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ORIGINAL ARTICLE

HER2-Low breast carcinoma: A newly recognised entity driving paradigm shifts in precision treatment

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Abstract

Introduction: Recent clinical trials have shown the usefulness of anti-HER2 (Human epidermal growth factor receptor 2) treatment in the newly described "HER2-low" subset of breast carcinoma, offering added treatment option to the once considered non-responders. This study aimed to identify this important new subset's demographic and pathological features in a Malaysian cohort. *Materials and Methods:* All newly and recurrent histopathologically-diagnosed invasive breast carcinomas encountered at the University of Malaya Medical Centre (UMMC) between January 2018 to December 2023 that satisfied inclusion criteria were enrolled. Patient demographics were retrieved from the histopathological requests. All haematoxylin and eosin (H&E) stained, immunohistochemically (IHC) stained HER2, oestrogen receptor (ER) and progesterone receptor (PR) together with HER2 amplification assayed by dual-colour dual-hapten in situ hybridisation (DDISH) sections for all cases were reviewed for histological type, histological grade, pathological stage, hormone receptors (HR encompassing ER and PR) and HER2 status. *Results:* 710 invasive breast carcinomas were finally included. HER2-low was noted in 48.2% (n = 342) of the carcinomas, while 191 (26.9%) were in the conventional HER2 positive category. HER2-low carcinomas demonstrated significantly lower (Grade 1 and 2) histological grade and were more commonly hormone status positive compared with both HER2 positive and negative groups. *Conclusion:* HER2-low constituted a significant number of breast carcinomas and appears to have its own unique features. The recognition of this category has expanded anti-HER2 treatment options to an additional 48% of breast carcinomas.

Keywords: Breast carcinoma, HER2; HER2-low

INTRODUCTION

Breast carcinoma was the most common malignancy recorded among Malaysians between 2017-2021, accounting for 17.6% of carcinoma patients.[1] It contributed to 38.9% of female malignancies and is the highest-incidence carcinoma amongst Malaysian females.[1] Encountered more commonly from age 40, a significant peak was observed in the 65-69 age group of females. About 50% of Malaysian breast carcinoma was diagnosed late (Stage 3 and 4, 28% and 22% respectively).[1,2]

HER2 (Human epidermal growth factor receptor 2)-positive breast carcinoma, i.e. breast carcinoma associated with amplification of the *HER2* gene, has been historically acknowledged to be more aggressive with unfavourable prognosis.[3,4] HER2 (also known as ErbB2) gene-amplified carcinoma forms approximately 15-30% of all breast carcinoma.[5-7] HER2, a proto-oncogene located on chromosome 17q21, encodes transmembrane receptors with tyrosine kinase activity. Several pathways, such as the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) and mitogen-activated protein kinase (MAPK) are triggered and activated by HER2, leading to promotion of cell survival, inhibition of apoptosis, and increase of cell proliferation and poor differentiation.[8-10] Several studies have revealed that HER2-targeted therapies have improved patients' survival rate and lessened recurrence risk, with HER2-positive cases being offered treatment, while HER2-

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negative cases, predicted to be unresponsive to anti-HER2 treatment, are typically not offered this intervention.[11,12] Recent clinical trials have, however, found that novel anti-HER2 therapy might benefit a new category of cases labelled as HER2-low positive, which were historically classified as HER2-negative.[13,14] The approval of Trastuzumab deruxtecan (T-DXd) represents a paradigm shift in oncology, as the first antibody-drug conjugate (ADC) indicated for patients with HER2-low breast carcinoma, based on the DESTINY Breast −04 trial.[13,15] Patients treated with this ADC have shown significantly reduced risk of disease progression and death, as well as improved progression-free survival. As such, recent guidelines have been published with recommendations to facilitate the identification of breast carcinoma with HER2-low positivity.[16,17]

Correlation of HER2-low breast carcinoma with clinico-pathological characteristics has been a recent focus in breast cancer research.[18,19] HER2-low breast carcinoma seems to be associated with hormone receptor positivity and is less aggressive with better prognosis when compared with HER2-negative breast carcinoma.[20,21] Our current study aims to identify HER2-low breast carcinoma in a Malaysian setting, to assess its clinico-pathological characteristics in comparison with HER2-positive and HER2-negative cases.

