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Association of HER2-low with clinicopathological features in patients with early invasive lobular breast cancer: an international multicentric study

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Abstract

Purpose The antibody–drug conjugate trastuzumab deruxtecan has proven to be not only efficient in patients with HER2+ breast cancers (BC), but also in those patients with so-called HER2-low BC. HER2-low tumors are well described in the general BC population, but not in patients with invasive lobular carcinoma (ILC). Here, we aimed at analyzing the association of HER2-low with clinicopathological features and survival outcomes in patients with early-stage pure ILC.

Methods A multicentric retrospective cohort of patients diagnosed with stage I-III estrogen receptor positive (ER+) HER2 negative (HER2-) ILC between 01/01/2000 and 12/31/2020 was assembled. HER2- disease was categorized further by immunohistochemical (IHC) score into HER2 0, HER2 1+ and HER2 2+ following time appropriate ASCO/CAP guidelines from 2007 onward and by local guidelines prior to 2007. The association of HER2-low (HER2 1+ and 2+) with clinicopathological variables was assessed using multinomial logistic regression. Survival analyses were performed to evaluate the association of HER2-low with disease-free (DFS), distant recurrence-free (DRFS) and overall survival (OS).

Results The data of 2098 patients with ER+ HER2- ILC was collected of which 1103 (52.6%) had a HER2-low tumor. Of these 716 (34.1%) had an IHC score of HER2 1+ and 387 (18.4%) of HER2 2+. In multivariable analysis, both tumor size of ≥ 2cm (OR: 1.37; 95%CI 1.01 – 1.87; *p*-value 0.042) and multifocality (OR: 1.55; 95%CI 1.11 – 2.15; *p*-value 0.009) were associated with HER2-low. HER2-low was associated with worse DFS (HR: 1.32; 95%CI 1.06 – 1.66; *p*-value 0.015) and OS (HR: 1.42; 95%CI 1.12 – 1.81; *p*-value 0.004) as compared to HER2 0. No association of HER2-low with DRFS was observed.

Conclusions HER2-low is present in more than half of the patients with early ER+ HER2- pure ILCs and is associated with larger tumor size and multifocality. HER2-low is associated with a worse DFS and OS as compared to HER2 0.

Keywords Invasive lobular carcinoma, HER2-low, Clinicopathological features, Survival

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Introduction

The development of the human epidermal growth factor receptor 2 (HER2) targeted antibody drug conjugate (ADC) trastuzumab-deruxtecan and the efficacy of this drug for tumors with lower expression levels of HER2 that lack HER2 amplification have caused a paradigm shift in how HER2 is evaluated in the clinic [1–3]. Although ASCO/CAP guidelines did include HER2 immunohistochemistry (IHC) scoring classifications from 0 to 3+, HER2 was until recently approached in a dichotomous way in clinical trials and clinical practice [4]. Tumors with a HER2 IHC score of 0, 1+ or 2+ and no *ERBB2* amplification were seen as HER2- and these patients were not eligible for anti-HER2 treatment [5, 6]. Tumors with a HER2 IHC score of 3+ and 2+ with *ERBB2* amplification were classified as HER2+ and patients with HER2+ tumors could be treated with therapies like trastuzumab, pertuzumab, HER2-specific tyrosine kinase inhibitors and trastuzumab-emtansine [5, 6].

Trastuzumab-deruxtecan is an anti-HER2 directed antibody linked via a cleavable linker to a topoisomerase I inhibitor payload [7, 8]. This drug not only proved to be efficient in HER2+ tumors [9, 10] but also in patients with tumors having HER2 IHC scores of 1+ and 2+ (HER2-low) without *ERBB2* amplification in the DESTINY-BREAST-04 trial [3, 8]. This new category of HER2-low has since then been explored by several research groups for its associations with other clinicopathological features and its relevance in breast cancer prognosis [11].

The prevalence of HER2-low in a population of patients with HER2- breast cancer is estimated to range from 35 to 65%, with most of these cases being estrogen receptor positive (ER+) [12–14]. The vast majority of HER2-low tumors present with an IHC score of 1+, found in approximately 87% of the cases [12, 14]. HER2-low has been associated with lower KI67 expression levels, more progesterone receptor (PR) positivity, higher androgen receptor expression, more lymph node involvement and lower histological grade in comparison to HER2 0 [14]. Moreover, patients with HER2-low were less likely to receive adjuvant chemotherapy regimens [14]. Others reported an association with older age at diagnosis and male breast cancers [15]. Considering the prognostic value of HER2-low, discordant results have been reported so far [11, 16, 17]. However, most of these trials were focusing on the global breast cancer population and not specifically on ER+ breast cancer.

Invasive lobular carcinoma (ILC) accounts for approximately 15% of all breast cancer diagnoses and most ILCs are ER+ and HER2 non-amplified [18, 19]. Understanding the relevance of HER2-low in patients with ILC is very important. However, the evidence regarding HER2-low in ILC is much scarcer as compared to invasive breast cancer of no-special type (IBC-NST), the most common form of breast cancer [20]. Additionally, the efficacy of trastuzumab-deruxtecan has not yet been formally evaluated for patients with ILC [21].

The association of HER2-low with clinicopathological features and prognosis has so far only been studied in two single center retrospective cohorts of 666 and 1103 patients with ILC, respectively [22, 23]. Within these retrospective analyses, the prevalence of HER2-low in patients with ILC was respectively 40% and 65% when both HER2+ and HER2- disease was included. In patients diagnosed with primary ER+ HER2- ILC, the prevalence was 40% and 70% respectively in both retrospective studies. Conflicting results on the association of HER2-low with clinicopathological features were reported. While one group did not find any association [23], the others did describe an association of HER2-low with older age, higher tumor grade and higher expression levels of ER as well as more non-classic variants of ILC [22]. As for prognosis, an association with worse disease-free survival (DFS) in HER2-low as compared to HER2- tumors was described in one of these studies [23].

Given the clinical relevance of the ILC population with HER2-low tumors and the conflicting results reported in the previous studies, we aimed, in the context of the European Lobular Breast Cancer Consortium (ELBCC), at providing more robust data by assembling a large multicentric retrospective cohort of more than 2,000 patients diagnosed with early-stage ER+ HER2- ILC to evaluate the association between HER2-low status, standard clinicopathological features and survival outcomes.

