, or PCR). Cases analyzed with multiplex ligation–dependent probe amplification were not included in this report because this variable was not included in the synoptic reporting module. HER2 status was scored according to international guidelines,[3,27,28] although different local protocols were used regarding the type of reflex testing, as depicted in Supplementary Figure S1.

For a subset of patients, the Ki-67 expression level, the density of stromal tumor-infiltrating lymphocytes (sTILs), and the results of the MammaPrint were also available.

Statistical Analysis

Descriptive statistics were performed for frequencies of categorical values. We calculated absolute and relative values for categorical variables. Median and range (25th and 75th percentiles) were calculated for nonnormally distributed continuous variables.

To assess the differences between nonamplified tumors, we compared baseline categorical characteristics of HER2-0 vs HER2-low cases with χ^2 tests. Combined IHC data from the biopsy and

the resection specimen were used to determine the HER2 category. If these variables differed, data from the resection specimen were used to correct for potential intratumor heterogeneity. For nonnormally distributed continuous variables, we performed the Mann–Whitney *U* test. Sankey diagrams, made in SankeyMATIC.com, were used to depict the distribution of HER2 status between multiple tumors in the same patient and the differences in HER2 expression between biopsy and resection. We performed a multivariable logistic regression model, including those variables that were statistically significant ($P < .005$) in univariate analysis and had complete data in each of the other variables. The overall survival distributions were estimated using the Kaplan–Meier test. The log-rank test was additionally applied to assess the difference in survival distribution between groups. We reported odds ratios (ORs) with 95% CIs for the variables included in the multivariable analysis. A *P* value of <.05 was considered statistically significant. Statistical analyses were done in SPSS (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0, IBM Corp).

Results

General Patient and Tumor Characteristics

A total of 65,035 patients diagnosed with invasive BC between January 2014 and March 2022 were retrieved, which resulted in a total number of 69,424 tumors. Approximately 99.2% ($n = 64,526$) of patients were women, and 0.8% ($n = 509$) were men. Most patients (67.2%, $n = 43,735$) underwent breast-conserving surgery, whereas 32.8% ($n = 21,299$) had a mastectomy. Most patients (89%, $n = 65,495$) had ER-positive BC, whereas only 8% of patients ($n = 5516$) had HER2-positive BC, and 4% had TNBC ($n = 2849$).

Tissue Used for ER and HER2 Evaluation: Biopsy vs Resection

The ER status was determined on the biopsy only (44%, $n = 33,723$), the resection specimen only (52%, $n = 40,191$), or both (4%, $n = 3245$). HER2 status was determined on the biopsy (38%, $n = 26,791$), the resection specimen (62% $n = 43,908$), or both (7%, $n = 4620$). Overall, 20,506 reflex tests were performed, which corresponded to 29% of the total number of specimens tested for HER2. The most utilized reflex test was fluorescence in situ hybridization (52%, $n = 10,659$), followed by chromogenic in situ hybridization/silver in situ hybridization (39%, $n = 8066$).

The tissue used for receptor status evaluation (biopsy vs resection) changed over the study period. In the first years (2014-2016), most tests were performed on the resection specimen, whereas in more recent years, tests were performed mainly on the tissue from core needle biopsies.

HER2 Classification: Biopsy vs Resection

Based on the biopsy, 35% ($n = 9360$) of tumors were scored as HER2 IHC 0, 42% ($n = 11,250$) as 1+, 18% ($n = 4762$) as 2+, and 5% ($n = 1419$) as 3+. In the resection specimen, 38% of tumors ($n = 16,755$) were scored as IHC 0, 39% ($n = 16,905$) as 1+, 16% ($n = 7141$) as 2+, and 7% ($n = 3107$) as 3+. This resulted in a HER2-low proportion of 57% ($n = 15,317$) in the biopsy and 52% ($n = 22,623$) in the resection specimen.