MATERIALS AND METHODS

(I) Recruitment of cases

All newly and recurrent histopathologically diagnosed invasive breast carcinomas, with or without metastasis, encountered at the University of Malaya Medical Centre (UMMC) during the 6-year period from January 2018 to December 2023 were retrieved from the Department of Pathology, University of Malaya Medical Centre's archives. These included invasive breast carcinoma from females and males. Both treatment naïve and post-neoadjuvant-treated cases were included.

The cases were identified using the Laboratory Information System (LIS). After curation to avoid duplication of cases, all histological material of the cases, including the haematoxylin and eosin (H&E) stained sections, as well as the sections immunohistochemically (IHC) stained for oestrogen receptor (ER) protein, progesterone receptor (PR) protein and HER2 oncoprotein, the dual-colour dual-hapten in situ hybridised (DDISH) HER2 slides for the relevant cases were also retrieved. All slides were reviewed. IHC had been performed on Ventana BenchMark XT and DDISH on the Ventana ULTRA automated system. The antibodies used for IHC assays for ER, PR and HER2 were Ventana SP1, 1E2 and 4B5, respectively. DDISH HER2 was carried out using the Ventana HER2 Dual ISH DNA Probe Cocktail. Histological type, Bloom and Richardson grade, ER, PR and HER2 status of the cases were re-confirmed. As pathological staging was not routinely carried out in most cases in our institution, this was carried out during the review. Information on clinical characteristics and history of neoadjuvant treatment were retrieved from the histopathological examination request forms. Carcinomas with IHC HER2 score of 2+ positivity not subjected to HER2 DDISH testing were excluded from the study.

(II) Histological type, Bloom and Richardson grade, ER and PR status, pathological staging

The histological typing of the breast carcinomas was based on the 2019 WHO Classification of Tumours of the Breast, 5th edition and histological grading was based on the Modified Bloom and Richardson Scoring System.[22,23] ER and PR status were categorised according to the ASCO /CAP 2020 guideline.[24] ER status is categorised into low positive when 1-10% of tumour nuclei stained positively, positive when more than 10% tumour nuclei stained positively and negative when less than 1% tumour nuclei stained, regardless of staining intensity. PR is classified into positive, when 1% or more tumour nuclei stained positively, and negative when less than 1% tumour nuclei demonstrated staining. Pathological staging was carried out according to American Joint Committee on Cancer Staging Manual, 8th edition (AJCC 8th ed.) staging system.[25]

(III) HER2 status

HER2 status of the cases were reviewed and re-evaluated using current ASCO/CAP and local Malaysian guidelines.[16,17] IHC HER2 score of 0 was classified as HER2-negative, and HER2-positive when IHC HER2 score was 3+, or 2+ (with positive DDISH HER2 gene amplification). The HER2-low category was defined as HER2 of 1+ on IHC staining or 2+ (with negative DDISH HER2 gene amplification). Representative examples of IHC scores (0, 1+, 2+, and 3+) in breast carcinoma are illustrated in Figure 1, while Figure 2 demonstrates DDISH-confirmed HER2 gene amplification in a case of breast carcinoma.