Methods

Patients

This retrospective analysis was approved by the ethics committee of the University Hospitals Leuven (S64063). Four European centers with expertise in diagnosing ILC participated in this analysis: University Hospitals Leuven, Leuven, Belgium; GZA Hospital Sint-Augustinus, Antwerp, Belgium; Institut Jules Bordet, Brussels, Belgium; Charité Universitätsmedizin, Berlin, Germany. In total, a multicentric international cohort of 2098 female patients diagnosed between January 2000 and December 2020 with non-metastatic pure (i.e. not mixed with other histological types) ER+ HER2- ILC was assembled. The following patient and tumor characteristics were collected: year of birth, age at diagnosis, body mass index (BMI) at primary diagnosis, menopausal state, diameter, nodal involvement, pathological TN classification, pathology-based multifocality, grade, ER-status, PR-status, and HER2-status. For each patient, the use of radiotherapy and/or systemic (neo)adjuvant therapies was registered. Furthermore, the following event-related data were

collected: locoregional, contralateral, and distant recurrence with the respective dates of recurrence, as well as death, date of death, and cause of death.

Histopathological characterization of the tumors

The abovementioned centers were chosen since there is an expertise in breast cancer research and a strong focus on ILC. The diagnosis of ILC depended on morphological characterization and if needed E-cadherin, β-catenin and/or p120-catenin IHC. Historically, available IHC scores from resection specimens of the primary tumors were used to define ER-status. The Allred score was used to determine ER positivity in GZA Hospitals Sint-Augustinus, Institut Jules Bordet, and for patients diagnosed from 2004 onward in University Hospitals Leuven. A score of 0–2 was seen as negative, a score of 5–8 was seen as positive, and a score of 3–4 was seen as unknown unless more information about the composition of the score was available, making tumors with ≥1% of positive cells with at least weak staining being interpreted as being ER +. For patients diagnosed between 2000 and 2003 at University Hospitals Leuven, the H-score and/or Allred score were used. In case no Allred score was available, an H-score of 1 was considered to be positive. Charité Universitätsmedizin reported the percentage of ER-staining cells with a cutoff of 1% for positivity. In all centers, HER2 was first assessed using IHC and scored according to the respective guidelines of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) at the time of diagnosis [4, 24, 25]. Differences between the guidelines have been summarized by Ahn et al [26]. Patients were divided into subgroups by time period of diagnosis in which different ASCO/CAP guidelines were applied (local guidelines 2000–2006; ASCO/CAP guidelines 2007: 2007–2013; ASCO/CAP guidelines 2013/2018: 2014–2020). A score of 0–1+ was seen as negative, and a score of 3+ was seen as positive. Tumors with a score of 2+ were further evaluated using fluorescence in situ hybridization (FISH) in Germany. In Belgium, all cases with scores 2+ and 3+ were tested by FISH to exclude or confirm HER2 amplification based on guidelines at the time of diagnosis. All cases that were either 1+ or were 2+ and had negative FISH were seen as HER2-low. In case of multifocality, the receptors' scores from the largest focus were considered within the analyses. Receptor status of other foci were not available for all multifocal tumors.

Statistical analyses

The following standard clinicopathological variables besides HER2-status were defined to be included in the association and survival analyses: age, BMI, grade, tumor size, nodal involvement, multifocality, and PR expression. HER2-status was both considered as a binary (HER2-low and HER2 0) and as a categorical variable (HER2 2+, HER2 1+ and HER2 0). The association of HER2 both as a binary and as a categorical variable with clinicopathological variables was evaluated using multinomial logistic regression models with 'HER2 0' as the baseline category. Model 1 was adjusted for the center (further referred to as a univariable model), and Model 2 was additionally adjusted for all considered variables (further referred to as a multivariable model).

DFS was defined as the time from diagnosis to the first event of either locoregional recurrence, contralateral recurrence, distant recurrence, or death; distant recurrence-free survival (DRFS) as the time from diagnosis to the first event of distant recurrence; and overall survival (OS) as the time from diagnosis to death from any cause. The median follow-up was calculated using the reverse Kaplan–Meier estimator. The Kaplan–Meier method was first used to estimate the rates of DFS and OS in patients of different HER2 categories. Crude cumulative incidence curves accounting for death without distant recurrence as the sole competing event were constructed for inspection of event rates of DRFS according to HER2 categories. Non-breast primary tumors and their related survival events could not be considered in our analyses as these data were not available. Models 1 (stratified by center) and 2 (adjusted for standard clinicopathological variables and treatment and stratified by center) Cox regression models were next performed to quantify the association of HER2 as a categorical variable with DFS and OS. DRFS was analyzed in the presence of death without distant recurrence as the competing risk using Fine-Grey subdistribution hazard regression models: Model 1 was adjusted for the center, and Model 2 was additionally adjusted for all considered variables including diagnostic time period. For simplicity, Models 1 and 2 will be subsequently referred to as univariable and multivariable models, respectively, in the text. Statistical analyses were performed using R version 4.1.1. All statistical tests were considered statistically significant when the p value <0.05 was a standard evidence criterion.

Results

Patient population

In total, 2098 patients diagnosed with early-stage ER+/HER2-ILC were included for these analyses. The median follow up of this cohort was 8.94 years. Of these 2098 patients, 995 (47.4%) had a primary tumor categorized as HER2 0 and 1103 (52.6%) as HER2-low. The majority of the HER2-low tumors had an IHC score of 1+ (64.9% *vs.* 35.1% HER2 2+). Patient and tumor characteristics of

patients with early-stage ER+/HER2- ILC who had either a tumor that was categorized as HER2 0, 1+ or 2+ are summarized in Table 1.

Since ASCO/CAP guidelines to evaluate HER2 expression have changed over time [26], we assessed how many patients per category were diagnosed in each time frame of the applied guidelines. For HER2 0, we observed a decreasing relative frequency over the different diagnostic periods (2000–2006: 39.4%, 2007–2013: 32.3% and 2014–2020: 28.3%). For HER2 1+ and 2+, the relative frequencies are lower in the period 2000–2006 (HER2 1+ : 2000–2006: 7.4%, 2007–2013: 51.3% and 2014–2020: 41.3% and HER2 2+ : 2000–2006: 5.9%, 2007–2013: 32.0% and 2014–2020: 62.0%).