In total, 4620 patients had a HER2 IHC score performed on both the biopsy and the resection. In a substantial proportion of these cases (39%, $n = 1788$), the IHC score changed from the biopsy to the resection, as shown in Supplementary Figure S2. The most common changes were from IHC 1+ to 0 ($n = 516/1788$), IHC 0 to 1+ ($n = 492/1788$), and IHC 2+ to 1+ ($n = 298/1788$). Furthermore, we reclassified these cases as HER2-0, low, and positive, as illustrated in Figure 1. Overall, from the 4323 analyzed cases, 1218 tumors (28%) had a different HER2 score from the biopsy to the resection. The most common change happened between HER2-0 in the biopsy to HER2-low in the resection ($n = 576/1218$) and HER2-low in the biopsy to HER2-0 in the resection ($n = 572/1218$).

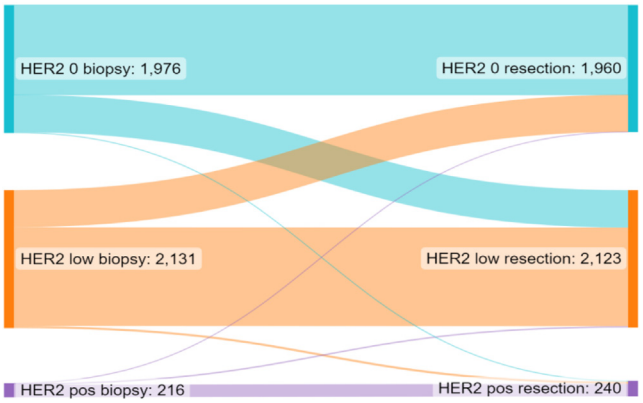

Figure 1.
Comparison between the level of HER2 expression (classified as HER2-0, HER2-low, or HER2-positive) in the needle biopsy and the corresponding resection specimen.

HER2 Expression in Patients With Multiple Tumors

In total, 4170 patients had >1 tumor reported in the resection specimen. Most of these 4170 patients (79%, $n = 3311$) had 2 tumors, whereas 21% ($n = 859$) of patients had ≥3 tumors.

When we analyzed the difference in HER2 IHC in patients with 2 tumors, we found that 37% ($n = 1529$) of the patients had a different IHC score in the second tumor compared with the first tumor. Approximately 49% ($n = 755/1529$) of these cases had 1 tumor classified as IHC 0 and another classified as IHC 1+, and 18% ($n = 270/1529$) had 1 tumor classified as IHC 0 and another as IHC 2+ or 3+ (Supplementary Figure S3).

Figure 2 presents the patients with 2 tumors reclassified as HER2-0, low, and positive. Most cases (72%, $n = 2907$) had the same HER2 classification in both tumors, whereas 28% ($n = 1132$) had a different HER2 classification. Of the patients who had a different HER2 classification between tumors, approximately 80% ($n = 916/1132$) of these cases had 1 tumor classified as HER-0 and another classified as HER2-low.

HER2 Expression in Male Patients

We retrieved data from 509 male patients, resulting in 516 tumors. Most of these patients (93.5%, $n = 476$) were aged ≥50 years, and the vast majority (94.3%, $n = 480$) had undergone a mastectomy Almost all tumors (98.4%, $n = 501$) were ER positive. Furthermore, 42% ($n = 155$) were HER2-0, 53% ($n = 272$) were HER2-low, and 5% ($n = 25$) were HER2 positive. From all the clinicopathologic features analyzed (data not shown), only the presence of angioinvasion was significantly higher in HER2-low BC compared with HER2-0 cases (16% vs 28%, $P = .008$).

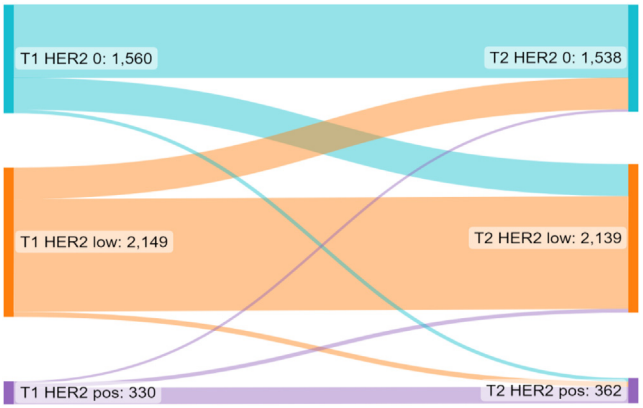

Figure 2.
Differences in the levels of HER2 expression between multiple tumors in the resection specimen (HER2-0, HER2-low, or HER2-positive). BC, breast cancer; ER, estrogen receptor.