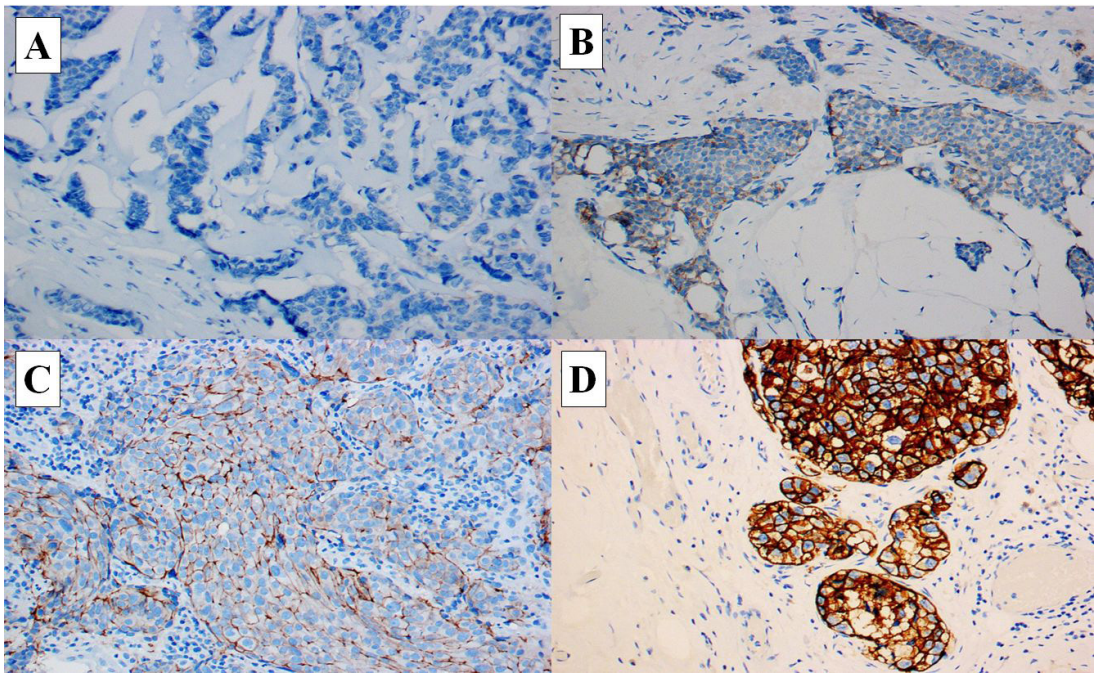

Figure 1. HER2 immunohistochemistry staining pattern (×200).
(a) HER2 (0) with no membrane staining detected. (b) HER2 (1+) with incomplete faint membrane staining. (c) HER2 (2+) demonstrates weak to moderate complete membrane staining. (d) HER2 (3+) shows intense and complete circumferential membrane staining

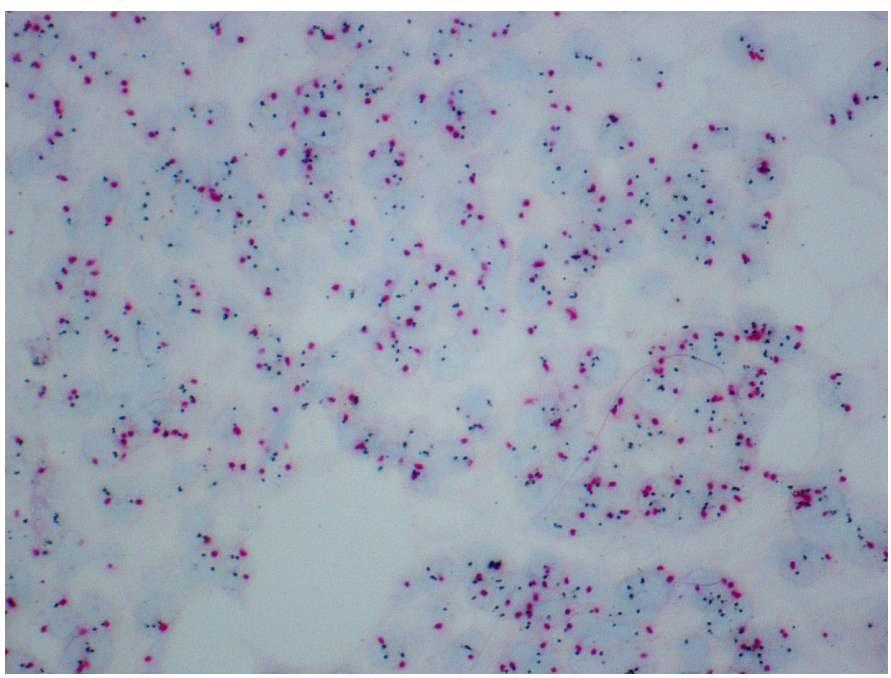

Figure 2. Breast carcinoma with HER2 amplification (dual-colour dual-hapten in situ hybridisation (DDISH) ×400). HER2 is represented by the silver-impregnated black signals while the red signals represent the chromosome 17 probes.

The criteria used for determining ER, PR and HER2 status in this study, are shown in Table 1.[16,17,24]

Statistical analysis

Statistical analysis was performed using Microsoft Excel (Home and Student version 2019). Analyses were conducted to evaluate patients' demographic and clinical characteristics, histological type, Bloom and Richardson grade, pathological stage, ER, PR, and HER2 status. Statistical significance was determined as $p < 0.05$.