Association of HER2-status with standard clinicopathological features

HER2 was first assessed as a binary variable (HER2-low *vs.* HER2 0). The association between HER2-status and the clinicopathological features was evaluated using regression analyses (Fig. 1). In the multivariable analysis, both tumor size of ≥2 cm and multifocality were associated with HER2-low. Histological grade 3 was associated with HER2-low in the univariable, but not in the multivariable analysis. No significant associations were seen for age at diagnosis, nodal involvement and PR expression.

Secondly, HER2 was approached as a categorical variable (HER2 0, HER2 1+ and HER2 2+). The regression analyses evaluating the association between clinicopathological features and both HER2 1+ *vs.* HER2 0 and HER2 2+ *vs.* HER2 0 are shown in Fig. 2. Considering HER2 1+ *vs.* HER2 0, an association between multifocality and HER2 was seen. No other associations were seen for this comparison. More clinicopathological features were associated with HER2 2+ (*vs.* HER2 0). Higher tumor grade, tumor size of ≥2 cm as well as multifocality were associated with HER2 2+ *vs.* HER2 0.

Association of HER2-status with outcome

We considered three endpoints for the survival analyses: DFS, DRFS and OS. As for the association analyses, we first compared the survival between patients with HER-low tumors *vs.* HER2 0 tumors. The Kaplan Meier curves suggest a worse DFS and OS (Fig. 3A and C, respectively) for HER2-low compared to HER2 0, while the cumulative incidence curves of DRFS (Fig. 3B) suggest no significant difference between HER2-low and HER2 0. In multivariable analyses, HER2-low was associated with worse DFS (HR: 1.322; 95%CI 1.055 − 1.657; *p*-value 0.015) and OS (HR: 1.423; 95%CI 1.122 − 1.805; *p*-value 0.004) as compared to HER2 0. Known prognostic factors like larger tumor size, higher tumor grade and nodal involvement were also independently associated with worse outcomes for DFS, DRFS and OS. Considering HER2 as a categorical variable, we observed that patients with HER2 1+ as compared to both HER2 2+ and HER2 0 had a worse DFS, while the curves for OS suggest a worse prognosis for both HER2 1+ and HER2 2+ as compared to HER2 0 (Fig. 4A and C respectively). Regarding DRFS, the cumulative index curve (Fig. 4B) suggests less events for patients with HER2 2+ tumors as compared to the two other groups. In the multivariable analyses, only HER2 1+ was associated with worse DFS (Fig. 5A), while both HER2 1+ and HER2 2+ were associated with worse OS (Fig. 5B). No associations between HER2 categories and DRFS (Fig. 5C) were observed in the multivariable analysis.

Discussion

Within our cohort of patients diagnosed with ER+/HER2- pure ILC, the prevalence of HER2-low was 52.6%. This prevalence lies in between the 40% and 70% of HER2-low tumors in the 599 and 1051 ER+/HER2- ILC tumors reported by the two recent publications, respectively [22, 23]. Overall, it has been suggested that the prevalence of HER2-low is somewhat lower in ILC as compared to IBC-NST [22, 27, 28]. In line with previous reports in the general breast cancer population [12, 14], the majority of the HER2-low tumors have an IHC score of 1+ in our cohort (64.9%).

The presence of HER2-low in more than half of the patients with ER+/HER2- pure ILC indicates a possible broadening of future treatment options for these patients. Although trastuzumab-deruxtecan is currently only approved in the metastatic setting for patients with HER2-low tumors (after prior treatment with chemotherapy in this setting) [29], clinical trials in neo-adjuvant setting are currently ongoing [30, 31]. Hopefully, the inclusion rate of patients with ILC will be documented in these clinical trials with efficacy sub analyses for ILC, which is now often not the case for patients with ILC [21].

For patients with primary HER2-low ILC progressing to metastatic disease, trastuzumab-deruxtecan may already provide a valid treatment option following results of the DESTINY-BREAST 04 trial [3]. Within the patients diagnosed with hormone receptor positive disease, progression-free survival (PFS) increased from a median of 5.4 months to 10.1 months for those receiving trastuzumab-deruxtecan (*vs.* treatment of physician's choice). The overall survival increased from a median of 17.5 months to 23.9 months. No specific data on efficacy for ILC are available [21]. Recently, trastuzumab-deruxtecan was tested in patients that had a tumor with HER2 IHC score of 0 that had incomplete or faint staining in

Table 1 Patient and tumor characteristics patients with ER +/HER2 − ILC by HER2 IHC score

Characteristic	*ALL*		*HER2 0*		*HER2 1+*		*HER2 2+*	
	N (Total = 2098)	**%**	**N (Total = 995)**	**%**	**N (Total = 716)**	**%**	**N (Total = 387)**	**%**
Age								
≤ 50	459	21.9	232	23.3	149	20.8	78	20.2
> 50	1639	78.1	763	76.7	567	79.2	309	79.8
Missing	0		0		0		0	
Menopausal state								
Premenopausal	470	24.3	259	27.5	123	19.5	88	24.5
Postmenopausal	1462	75.7	684	72.5	507	80.5	271	75.5
Missing	166		52		86		28	
Diagnostic period								
2000–2006	468	22.3	392	39.4	53	7.4	23	5.9
2007–2013	812	38.7	321	32.3	367	51.3	124	32.0
2014–2020	818	39.0	282	28.3	296	41.3	240	62.0
Missing	0		0		0		0	
BMI								
Underweight (< 18.5)	59	3.0	34	3.6	18	2.8	7	1.8
Lean (18.5–24.9)	1008	51.3	471	50.4	353	54.6	184	47.8
Overweight (25–29.9)	578	29.4	288	30.8	160	24.8	130	33.8
Obese (≥ 30)	321	16.3	142	15.2	115	17.8	64	16.6
Missing	132		60		70		2	
Tumor size (pT)								
< 2 cm	793	40.2	423	43.3	272	41.3	98	29.1
≥ 2 cm	1179	59.8	553	56.7	387	58.7	239	70.9
Missing	126		19		57		50	
Nodal involvement (pN)								
No	1179	60.8	597	62.3	390	60.6	192	57.0
Yes	760	39.2	361	37.7	254	39.4	145	43.0
Missing	159		37		72		50	
Histological grade								
1 and 2	1844	92.8	915	93.3	623	93.5	306	90.3
3	142	7.2	66	6.7	43	6.5	33	9.7
Missing	112		14		50		48	
PR expression								
PR+	1715	86.4	792	85.4	592	85.5	331	90.4
PR-	270	13.6	135	14.6	100	14.5	35	9.6
Missing	113		68		24		21	
Focality								
Unifocal	1056	77.2	625	82.1	241	71.9	190	70.1
Multifocal	311	22.8	136	17.9	94	28.1	81	29.9
Missing	731		234		381		116	
Radiotherapy								
Yes	1657	81.0	806	82.5	538	78.5	313	81.5
No	389	19.0	171	17.5	147	21.5	71	18.5
Missing	52		18		31		3	
Chemotherapy								
Yes	517	25.0	253	25.7	176	25.3	88	22.8
No	1549	75.0	732	74.3	519	74.7	298	77.2
Missing	32		10		21		1	
Endocrine treatment								
Yes	1960	97.8	924	96.4	660	99.0	376	99.2
No	45	2.2	35	3.6	7	1.0	3	0.8
Missing	93		36		49		8	