Clinicopathologic Characteristics: HER2-0 vs HER2-Low

Table 1 presents the clinicopathologic characteristics of HER2-low and HER2-0 cases. In this analysis, we used the ER and HER2 scores from the resection specimens if available. If these data were missing on the resection specimen, we used the biopsy results. Overall, most absolute differences between HER-low and HER2-0 cases were limited. In brief, patients with HER2-low tumors underwent breast-conserving surgery slightly less often compared with patients having HER2-0 tumors ($P < .001$). In addition, the histologic subtype differed between both groups; HER2-low tumors were more often subtyped as no special type, whereas the proportion of lobular carcinomas was higher in the HER2-0 group ($P < .001$). HER2-low tumors were associated with a significantly higher expression of ER ($P < .001$) and PR ($P < .001$) compared with HER2-0 tumors. In addition, the proportion of high-grade tumors was somewhat lower in HER2-low cases than that in the HER2-0 group ($P < .001$). However, the proportion of cases with nodal metastases was slightly higher in HER2-low cases than that in HER2-0 cases ($P < .001$).

Clinicopathologic Characteristics: HER2-0 vs HER2-Low, Stratified by ER

As presented in Table 2, most of the absolute differences between HER2-low and HER2-0 cases within the ER+ and ER− cohorts were very limited. Within the ER+ cohort, HER2-low cases were more often completely removed after breast-conserving surgery compared with HER2-0 cases ($P < .001$) in univariate analyses, which could be related to the lower proportion of lobular carcinomas ($P < .001$). In addition, HER2-low tumors were associated with more angioinvasion ($P < .001$), a slightly higher risk of recurrence as determined by the MammaPrint ($P = .049$), more mitoses ($P < .001$), lower PR expression ($P < .001$), and higher positive nodal status ($P = 0.002$) compared than HER2-0 BC. The expression of ER was slightly higher in HER2-low cases ($P < .001$ in the categorical analyses). After multivariable analysis, lumpectomy being the only type of surgery (OR, 0.88; CI 95%, 0.83-0.92; $P < .001$), lobular histologic subtype or other (OR, 0.73; CI 95%, 0.69-0.78; $P < .001$), higher ER percentage (OR, 1.003; CI 95%, 1.001-1.005; $P = 0.01$), and lower PR expression (OR, 0.99; CI 95%, 0.998-0.999; $P < .001$) were associated with HER2-low within the ER+ cohort.

On the other hand, within the ER− cohort, patients with low levels of HER2 expression were older than that with HER2-0 tumors ($P < .001$) in univariate analyses, with a higher proportion of patients undergoing mastectomy ($P < .001$). In addition, in the HER2-low group, the proportion of tumors classified as no special type or lobular carcinoma was higher, whereas more cases were classified as other histologic subtypes in the HER2-0 group ($P < .001$). HER2-low tumors were less often grade 3 ($P < .001$) with a lower number of mitosis ($P < .001$) and a lower Ki67 expression ($P = .013$) than HER2-0 tumors. Similar to the ER+ cohort, these tumors were associated with positive nodal status ($P = .019$). After multivariable analysis, only age >50 years (OR, 0.76; CI 95%, 0.58-0.98; $P = .040$), lumpectomy (OR, 1.36; CI 95%, 1.12-1.65; $P = .001$) and tumor grade 3 (OR, 1.73; CI 95%, 1.38-2.18; $P < .001$) showed an association with HER2-low in the ER− cohort.

Survival Analysis: HER2-Low vs HER2-0 Cases, Stratified by ER

A total number of 53,833 patients (including 48,846 ER+ patients and 4987 ER− patients) had overall survival data and detailed HER2 status (0 and low). The median follow-up time of the total study cohort was 45.9 months. After adjusting for ER status, the median follow-up time was 46.6 months for the ER+ cohort and 45.6 for the ER− cohort. Patients with HER2-low had a slightly longer overall survival than patients with HER2-0 (93.7 vs 92.7 months, $P < .001$). However, when adjusted for ER status, there was no statistical overall survival difference between HER2-low and HER2-0 BC, neither in the ER+ cohort (94.4 vs 94.06 months, $P = .144$) (Fig, 3A) nor in the ER− cohort (83.6 vs 84.5 months, $P = .422$) (Fig. 3B).

