

The Prevalence of HER2 Positivity In HER2 Borderline Tumors In Iranian Breast Cancer Patients And Predictive Variables

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Abstract

Background: Human epidermal growth receptor-2 (HER2) gene amplification is an important predictive and prognostic factor in breast cancer treatment. However, the expression of HER2 by immunohistochemistry (IHC) is determined as borderline in some cases and confirmation of the HER2 status by in situ hybridization either fluorescent (FISH) or bright field chromogenic (CISH) is necessary for correct treatment decision-making. Considering the high cost of FISH and CISH, we aimed to investigate whether the HER2 status could be predicted by other histological and cellular characteristics of the tumor by evaluating the association of these characteristics with the actual tumor HER2 status.

Methods: Data of 438 breast cancer patients with IHC-determined HER-2 borderline disease was evaluated retrospectively. FISH or CISH results, pathologic tumor size and type, node involvement, Ki67%, presence of estrogen and progesterone receptor (ER, PR), HER2 status, lymphovascular invasion (LVI), perineural invasion (PNI), and stage were retrieved from clinic records.

Results: Seventy-four (16.9%) patients had positive results for HER2 status with FISH or CISH. Logistic regression analysis showed that the pathologic size had a positive association with HER2 positivity with an OR equal to 1.03 (Odds ratio (OR):1.03, 95% CI: 1.01-1.05). In addition, the adjusted OR illustrated a statistically significant association between HER2 positivity and PR negativity (OR=2.14, 95% CI: 1.14-4.02). The invasive lobular carcinoma histology had a reverse association with HER2 positive status, with a borderline significance level (OR=0.15, 95% CI: 0.02-1.18).

Conclusion: We could not find an applicable model to predict the actual status of HER2 in borderline cases and still, we have to recommend further assay by FISH or CISH in these patients.

Background

In usual circumstances, human epidermal growth receptor-2 (HER2) has an important role in normal cell growth and differentiation. However, amplification of the HER2 gene leads to overexpression of the receptor, and subsequently can result in the development of many types of cancer; including breast cancer [1]. Amplification of HER2 had previously been reported in 25 to 30% of primary breast cancers, but following refinement of test performance parameters the frequency of HER2 positivity has been shown to be between 13 to 20% [2]. A systematic review from Iran with a significant heterogeneity among the included articles showed the rate of HER-2 positive breast cancer to vary from 23.3% to 81% [3]. Several studies have suggested that the HER2 subtype of breast cancer is associated with an aggressive course, higher relapse and mortality rate, and reduced level of estrogen and progesterone receptors [4, 5]. A separate study by Kadivar et al. reported that the prevalence of HER2 subtype in Iranian women with breast cancer was 11.9%, and showed that vascular invasion and higher grade tumors were more prevalent in this subtype of the cancer [6]. Moreover, with the exception of lymph node involvement, survival analysis has shown that HER2 amplification is the best predictive factor for the clinical outcome [7].

Roses and co-workers showed that although high nuclear grade, large lesion size, and HER2 overexpression in ductal carcinoma *in situ* (DCIS) were associated with invasive disease on univariate analysis, HER2 is the only significant predictor for the presence of invasive breast cancer. Therefore, targeting HER2 in an early stage of the disease might prevent disease progression [8]. Thus, a precise HER2 test result is necessary for the accurate prediction of disease progression and before any anti-HER2 therapy.

In routine practice, the expression of HER2 is determined by immunohistochemistry (IHC) as +1 (negative) and +3 (positive). More than 10% complete strong membrane staining is defined as a positive result. However, some cases will be scored + 2 for HER2, as borderline tumors which cannot be classified as HER2 positive or negative. In situ hybridization, either fluorescent (FISH) or bright field chromogenic (CISH) is recommended in borderline cases to confirm the presence of HER2 gene amplification [7]. Dual probe FISH analysis remains the most useful test which should be applied in all cases when the immunostaining is doubtful or has a technical artifact [9].