Ethical approval

The study was approved by the Institutional Review Board (UMMC-MREC-ID: 2023516-12445) and carried out in compliance with the Declaration of Helsinki. Written informed consent for breast cancer resection was obtained from all patients. The Medical Research Ethics Committee/Institutional Review Board of UMMC permitted waiver of additional patient consent for retrospective studies on archived material of an observational nature, as in this study. Patient confidentiality was safeguarded by restricting data access of data exclusively to the authors of this work.

RESULTS

Demographic profile

In total, 762 invasive breast carcinomas were retrieved from the archives. Fifty-two were excluded as HER2 DDISH testing was not performed although IHC showed HER2 score 2+. A total of 710 invasive breast carcinomas, from 705 patients (703 female; 2 male), were finally included in the study. Five patients had bilateral primary breast carcinomas with differing histological types and hormone receptor status. The demographic profile of the patients is shown in Table 2. Of the 705 patients, 367 (52.1%) were Chinese, 206 (29.2%) Malay, 107 (15.2%) Indian and 25 (3.5%) of other ethnicity. The mean±SD age was 59.88 ±12.3 years, while the ages ranged from 28 to 92 years. More than half of the patients studied (n=408, 57.8%) were in 50-69-years age group. In total, 562 patients (79.7%) were 50 years old or more, and 143 (20.3%) were less than 50 years old.

Histological Type, Bloom and Richardson Grade and Pathological Stage

Of the invasive breast carcinomas, 667(93.9%) were of no specific type (NST) and this formed the main histological type. This was followed by 20 (2.8%) lobular, with other specific types mucinous, metaplastic, micropapillary and adenoid cystic carcinoma totalling 23 (3.3%). Eighty-five (12.0%) were grade 1, 341 (48.0%) grade 2 and 284 (40.0%) grade 3. Pathological staging could only be carried out in 403 (56.8%) of the carcinomas, while no records of the surgical resections were available in our institution in 307. Of the 403 staged carcinomas, 121 (30.0%) were in pathological stage I, 158 (39.2%) stage II, 77 (19.1%) stage III and 47 (11.7%) stage IV.

ER and PR positivity and Hormone Receptor (HR) Status

Of all carcinoma, 500 (70.4%) carcinomas were ER-positive, 201 (28.3%) ER-negative, and 9 (1.3%) ER-low-positive. In total, 413 (58.2%) were PR-positive and 297 (41.8%) PR-??negatives. Finally, 189 (26.6%) were considered HR negative i.e. both ER and PR were negative, while 521 (73.4%) were HR-positive, characterised by ER positivity (including low positivity) and/or PR positivity.

HER2 status

For HER2 immunohistochemical analysis, 177 (24.9%) of the carcinomas were scored as 0, 266 (37.5%) as 1+, 105 (14.8%) as 2+ and 162 (22.8%) as 3+. Among the 105 carcinomas with a 2+ score, 76 (72.4%) showed no *HER2* gene amplification, while 29 (27.6%) demonstrated amplification on DDISH testing. Based on current HER2 reporting guidelines, 342 (48.2%) carcinomas were HER2-low and 191 (26.9%) HER2-positive, both categories now considered potentially amenable to treatment.[16,17] This contrasts with only 191 carcinomas (26.9%) been considered HER2-positive (HER2 IHC 3+ or IHC 2+ with amplification of HER2 on DDISH) and eligible for treatment under the earlier 2018 HER2 reporting guideline.[26]

The histopathological type, Bloom and Richardson grading, hormone receptor (HR) status, HER2 status and pathological stage of the invasive breast carcinoma are shown in Table 3.

Association of HER2 status with ethnicity, age, histological type, Bloom and Richardson grade, hormone receptor (HR) status and pathological stage.

Association of HER2 status with ethnicity, age, histological type, Bloom and Richardson grade, hormone receptor status and pathological stage of the invasive breast carcinomas are shown in

Table 1: Criteria for determination of oestrogen receptor (ER), progesterone receptor (PR), HER2 status using immunohistochemical (IHC) and dual-colour dual-hapten in situ hybridisation (DDISH) methods