Discussion

The DESTINY-Breast-04 study showed that treatment with the novel ADC T-DXd significantly improved progression-free and overall survival in patients with HER2-low locally advanced or metastatic BC.[4,8] Because data regarding the potential treatment benefit for HER2-0 tumors are currently lacking, only patients with HER2-low tumors are now eligible for these novel HER2-targeting drugs. For this reason, it is important to obtain more information about the incidence, clinicopathologic characteristics, and outcome of HER2-low tumors, not only from clinical trials but also from real-world data. Therefore, the purpose of this study was to assess whether HER2-low BC differed from HER2-0, adjusted by ER status.

In our cohort, general patient characteristics, such as sex, age, and type of surgery were similar to previous studies.[9,13,15,16,18] Regarding the receptor status, a relatively lower proportion of HER2-positive cases (8%) and TNBC (4%) was observed in this cohort compared with other studies,[10,15,20,29] which is likely explained by the exclusion of patients undergoing neoadjuvant chemotherapy in this study. We also included a group of male patients ($n = 509$). The proportion of HER2-low BC was similar to the female cohort (53% in men compared with 54% in women), whereas the proportion of HER2-0 was higher (42% in men compared with 38% in women). In line with previous literature, the proportion of HER2-positive cases is low in male BC compared with female BC.[30] A few studies have analyzed HER2-low expression in the male population. Schettini et al[9] found that within the male

Table 1
Comparison of clinicopathologic characteristics between patients with HER2-0 and HER2-low BC

Clinical characteristics of patients (*n*, %)	HER2-0 (*n* = 21,818)	HER2-low (*n* = 32,442)	*P* value
Sex			
Female	21,663 (99.3)	32,170 (99.2)	.098
Male	155 (0.7)	272 (0.8)	
Missing	0	0	
Age at diagnosis (y)			
<50	2917 (13)	4327 (13)	.914
≥50	18,901 (87)	28,115 (87)	
Missing	0	0	
Type of surgery			
Lumpectomy	15,209 (70)	21,913 (68)	<.001
Mastectomy	6609 (30)	10,528 (33)	
Missing	0	1	
Tumor characteristics (*n*, %)	HER2-0 (*n* = 23,482)	HER2-low (*n* = 34,865)	
Diameter invasive breast cancer (median, range)	1.4 (0.9-2.1)	1.4 (0.9-2.1)	.028
Missing	51	57	
Histologic type			
No special type	18,979 (81)	29,321 (84)	<.001
Lobular	4142 (18)	5276 (15)	
Other	354 (2)	263 (1)	
Missing	7	5	
Grade of invasive component			
1	7241 (31)	11,467 (33)	<.001
2	11,353 (49)	17,468 (51)	
3	4448 (19)	5478 (16)	
Missing	440	452	
Angioinvasion			
Yes	2169 (10)	3510 (11)	.012
Uncertain	602 (3)	868 (3)	
No	18,787 (87)	27,983 (87)	
Missing	1924	2504	
ER status			
Positive	20,633 (88)	32,822 (94)	<.001
Negative	2849 (12)	2043 (6)	
Missing	0	0	
PR status			
Positive	17,237 (73)	27,466 (79)	<.001
Negative	6233 (27)	7386 (21)	
Missing	12	13	
MammaPrint			
Low risk	490 (65)	936 (61)	.121
High risk	270 (36)	595 (39)	
Missing	22,722	33,334	
Mitosis (median, range)	3 (1-9)	3 (2-8)	.067
Missing	433	448	
Ki67 (median, range)	10 (5-20)	10 (5-20)	<.001
Missing	21,218	32,448	
sTILS (median, range)	2 (1-5)	2 (1-5)	.6
Missing	23,313	34,660	
Resection margins invasive component (*n*, %)[a]			
Free	10,140 (90)	14,789 (91)	<.001
More than focally not free	388 (3)	489 (3)	
Focally not free	769 (7)	939 (6)	
Missing	12,185	18,648	
Axillary nodal status			
Positive	4505 (26)	7000 (28)	<.001
Negative	12,618 (74)	18,088 (72)	
Missing	6359	9777	