BMI body mass index, *ER* estrogen receptor, *HER2* human epidermal growth factor receptor 2, *ILC* invasive lobular carcinoma, *N* Number, *PR* progesterone receptor

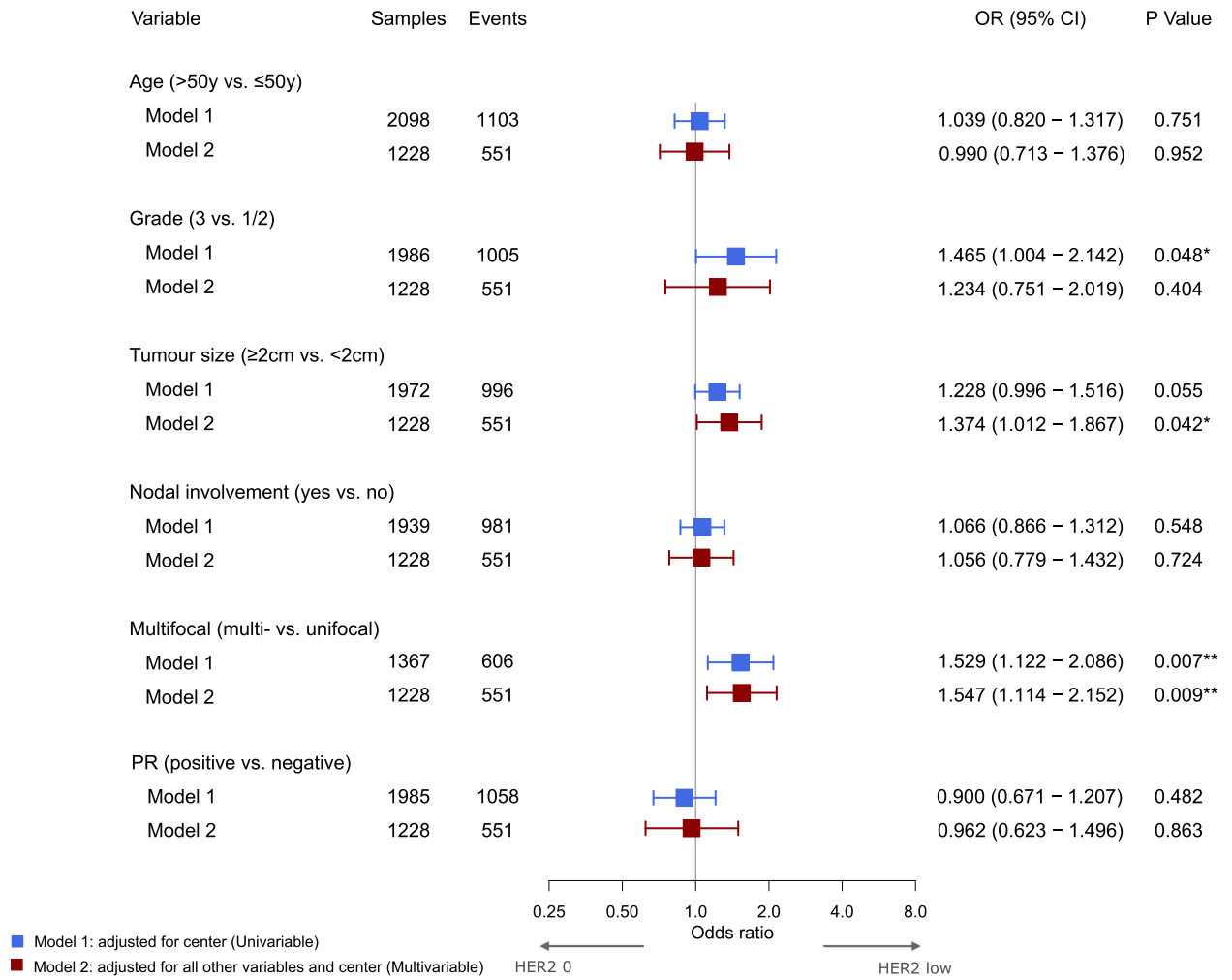

Fig. 1 Association of clinicopathological features of ER+/HER2- ILC with HER2-low vs. HER2 0. Forest plot illustrating the association of clinicopathological features of ER+/HER2 – ILC with HER2 as outcome variable and HER2 assessed as binary variable. ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; ILC: invasive lobular carcinoma; OR: odds ratio; PR: progesterone receptor; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

≤10% of the tumor cells, these tumors are categorized as HER2-ultralow and are a subset of the HER2 0 tumors. The first results of use of trastuzumab-deruxtecan for HER2-ultralow tumors in the metastatic setting were recently published and are promising [32]. Increase in median PFS of trastuzumab-deruxtecan when compared to treatment of physician's choice was comparable between HER2-low (13.2 months *vs.* 8.1 months respectively) and HER2-ultralow (13.2 months *vs.* 8.3 months respectively). In our dataset, as no information was available on the intensity of staining and percentage of cells staining for HER2, the HER2-ultralow category could not be defined and thus not be studied separately. An important element we should consider in the metastatic setting is the heterogeneity in HER2 expression levels between primary tumor and metastatic lesions as well as the heterogeneity of HER2 expression between different metastatic lesions from a same patient [14, 33]. This complicates the assessment of eligibility for treatment with trastuzumab-deruxtecan.