In our country, IHC is the first step in HER2 detection. Further assay of HER2 borderline tumors is performed via FISH or CISH tests; which are expensive and time-consuming, and also unavailable in many centers. In some cases due to economic issues, further evaluation with FISH assay is not performed or is carried out with a significant delay. For these patients, developing a predictive model that could properly estimate the result of FISH in HER2-borderline breast cancer would be very useful and prevent treatment delays. Therefore, this study was designed to determine the prevalence of HER2 positivity in HER2 borderline tumors in Iranian breast cancer patients and to study the correlation of histopathologic tumor characteristics with these positive HER2 results.

Methods

This Study was conducted accordance with Declaration of Helsinki and the Institutional Research Board (No# 97-03-218-40456) and the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1397.890) approved the study. The selected records belonged to all breast cancer patients attending one private clinic between 2010 and 2020 (during 10 years), and who had a borderline HER2 status. All patients who had the results of FISH or CISH in their data profile were included in the study for final analysis. The clinicopathologic characteristic including patient age, family history, laterality of the tumor, tumor grade according to the modified Bloom-Richardson classification, lymphovascular invasion (LVI), perineural invasion (PNI), pathologic tumor size and type, presence of distant metastases and disease stage, node involvement, Ki67%; estrogen receptor (ER), progesterone receptor (PR), and final HER2 status results, were retrieved from clinic records.

We used the SPSS software (SPSS, Version 20, SPSS, Inc., IL, USA) to perform statistical analysis. We tested differences in means by the Student's t-test in HER2 positive and negative cases. Categorical variables were compared by the Chi-square (χ^2) test. A two-sided p-value of less than 0.05 was considered significant. Multivariable binary logistic regression was performed to estimate the odds ratio

(OR) and confidence intervals (CI) for the association between histopathologic variables and the HER2 status. Variables were selected a priori for inclusion in the multivariable model on the basis of the association with HER2 status in univariable analyses (P-value less than 0.1).

Results

Among the patients whose IHC had revealed HER-2 borderline disease, 438 had FISH or CISH results in their records and were included in the final analysis. The mean age of the patients was 50.75 ± 12.17 , ranging from 26 to 83 years. Table 1 represents the tumor characteristics of all breast cancers in the study population. Family history was positive in 147 (33.6%) of cases, including 58 (13.2%), 55 (13.2%) and 34 (7.8%) for first, second and third degree relatives, respectively.

In the total population, 16 cases had metastasis and the most common sites of metastases were lung, bone, brain, and liver. Seventy-four (16.9%) patients had final positive results for HER2 status and the remaining ($n = 364$, 83.1%) had negative results. Table 2 compares the tumor and patient characteristics of the cases with positive and negative HER2 status. Our results showed that larger tumor size and PR negativity were more prevalent in HER2 positive cancers. The results of the logistic regression analysis considering pathologic tumor size and type, Ki67 ($<15\%$ and $\geq 15\%$), metastasis, and PR as independent variables are shown in Table 3. HER2 positivity had a significant association with pathologic tumor size with OR equal to 1.03 (95% CI: 1.01-1.05, P-value = 0.006). In addition, the adjusted OR illustrated a statistically significant association between HER2 positive and PR negative (OR = 2.14, 95% CI: 1.14-4.02, P-value = 0.02) features. Contrarily, our result showed the invasive lobular carcinoma (ILC) had a reverse association of borderline significant level (OR = 0.15, 95% CI: 0.02-1.17, P-value = 0.07) with HER2 positive status. None of the other variables showed any association with HER2 status.

Discussion

In this study, breast cancers with borderline HER2 were evaluated in Iranian patients, and we sought to develop a predictive model for estimation of the actual HER2 status in these cases. In the present study, the prevalence of HER 2 amplification was 16.9% (74 out of 438) in patients with IHC-based borderline HER2 results. Our results revealed a positive association between a final positive HER2 status and tumor size as well as with PR negativity, and a reverse association with ILC.