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; sTILs, stromal tumor-infiltrating lymphocytes.
[a] The analysis of resection margins was restricted to patiensts who had undergone lumpectomy.

cohort, HER2-low tumors were more frequent in older patients and showed more axillary lymphnode involvement than those with HER2-0. Although we did not observe a difference in nodal involvement between HER2-0 and HER2-low BC cases in our male population, we reported more angioinvasion in HER2-low tumors than that in HER2-0 tumors.

We demonstrated that a high proportion of biopsies that were scored as HER2-0 changed to HER2-low in the resection specimen

Table 2
Comparison of clinicopathologic characteristics between patients with HER2-0 and HER2-low BC, according to ER status

Clinical characteristics of patients (*n*, %)	ER+ (*n* = 48,846)				ER− (*n* = 4,987)			
Sex	HER2 0 (*n* = 18,800)	HER2 low (*n* = 30,046)	*P* value univariate	*P* value multivariate	HER2-0 (*n* = 3097)	HER2-low (*n* = 1890)	*P* value univariate	*P* value multivariate
Female	18,648 (99)	29,774 (99)	.262		3096 (100)	1890 (100)	.435	
Male	152 (1)	272 (1)			1 (0)	0 (0)		
Missing	0	0			0	0		
Age at diagnosis (y)								
<50	2432 (13)	4062 (14)	.065		507 (16)	237 (13)	<.001	.04
≥50	16,368 (87)	25,984 (86)			2590 (84)	1653 (88)		
Missing	0	0			0	0		
Type of surgery								
Lumpectomy	13,327 (71)	20,510 (68)	<.001	<.001	1854 (60)	1045 (55)	.001	.001
Mastectomy	5473 (29)	9535 (32)			1243 (40)	845 (45)		
Missing	0	1			0	0		
Tumor characteristics (*n*, %)	(*n* = 20,633)	(*n* = 32,789)			(*n* = 2,849)	(*n* = 2,005)		
Diameter (median, range)	1.4 (0.9-2)	1.4 (0.9-2.1)	<.001	.655	1.7 (1.1-2.7)	1.8 (1.2-2.6)	.8	.691
Missing	48	55			3	2		
Histologic type								
No special type	16,479 (80)	27,505 (84)	<.001	<.001	2500 (88)	1760 (88)	<.001	.544
Lobular	4073 (20)	5170 (16)			69 (2)	99 (5)		
Other	74 (0.4)	109 (0.3)			280 (10)	146 (7)		
Missing	7	5			0	0		
Grade of invasive component								
1	7152 (35)	11,388 (35)	<.001	.083	89 (3)	64 (3)	<.001	<.001
2	10,794 (53)	16,857 (52)			559 (20)	584 (30)		
3	2339 (12)	4129 (13)			2109 (77)	1321 (67)		
Missing	348	415			92	36		
Angioinvasion								
Yes	1733 (9)	3175 (10)	<.001	.089	436 (17)	330 (18)	.452	.879
Uncertain	484 (3)	794 (3)			118 (5)	74 (4)		
No	16,713 (88)	26,464 (87)			2074 (79)	1454 (78)		
Missing	1703	2356			221	147		
MammaPrint								
Low risk	482 (66)	920 (61)	.049		4 (31)	0 (0)	.101	
High risk	252 (34)	579 (39)			9 (69)	7 (100)		
Missing	19,899	31,290			2836	1998		
Mitosis (median, range)	3 (1-7)	3 (1-8)	<.001	.646	18 (10-30)	15 (7-27)	<.001	.073
Missing	344	411			89	36		
Ki67 (median, range)	10 (5-18)	10 (5-20)	.138		50 (25-75)	40 (17-65)	.013	
Missing	16,756	27,752			2629	1886		
sTILS (median, range)	2 (1-5)	2 (1-5)	.237		5 (1-22)	1 (1-5)	.179	
Missing	20,485	32,593			2828	1996		
ER percentage (median, range)	100 (95-100)	100 (98-100)	.262	.01	0	0	<.001	.082
Missing	47	54			746	473		