In our dataset, we found a significant association of the HER2-low status with larger tumor size as well as with multifocality. The results on multifocality might be biased however, since only the characteristics of the largest tumor focus were considered in our dataset, therefore not excluding presence of HER2 0 in the other smaller foci. A single center retrospective analysis of 666 patients with ILC (599 with ER +/HER2- ILC) performed at the University of California, San Francisco, did not find any significant difference

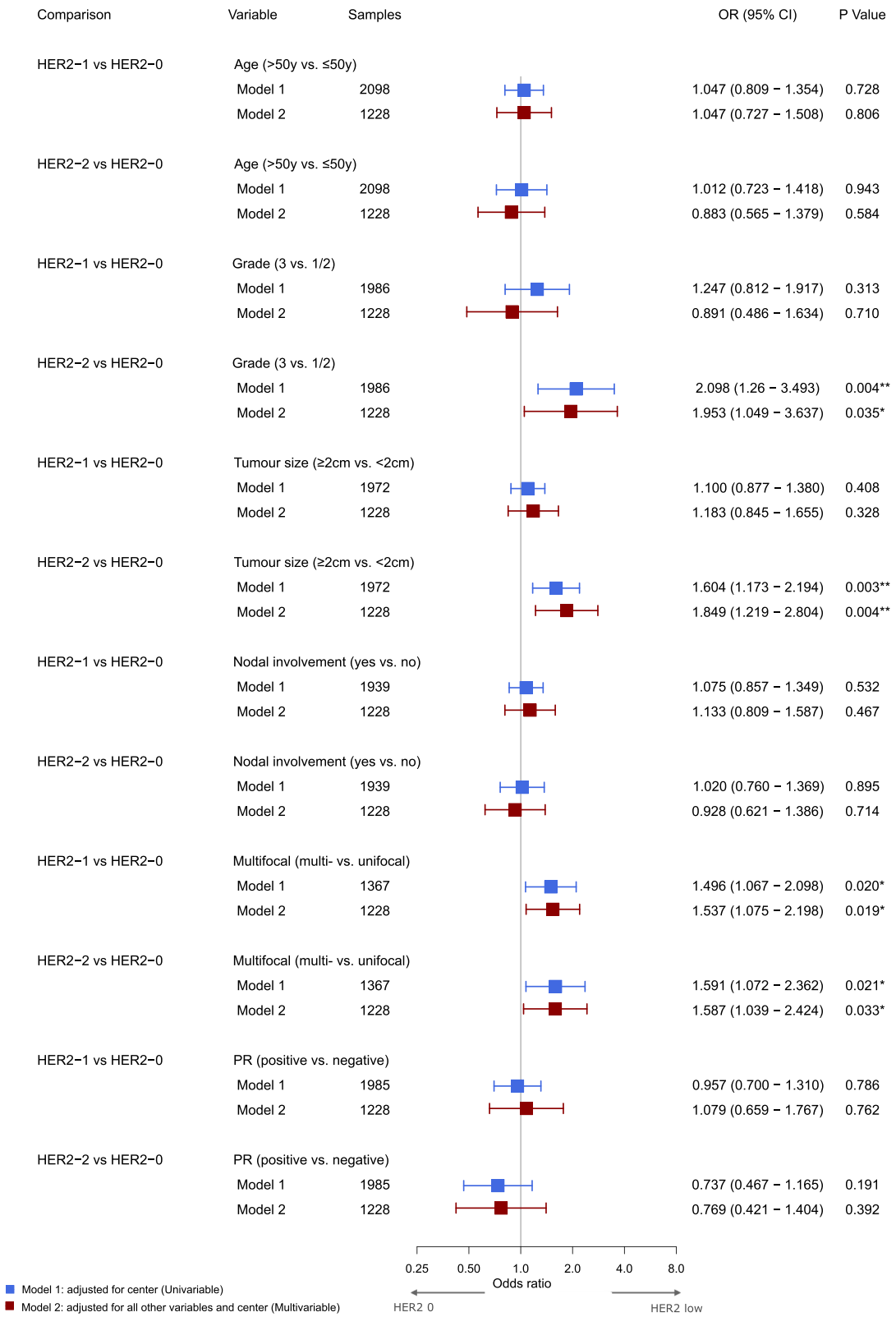

Fig. 2 Association of clinicopathological features of ER+/HER2- ILC with HER2 IHC 1 and 2 vs. 0. Forest plot illustrating the association of clinicopathological features of ER +/HER2− ILC with HER2 as outcome variable and HER2 assessed as categorical variable. ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; ILC: invasive lobular carcinoma; OR: odds ratio; PR: progesterone receptor; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