The prevalence of HER2 positivity in borderline HER2 in our study was similar to a large cohort of patients in India, which was estimated as 14.6% [5]. However, in numerous studies, the rate of HER2 amplification by FISH in IHC borderline tumors was higher than the present study, from 27.5% to 70% [10-14]. The reason for such a high reported rate was explained in some of the publications. One study by Okaly et al. [15] in 2019 reported that more than half of the patients (54%: 72 out of 134) with borderline HER on IHC had HER2 amplification on FISH due to a possible referral bias. Similarly, Panjwani et al. [16] explained the high rate of HER2 amplification which was 66.6% (24/36) in IHC borderline cases by a high load of referral cases, quality of tissue fixation, method of processing, and duration of storage. In fact, some of

this variability could be justified by interobserver and intraobserver variation in IHC interpretation and the evolution of HER2 practice guidelines.

Of note, all of these studies were conducted before the update of the American Society of Clinical Oncology (ASCO)/ College of American Pathologist (CAP) practice guideline in 2018, and applying this guideline may decrease the rate of HER2 positivity [17]. Wei et al. [18] studied the quantitative impact of 2018 ASCO/ CAP guidelines on HER2 status and showed an average of 9% reclassification in overall HER2 status with a net increase in negative HER2 designation

The present study found an association between HER2 amplification by FISH with tumor size. Limited studies have evaluated the association between tumor size and HER2 amplification by FISH in IHC borderline cases. Taucher et al. [19] found that tumor size and HER-2 status were inversely associated, in their study 32.8% (n=22) of 67 patients with tumors larger than 5 cm were HER-2 positive. In contrast, another investigation by Prati et al. [10] did not find any association between HER2 status determined by FISH with tumor size as well as nodal status, presence of LVI, and patient's age. Only tumor grade, P53 positivity, and negative hormone receptors had an association with HER2 positivity in their study. The difference of these results with our study may be explained by the larger size of tumors in our patients which is due to the absence of a breast cancer screening program in our country. The average tumor size of cases in our study was 23.77 ± 12.54 mm which is certainly higher than the other studies.

Our study showed an association between PR negative and HER2 positive characteristics in HER2 IHC borderline patients. Several studies have been conducted about the association of HER2 amplification by FISH and hormone receptor status [10, 13-15, 19-25]. The results of some studies are consistent with ours [10, 15, 19, 21, 26] and others are not [13, 14, 22, 25]. Prati et al. [10] evaluated 200 cases and reported that hormone receptor-positive tumors had a 9.6% incidence of HER2 overexpression and this rate rose to 31.2% for hormone receptor-negative tumors. The evaluation of 134 cases of breast cancer by Okaly et al. [15] showed that ER and PR negative tumors had a 74% and 69% rate of HER2 amplification, respectively. Also Toucher et al. [19] evaluated HER2 status in 923 patients with breast cancer and found that HER2 overexpression was correlated with negative ER/PR and grade III lesion, and young age. In another study on 256 invasive breast cancers, HER2 positive status was significantly associated with negative ER [21]. An association between HER2 overexpression by FISH with negative ER, PR status, negative P53, and high Ki67 labeling index was reported by one study on 100 breast invasive ductal carcinomas [26]. Konecny et al. [22] in 2003 showed that even when tumors were positive for both hormones receptor and HER2, the level of ER/PR were lower than those tumors which had non- amplified HER2 by FISH. In contrast to the previous studies, Shaikh et al. [25] study in Pakistan on 118 breast cancer patients confirmed the relationship between ER and PR positivity, and HER2 overexpression. Also, the result of a study by Guo et al. [14] showed that 43 out of 139 (30.9%) borderline HER2 cases had positive results using the FISH test, and that ER positivity, PR positivity, and tumor grade were three predictive factors that could estimate the probability of positive HER2 results by FISH. On the other hand, there is a study on 108 cases of breast cancer that showed no significant association between hormonal receptor status and HER2 status [13].