Levels of expression of ER								
0-9	0 (0)	0 (0)	<.001		2098 (99.8)	1526 (99.6)	.404	
≥10 to 49	401 (2)	467 (1)			5 (0.2)	6 (0.4)		
≥50 to 100	20,185 (98)	32,268 (99)			0 (0)	0 (0)		
Missing	47	54			746	473		
PR percentage (median, range)	90 (40-100)	80 (30-100)	<.001	<.001	0	0	.111	
Missing	434	621			584	371		
Levels of expression of PR								
0-9	3021 (15)	4722 (15)	<.001		2198 (97)	1582 (97)	.685	
≥10 to 49	3219 (16)	5856 (18)			53 (2)	44 (3)		
≥50 to 100	13,959 (69)	21,590 (67)			14 (1)	8 (1)		
Missing	434	621			584	371		
Resection margins invasive component[a]								
Free	9099 (89)	14,146 (91)	<.001		1041 (93)	601 (92)	.877	
More than focal not free	358 (4)	469 (3)			30 (3)	20 (3)		
Focal not free	719 (7)	907 (6)			50 (5)	30 (5)		
Missing	10,457	17,267			1728	1354		
Axillary nodal status								
Positive	3996 (26)	6588 (28)	.002	.066	509 (26)	403 (29)	.019	.5
Negative	11,151 (74)	17,068 (72)			1467 (74)	966 (71)		
Missing	5486	9133			873	636		

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; sTILs, stromal tumor-infiltrating lymphocytes.
[a] The analysis of resection margins was restricted to patients who had undergone lumpectomy.

or vice versa. A study from Miglietta et al[12] found similar disagreement in HER2-0 and HER2-low proportions between biopsies and surgical resections. These discordances could be because of tumor heterogeneity, but preanalytical and postanalytical factors may also play a critical role. Particularly, the higher proportion of HER2-low tumors in the biopsy compared with the resection (57% vs 52%) may be related to more rapid and thorough fixation.[31] Furthermore, several authors have highlighted the low-scoring accuracy of pathologists to differentiate between IHC 0 and IHC 1+, with approximately 70% of interobserver discordance between these cases.[9,32,33] We noticed that a substantial proportion of cases had a different HER2 status between multiple tumors, mainly a difference between HER2-0 and HER2-low. This could be explained by intertumor heterogeneity or intraobserver variability.[34] The different scores of HER2 expression in core biopsies, resection specimens, and multiple tumors could impact the optimal selection of patients if only patients with HER2-low tumors remain eligible for T-DXd treatment. As mentioned above, data regarding treatment effects in patients with HER2-0 tumors are limited. If future research also suggests relevant treatment responses in patients with HER2-0 tumors, our data could be placed in another perspective. On the other hand, HER2 testing on multiple tumor samples is likely to result in a HER2-low tumor if the initial test shows a HER2-0 tumor. The DESTINY-Breast-04 study did allow primary tumor sample results if the metastatic sample was unavailable, so a nonzero result of the primary tumor could be used to qualify for T-DXd.[35]

Additionally, we compared several clinicopathologic characteristics between HER2-0 and HER2-low tumors adjusted by ER status. Within the ER+ cohort, HER2-low BC was associated with several unfavorable pathologic features. However, their clinical relevance is questionable because the absolute differences were very small. Previously, several authors analyzed the differences between HER2-low and HER2-0 BC, adjusted to ER+. In contrast to our results, Horisawa et al[20] found an association between lower tumor grade and HER2-low BC. Furthermore, Tan et al[16] found an association between HER2-low tumors and higher presence of nodal metastases and higher ER expression. Interestingly, we found that the PR expression was lower in HER2-low tumors. This feature has been described in other studies, although no statistical significance was found.[15,17] On the other hand, within our ER− cohort, HER2-low tumors seem to be statistically associated with mainly favorable features compared with HER2-0 cases in univariate analysis, including lower grade, less mitosis, and lower Ki67 expression, excepting positive nodal status. Similar results have been presented in previous studies.[16,18,36] Furthermore, akin to our results, van den Ende et al[36] also described that HER2-low tumors had higher positive nodal status within the ER− cohort. Besides (although not statistically significant, possibly due to a limited number of cases with available data), the density of sTILS was lower in HER2-low cases within the ER− cohort. These results are in line with our previous studies.[10,36]