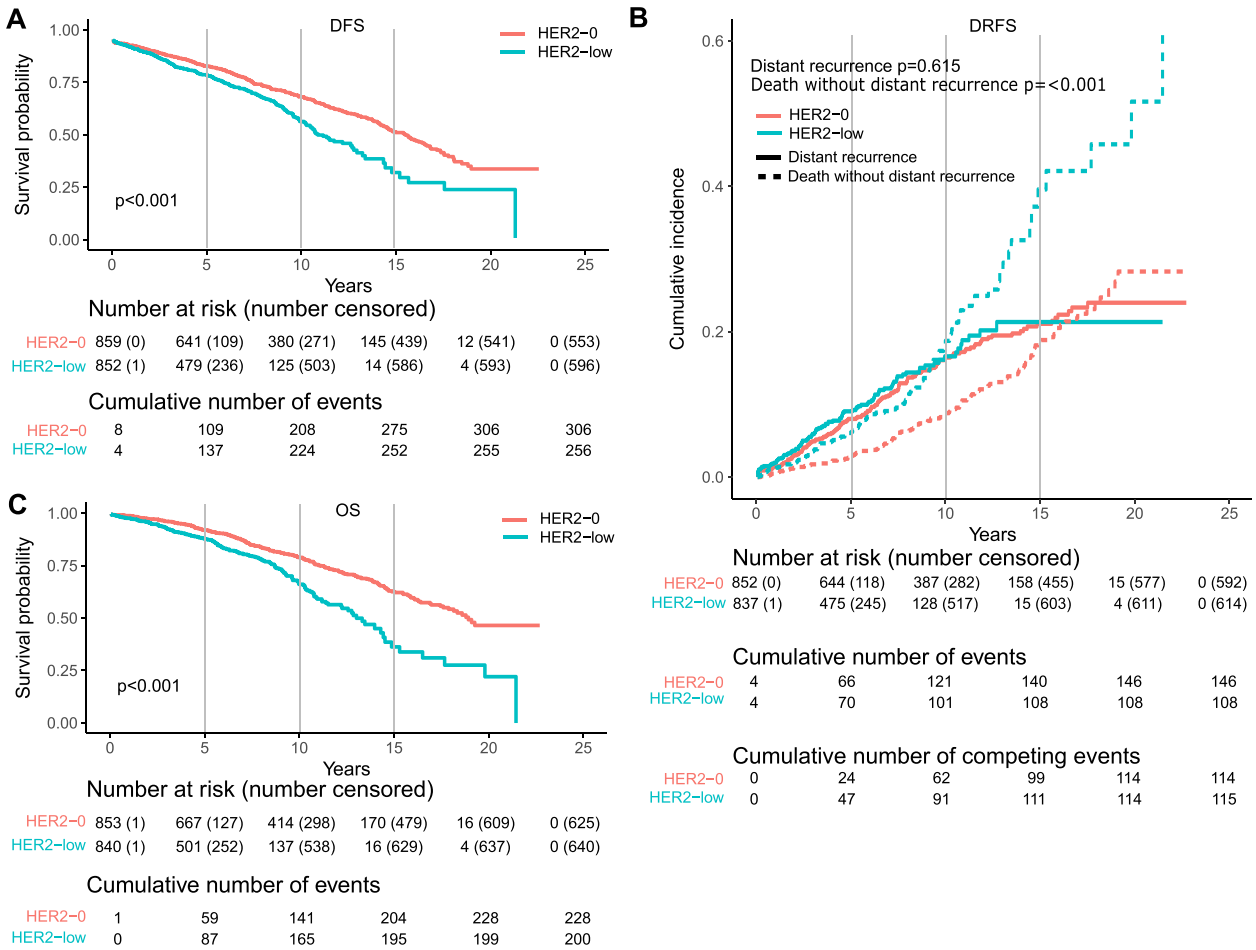

Fig. 3 DFS, DRFS, and OS by HER2-low *vs.* HER2 0 in patients with ER+/HER2 – ILC. Panels **A** and **C** show the Kaplan–Meier curves of, respectively, disease-free survival and overall survival probabilities in patients with HER2-low *vs.* HER2 0 tumors; panel **B** shows the cumulative incidence curves of distant recurrence-free survival events in patients with HER2-low *vs.* HER2 0 tumors. DFS: disease-free survival; DRFS: distant recurrence-free survival; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; ILC: invasive lobular carcinoma; OS: overall survival

in clinicopathological features between HER2-low and HER2 0 [23]. Another single center analysis from Institut Curie, France, focusing on ILC ($n = 1103$ patients with 1051 ER +/HER2- ILC), did report an older age at diagnosis for HER2-low, as well as more non-classic variants of ILC and higher-grade and more proliferative tumors [22]. It should be noted that all the observations they described were at the univariable level. They also noted an enrichment in *ERBB3* mutations in the HER2-low group. In our cohort, we did see an association between HER2-low and high grade at the univariable level, but this association lost significance in the multivariable model. We could not investigate the association with the ILC subtypes or somatic alterations as this information was not available. The comparison of the associations we observed between the HER2-status and the different variables and those observed in the other two studies should be taken with caution as not the same variables were studies across the three studies and as we only focused on ER+ HER2- ILC, while the other studies considered all HER2- ILC. Also, in the other two studies, no comparisons were made between HER2 1+ and HER2 0 or HER2 2+ and HER2 0 [22, 23]. When assessing these 2 scores separately, our results suggested an association of higher tumor grade, larger tumor size and multifocality with HER2 2+, while only multifocality seemed to be associated with HER2 1+.

Regarding the association of the HER2-status with clinical outcome, we observed a worse DFS and OS, but not DRFS for patients with HER2-low ILC as compared to patients with HER2 0. This is in line with findings from Rothschild et al [23]., although they only investigated DFS as an endpoint. In the French retrospective analysis, Djerroudi et al. described a lower risk of local recurrence for patients with HER2-low ILC, but did not find any association with breast cancer specific survival and