Like other studies, HER2 positive ILC was very rare in our study. We had 28 ILC patients and only one of them which was a pleomorphic ILC had a positive HER2 result by FISH. She was a 62 years old lady at the time of diagnosis whose tumor recurred with multiple lung, liver and peritoneal metastases 10 years after the initial treatment. Our observation does agree with others that most cases of ILC with HER2 overexpression represent the pleomorphic variant [27]. Kee et al. [28] reported a higher prevalence of HER2 positive classic type ILC (10.8%) compared with 1-6% in previous ILC case series. In Prati et al. [10] study only three cases of 31 (9.7%) ILCs out of 200 breast cancers had positive HER2 results by FISH. Consistently, HER2 positive classic type ILC as a rare entity was strongly associated with the absence of PR expression in another study [29].

Several studies [10, 19, 21, 23, 30] have revealed the association of poor grade and FISH positivity. Evidence about the low probability of FISH positivity in a low-grade tumor is strong enough to convince some researchers that HER2 assessment may be considered unnecessary in a subgroup of low-grade tumors [10, 19, 31]. In a study on 177 cases of well-differentiated breast cancers that were HER2 borderline on IHC, the rate of HER2 amplification by FISH was 1.7% (3/177) and all three HER2 positive tumors had low levels of amplification [32]. The prevalence of HER2 positivity among patients with well-differentiated tumors was reported from 0% in some studies [21, 24, 30], to less than 5% [10, 33, 34], and less than 10% in Taucher's study [19]. In the latter, the likelihood of HER2 positivity was 6.1% in hormone-receptor-positive patients with grade I and II tumors [19]. Similarly in our study, 10.7 % of patients with low-grade tumors had HER2 amplification by FISH but a statistically significant association between grade and HER2 amplification was not found. However, in our study, the grade of the tumor had not been mentioned on core biopsy samples in many patients, and the pathologists were not able to determine it on many surgical lumpectomy specimens because of complete or near-complete response to neoadjuvant chemotherapy. The missing information about the grade of tumors in this subgroup of patients might have altered our results.

Our study had some advantages. First, our sample size was large enough to find an association, in contrast with many of the previous studies. The second advantage was that all FISH tests had been done by a dedicated referral laboratory in our country. This study had also an important limitation due to missing data about grade, LVI, and PNI, especially in patients who had undergone neoadjuvant chemotherapy which may alter the results.

Conclusions

In conclusion, in HER2 borderline breast cancer, the rate of HER2 positivity is significantly increased with tumor size and PR negativity, and decreased in ILC histology. We couldn't find any applicable model or algorithm to predict the FISH results in borderline HER2 breast cancer in practice, and despite financial issues, we have to recommend further assay by FISH or CISH in all HER2 borderline patients.

Abbreviations

ASCO: American Society of Clinical Oncology

CAP: College of American Pathologist

CI: Confidence interval

CISH: Chromogenic in situ hybridization

DCIS: Ductal carcinoma *in situ*

ER: Estrogen receptor

FISH: Fluorescent in situ hybridization

HER2: Human epidermal growth receptor-2

IHC: Immunohistochemistry

LVI: Lymphovascular invasion

OR: Odds ratio

PNI: Perineural invasion

PR: Progesterone receptor

Declarations

Ethics approval and consent to participate: The Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1397.890) approved the study. Since in this study we reviewed the medical records of patients, informed consent was not necessary.

Consent for publication: Not Applicable

Availability of data and materials: The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Tables

Table 1. Total characteristics of study population (n = 438).