Regarding outcome, there was no statistically significant difference in overall survival between HER2-low and HER2-0 cases, neither in the ER+ group nor in the ER− group. This seems to be in line with our findings, where no substantial absolute clinicopathologic differences were found between HER2-low and HER2-0 in any of the cohorts. Previous literature concerning the outcome of ER+, HER2-low BC shows conflicting data. Similar to our results, several other studies have not found differences in overall survival between HER2-low and HER2-0 within the ER+ cohort.[10,16,37] In contrast, Mutai et al[17] found that HER2-low expression was associated with better overall survival, but only in women with high genomic risk

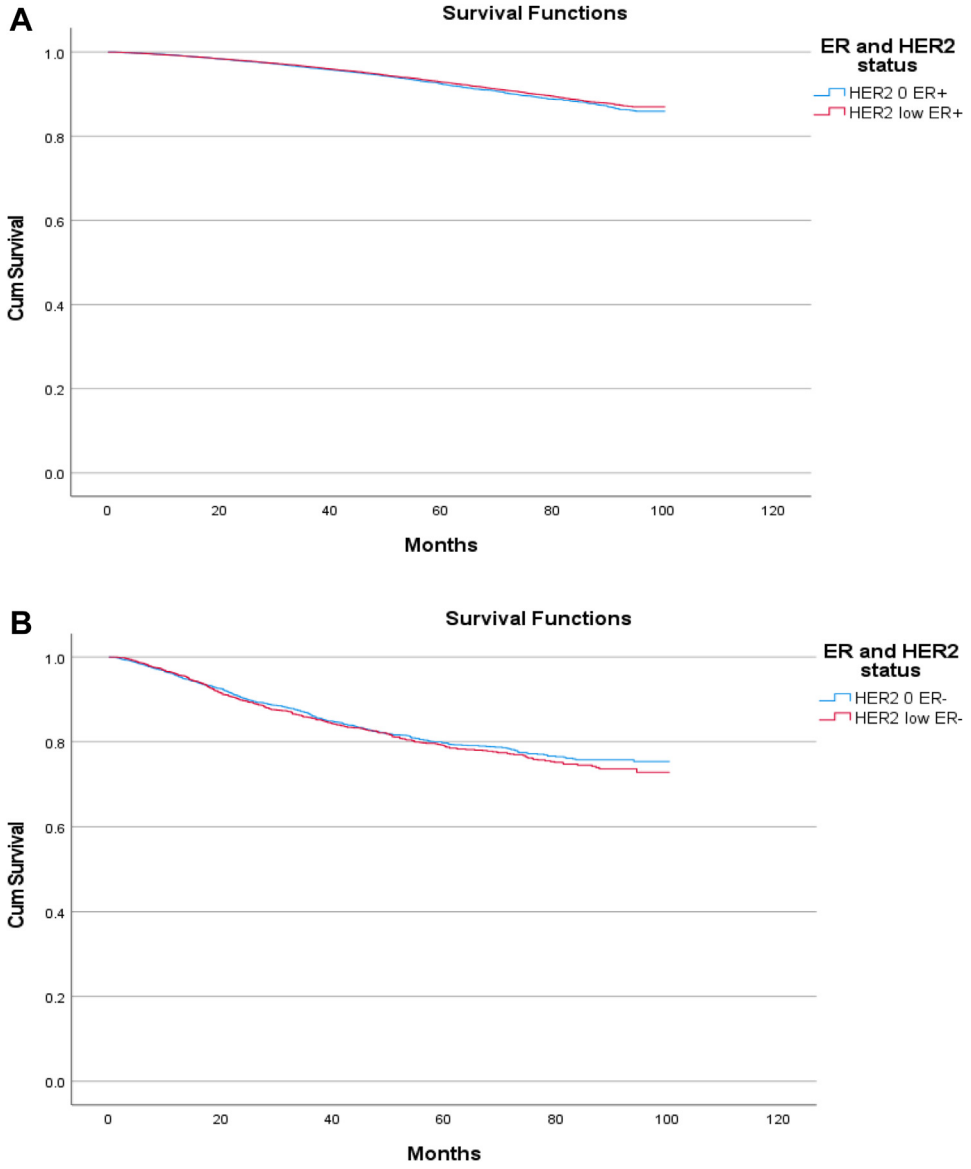

Figure 3.
(A) Overall survival of patients with ER+ BC, HER2-low vs HER2-0 cases ($P = .114$). (B) Overall survival of patients with ER- BC, HER2-low, vs HER2-0 cases ($P = .422$). BC, breast cancer; ER, estrogen receptor.

(oncotype recurrence score > 25). In line with our results in the ER− group, many other research studies found no statistical differences in overall survival between HER2-low and HER-0 tumors within the ER− cohort.[18–20,29,36,38] In contrast, Denkert et al[10] and Tan et al[16] found that HER2-low tumors had a longer overall survival than those with HER2-0 BC, in the HR− cohort. However, it is challenging to compare our results with previous studies, because most of them included a high proportion of patients previously treated with neoadjuvant chemotherapy or other types of treatment, whereas our study only focused on patients without neoadjuvant treatment.[10,16,20,36,38]

One of the biggest strengths of this study was a large size and the nationwide nature of this cohort with real-world data, which gives a realistic perspective of the tumor characteristics found in daily clinical practice. Our population included only patients without neoadjuvant treatment, which provided a uniform population but also resulted in low proportions of HER2-positive and TNBC cases. The nationwide (and therefore multicenter) character of this study corrects for any potential interobserver variability related to HER2 IHC interpretation, which might be a weakness in monocenter studies that affect the global HER2-low and HER2-0 tumor proportions. Our study also had several weaknesses. First, we only analyzed HER2 data of the primary tumor, whereas the level of HER2 expession of the distant metastases could also be clinically relevant. Second, we used the reflex test results (amplified or not amplified) to determine the HER2 status in cases with IHC 2+. However, due to the lack of detailed data from the reflex test, it was not possible to adjust the result of the reflex test according to the latest update of the American Society of Clinical Oncology/College of American Pathologists guidelines,[3] which could potentially have a minor impact on the proportion of HER2-low and HER2-positive cases. In addition, during this study period, there was no clinical