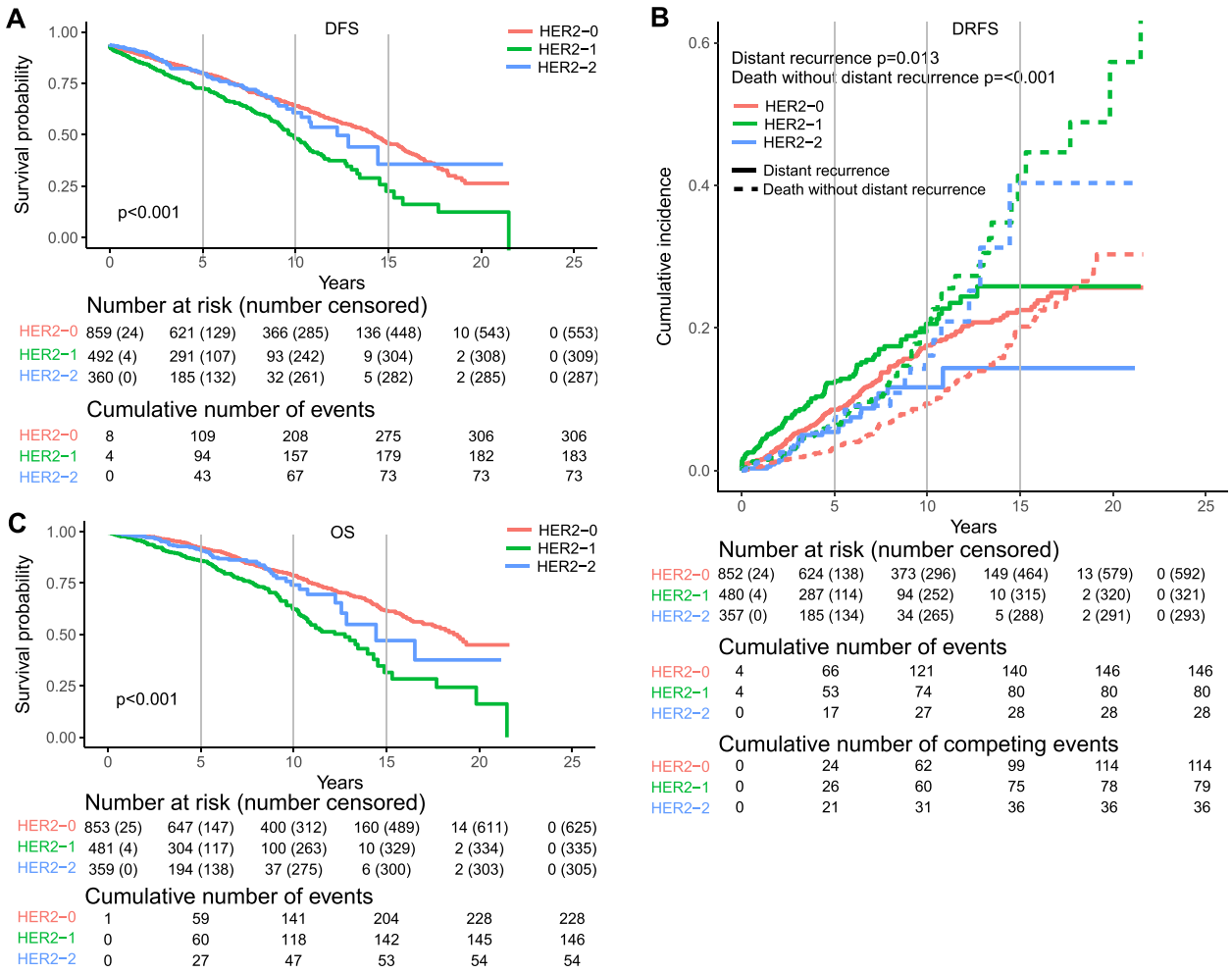

Fig. 4 DFS, DRFS, and OS by HER2 categories in patients with ER+/HER2 − ILC. Panels **A** and **C** show the Kaplan–Meier curves of, respectively, disease-free survival and overall survival probabilities in patients with tumors of different HER2 categories; panel **B** shows the cumulative incidence curves of distant recurrence-free survival events in patients with tumors of different HER2 categories. DFS: disease-free survival; DRFS: distant recurrence-free survival; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; ILC: invasive lobular carcinoma; OS: overall survival

distant metastases-free survival [22]. Their results should however be considered with caution given that these analyses only included 88 patients, both with ER+ and ER- disease, and given that no multivariable analyses were performed. When we considered the separate categorical HER2 IHC-scores, HER2 1+ was associated with both worse DFS and OS, while HER2 2+ was only significantly associated with worsened OS as compared to HER2 0.

(See figure on next page.)

Fig. 5 Association of clinicopathological features of ER+/HER2 − ILC with DFS, DRFS, and OS. Panels **A** and **C** show the forest plots presenting the association of HER2 with disease-free survival and overall survival, respectively, quantified by Cox models: Model 1 was adjusted by center and time period of diagnosis, and Model 2 was adjusted for all included variables, center and time period of diagnosis. Panel **B** shows the forest plots presenting the association of HER2 with distant recurrence-free survival quantified by Fine-Grey regression models: Model 1 was adjusted for center and time period of diagnosis, and Model 2 was adjusted for all included variables, center and time period of diagnosis. On the left side of panels **A**, **B**, and **C**, the results are shown with HER2 considered as a binary variable (HER2-low *vs.* HER2 0); on the right side of panels **A**, **B**, and **C**, the results are shown with HER2 considered as a categorical variable (HER2 2+, HER2 1+ and HER2 0). DFS, disease-free survival; DRFS, distant recurrence-free survival; HER2: human epidermal growth factor receptor 2; HR: hazard ratio; ILC: invasive lobular carcinoma; OS, overall survival; PR: progesterone receptor; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