Age (yrs)	50.75 ± 12.17 (26-83)
Pathologic Tumor size (mm)	23.77 ± 12.54 (2-80)
Ki67%	26.73 ± 19.33 (1-90)
Laterality	
Right	206 (47)
Left	232 (53)
Node Involvement	
Positive	190 (43.4)
Negative	230 (52.5)
Unknown	18 (4.1)
Metastasis	
Yes	16 (3.7)
No	422 (96.3)
Tumor Grade	
1	32(7.3)
2	266(60.7)
3	112 (25.6)
Missing	28 (6.4)
Lymphovascular Invasion (LVI)	
No	172 (39.3)
Yes	194 (44.3)
Unknown	72 (16.4)
Perineural Invasion (PNI)	
No	204 (46.6)
Yes	87 (19.9)
Unknown	147 (33.6)
Breast Cancer Type	
IDC	367 (83.8)
ILC	28 (6.4)
Missing	43 (9.8)

Hormone Receptor	
ER +	368 (84)
PR +	334 (76.3)

Data are presented as mean \pm Standard deviation and number (percentage), when appropriate. IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; ER: estrogen receptor(ER), PR: progesterone receptor.

Table 2. Comparison of variables between HER2 positive and negative patients.

Variable	HER2 Positive (n=74)	HER2 Negative (n=364)	P-value
Age	50.62 ± 11.62	50.78 ± 12.30	0.92
Pathologic Tumor Size	27.12 ± 13.81	23.13 ± 12.19	0.02
< 10mm	0 (0)	29 (8.4)	
≥ 10mm	67 (100)	318 (91.6)	0.01
Ki67%	30.03 ± 17.90	26.14 ± 19.54	0.15
< 15	13 (17.6)	96 (26.4)	
≥ 15	61(82.4)	268 (73.6)	0.11
Breast Cancer Type			0.06
IDC	63 (98.4)	304 (91.8)	
ILC	1 (1.6)	27 (8.2)	
Positive Family History			0.62
Yes	23 (31.1)	124 (34.1)	
No	51 (68.9)	240 (65.9)	
Node Involvement			0.45
Yes	35 (49.3)	155 (44.4)	
No	36 (50.7)	194 (55.6)	
Metastasis			0.12
Yes	5(6.8)	11 (3)	
No	69 (93.2)	353 (97)	
Tumor Grade			0.13
1	4 (6)	28 (8.2)	
2	38 (56.7)	228 (66.5)	
3	25 (37.3)	87(25.4)	
Lymphovascular invasion (LVI)			0.44
Positive	34 (57.6)	160 (52.1)	
Negative	25 (42.4)	147 (47.9)	
Perineural invasion (PNI)			0.39
Positive	11 (24.4)	76 (30.9)	

Negative	34 (75.6)	170 (69.1)	
ER			0.36
Positive	58 (81.7)	310 (85.9)	
Negative	13 (18.3)	51 (14.1)	
PR			0.01
Positive	47 (67.1)	287 (80.6)	
Negative	23 (32.9)	69 (19.4)	

IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; ER = estrogen receptor; PR = progesterone receptor.

Table 3. Results of the univariable and multivariable analysis considering HER2 status as a dependent variable.

	Univariable Analysis		Multivariable Analysis	
	Crude OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value
Pathologic size	1.02 (1.01-1.04)	0.02	1.03 (1.01-1.05)	0.006
Ki67% ($\geq 15/ <15$)	1.68 (0.88-3.20)	0.11	1.31 (0.65-2.65)	0.73
Metastasis (Yes/No)	2.33 (0.78-6.90)	0.13	1.88 (0.54-6.61)	0.33
PR (Negative/Positive)	2.04 (1.16-3.58)	0.01	2.14(1.14-4.02)	0.02
Breast Cancer Type (ILC/IDC)	0.18 (0.02-1.34)	0.09	0.15 (0.02-1.18)	0.07

Variables were entered into the multivariable models based on p-value in univariate analysis (p-value <0.15). Therefore, pathologic size (mm), Ki67 category ($\geq 15/ <15$), surgical pathology (IDC and ILC), metastasis, and PR (Negative/Positive) were entered in the model.