significance of differentiating between IHC 0 and IHC 1+, so pathologists likely gave more attention to the difference between 1+ vs 2+ and 2+ vs 3+ in this era. Finally, the outcome was restricted to overall survival with a limited follow-up time, which limited our outcome analyses.

In conclusion, in this large retrospective nationwide study, we demonstrated that there is considerable variability in HER2 classification (HER2-0 vs HER2-low), not only between the core biopsies and the corresponding resection specimens of the same patient but also between multiple tumors. This variability could impact optimal patient selection for treatment with T-DXd in case only patients with HER2-low BC remain candidates for this therapy. The observed variability could be a plea for multiple tumor sample testing in case of an initial HER2-0 result. After multivariate analysis, HER2-low tumors were associated with histologic subtype, a higher ER, and lower PR expression in the ER+ cohort, whereas within the ER− cohort, HER2-low tumors were associated with a lower tumor grade. However, the lack of substantial absolute differences in clinicopathologic features between HER2-low and HER2-0, combined with the limited follow-up time, could explain why there are no clinically relevant differences in survival observed, in either ER+ or ER− cases.

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Author Contributions

C.D., M.B., and X.B. contributed to the study concept and design. C.D., X.B., and E.A. performed the development of the methodology. X.B. and C.D. performed the writing. All authors participated in the acquisition, analysis and/or interpretation of data, and statistical analysis. All authors performed an exhaustive reviews and revisions of the paper. All authors read and approved the final paper.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Declaration of Competing Interest

C. van Deurzen was involved in an advisory board of Astra Zeneca/Daiichi Sankyo and received research funding from AstraZeneca.

Ethics Approval and Consent to Participate

According to the Code of Conduct of the Federation of Medical Sciences in the Netherlands, no informed consent or ethical approval was needed for this study. This study was performed following the Declaration of Helsinki.

Supplementary Material

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