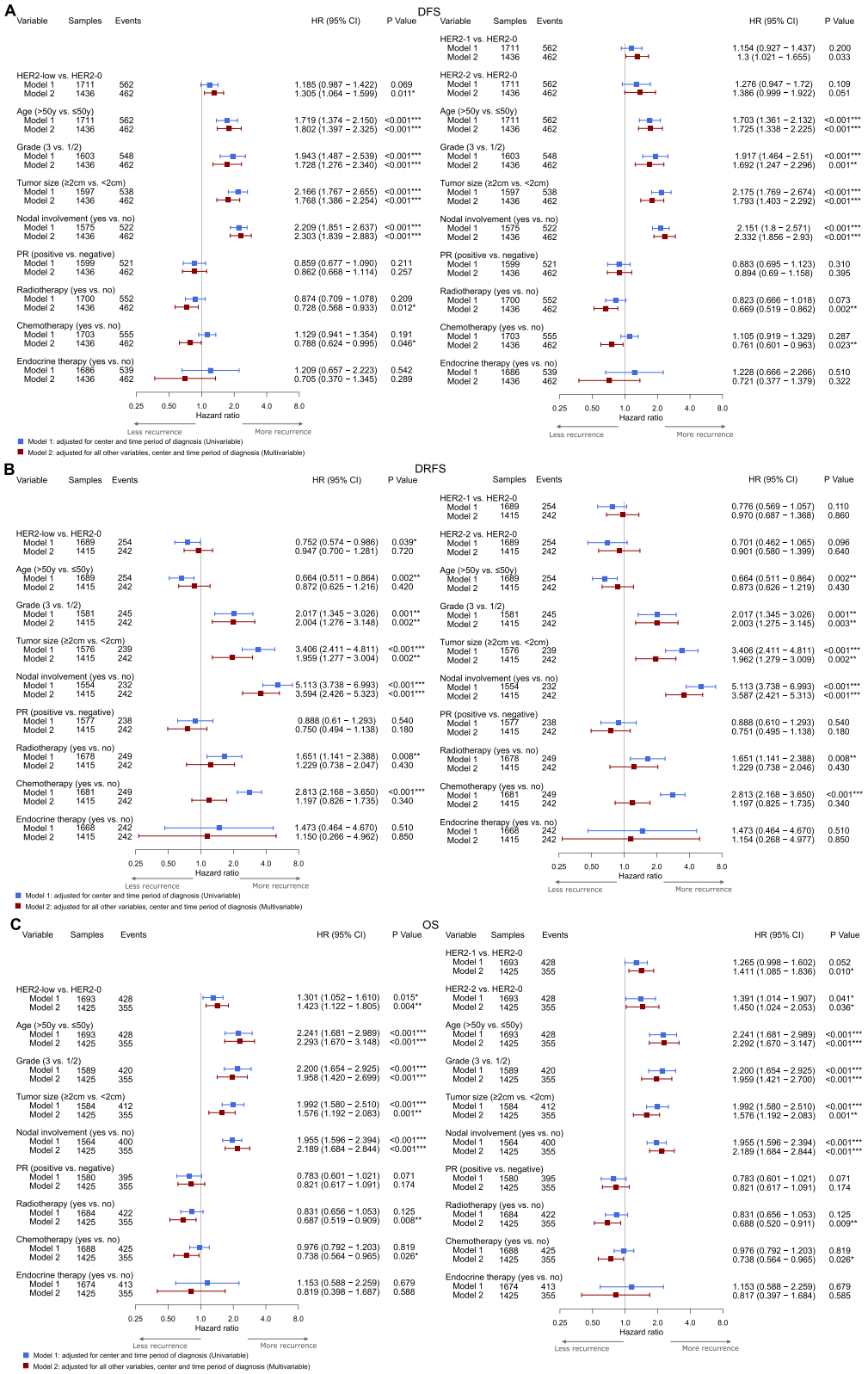

Fig. 5 (See legend on previous page.)

This study presents several limitations, some of which are inherent to the retrospective nature of the analyses. First, there is a lack of central pathology both for confirmation of ILC as well as for assessment of HER2 scoring. It is clear from a recent survey that pathological diagnosis of ILC can greatly differ between centers regarding the use of E-cadherin immunohistochemistry or not, as well as for the antibodies used [34]. For previous trials, we have learned that only 60–66% of locally diagnosed ILCs are confirmed by central pathology [35, 36]. However, here, we have selected specific centers that have a special interest in ILC diagnosis and treatment which present with pathology department specialized in differentiating ILC from other breast cancer subtypes. Second, no central pathology for HER2 could be performed for this large multicentric cohort of patients since archived material were no longer available for all included patients. Available, historical, clinical data needed to be considered to categorize the tumors according to HER2 scoring. It is however known that ASCO/CAP guidelines have changed over time and therefore adjustments for diagnostic period where implemented in the analyses [4, 25]. In a recent retrospective analysis, the interobserver variability ranged between Krippendorff's α of 0.56 and 0.73 to distinguish between HER2 0 (including HER2-ultralow) and HER2-low by use of the ASCO/CAP 2018 guidelines and modified ASCO/CAP 2007 guidelines respectively [37]. The historically used guidelines were not developed to differentiate between HER2 0 and HER2-low and interpretation of the IHC scores for HER2 in those time periods could therefore bias the results [38]. This might explain the lower rates of HER2 1+ and 2+ between 2000 and 2006 since pathologists were applying local guidelines and there was no clinical interest in differentiating between the different negative scores of HER2. It is unknown if for all patients HER2 was assessed on the resection specimen of the primary surgery or that for some the core needle biopsy was used to define HER2-status. Third, still concerning pathology, there is the variability between centers in scoring systems for ER and PR as described in the methods. Finally, the large inclusion period (2000–2020) also presents with the challenge of changing diagnostic tools and treatment regimens over time, impacting patient's survival, however different diagnostic periods were considered in the survival analyses. Also, cancer specific survival could not be assessed since data on cause of death was not available for all included centers. Since data on KI67 was not available in our dataset, it could not be included in the analyses. It is however known that KI67 has prognostic value for ILC [39]. In a retrospective analysis, a KI67 level of more than four percent was associated with worse DFS and OS [39]. Additional variables of interest assessed in other retrospective analyses like levels of stromal tumor infiltrating lymphocytes and association with classic *vs.* non-classic ILC could not be assessed [22], since data on these variables is missing from the dataset of several of the considered centers. We however admit that the inclusion of these variables could have impacted our results. Finally, no data was available on indication setting and type of radiotherapy schemes, chemotherapy and endocrine regimens. Nonetheless, due to the multicentric approach, we were able to bring together data on 2098 patients with ER+/HER2- ILC presenting one of the largest available datasets on the association of HER2-low with clinicopathological features and survival in patients with ILC.

To conclude, more than half of the patients with ER+/HER2- pure ILC may currently have primary tumors that are nowadays considered to be HER2-low. This opens perspectives towards treatment with novel HER2 directed ADCs like trastuzumab-deruxtecan, although these drugs are not available yet in neo-adjuvant and adjuvant setting. Associations between larger tumor size and multifocality with HER2-low were seen in our cohort and had not been described previously. Conflicting results have been found about the impact of HER2-low on survival outcomes, ours indicating worse prognosis. Validation of these results in other cohorts of patients with ILC with central pathology review are therefore warranted to define the relevance of HER2-low in patients with ILC compared to patients with IBC-NST.

Abbreviations

ADC	Antibody drug conjugate
ASCO/CAP	American Society of Clinical Oncology/College of American Pathologists
BC	Breast cancer
BMI	Body mass index
DFS	Disease-free survival
DRFS	Distant recurrence-free survival
ELBCC	European Lobular Breast Cancer Consortium
ER	Estrogen receptor
FISH	Fluorescence in situ hybridization
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
IBC-NST	Invasive breast cancer of no-special type
IHC	Immunohistochemical
ILC	Invasive lobular carcinoma
OR	Odds ratio
OS	Overall survival
PFS	Progression-free survival
PR	Progesterone receptor

Authors' contributions

Concept: KVB, H-LN and CD; Methodology: KVB, H-LN, FR, EB, GF and CD; Project administration: KVB, MM and CD; Investigation: KVB, H-LN, FR, MMK, GNM, PV and CD; Resources: KVB, H-LN, FR, GZ, MMK, GNM, PV, LD, ADD, EDA, DL, MM, EB, HW, AS, IN, PN, GF and CD; Data Curation: KVB, H-LN, FR, MMK, GNM, PV and CD; Writing Original Draft: KVB, H-LN and CD; Writing Review & Editing: KVB, H-LN, FR, GZ, MMK, GNM, PV, LD, ADD, EDA, DL, MM, EB, HW, AS, IN, PN, GF and CD; Visualization: KVB and H-LN; Funding Acquisition: KVB, MM and CD.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Leuven and the Ethics Committees of all involved centers.

Consent for publication
Individual informed consent for participation in this retrospective analysis was waivered by the ethics committees.

Competing interests
The authors declare no competing interests.

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