ER/ PR/ HER2	Status	Criteria
ER (IHC)	Low Positive	1-10% tumour nuclei stained.
	Positive	more than 10% tumour nuclei stained.
	Negative	none or less than 1% tumour nuclei stained.
PR (IHC)	Positive	1% or more than tumour nuclei stained.
	Negative	none or less than 1% tumour nuclei stained.
HER2	Positive	IHC (3+) or DDISH Group 1 or IHC (2+) with DDISH Group 1 or DDISH Group 3
	Low	IHC (1+) or IHC (2+) with DDISH Group 2 or DDISH Group 4
	Negative	IHC (0) or DDISH Group 5

HER2: IHC (3+): complete and intense membranous staining more than 10% of tumour cells; (2+): weak to moderate complete membranous staining in more than 10% of tumour cells or complete and intense membranous staining in ≤10% of tumour cells; (1+): incomplete membranous staining (faint/barely perceptible) more than 10% of tumour cells; (0): no staining or incomplete membranous staining (faint/barely perceptible) in ≤10% of tumour cells. DDISH: Group 1: HER2/CEP17 ratio ≥2.0 with ≥4.0 HER2 signals/cell; Group 2: HER2/CEP17 ratio ≥2.0 with <4.0 HER2 signals/cell; Group 3: HER2/CEP17 ratio <2.0 with ≥6.0 HER2 signals/cell; Group 4: HER2/CEP17 ratio <2.0 with ≥4.0 to <6.0 HER2 signals/cell; Group 5: HER2/CEP17 ratio <2.0 with <4.0 HER2 signals/cell. NST, no specific type

TABLE 2: Demographic profile of study patients with invasive breast carcinoma

Demographic Profile (n=705)		No of cases (%)
Gender	Female	703 (99.7%)
	Male	2 (0.3%)
Ethnicity	Chinese	367 (52.1%)
	Malay	206 (29.2%)
	Indian	107 (15.2%)
	Others	25 (3.5%)
Age grouping (year)	20-29	1 (0.1%)
	30-39	45 (6.4%)
	40-49	97 (13.8%)
	50-59	178 (25.2%)
	60-69	230 (32.6%)
	70-79	128 (18.2%)
	80-89	25 (3.5%)
	90-99	1 (0.1%)
Age (year)	<50	143 (20.3%)
	≥ 50	562 (79.7%)

Table 4. Table 5 compares the ethnicity, age, histological type, Bloom and Richardson grade, hormone receptor status and pathological stage of invasive breast carcinomas with HER2-negative and HER2-low while Table 6 compares the above between HER2 positive and HER2-low.

Age, histological type, Bloom and Richardson grade, and hormone receptor status showed significant difference ($p<0.05$) between invasive breast carcinomas that were HER2-negative, HER2-low and HER2-positive (Table 4). HER2-low breast carcinoma was more frequently seen in older patients (≥ 50 years old), as compared to the HER2-positive group. Although it did not reach significance, HER2-low breast carcinoma also appeared to be slightly older than those with HER2 negativity.

Compared with HER2-positive invasive breast carcinomas, NST type was less represented among the HER2-low and HER2-negative, with no significant difference in histological type propensity between the latter two. HER2-low carcinomas were of lower grade (Bloom and Richardson grade 1 and 2) compared with HER2-positive and HER2-negative cases. Hormone receptor positivity was also detected more frequently in HER2-low invasive breast carcinomas compared with HER2-negative and HER2-positive cases.

DISCUSSION

As expected, the majority (99.7%) of patients recruited for this study were female. The age of the patients was (mean±SD =59.88 y ±12.3 years), similar to that from other studies.[27,28] In this study, the breast carcinomas were encountered more commonly in women 50-years-old or more, similar to The Malaysian National Cancer Registry.[1,29] The ethnic distribution observed in this study, with Chinese patients forming the majority followed by Malays and Indians, is consistent with the findings of the local cancer registry report.[1,29]

48.2% of the invasive breast carcinomas in this study were classified as HER2-low. This rate falls within the 31-55% previously reported.[11,14,20,30-32] While noteworthy that it appears that HER2-low is as common and important a group among Malaysian breast carcinomas, it is far more important to recognise the fact that this significantly large number of patients, previously deemed unresponsive to anti-HER2 treatment, now have a treatment option shown to reduce the risk of disease progression and death, as well as improved progression-free survival.[16,17] To further reiterate its importance, when previously only about slightly more than a quarter of patients would be treated with anti-HER2, now at least three-quarters can benefit

TABLE 3: Histological type, Bloom and Richardson grade, Hormone Receptor (HR) status, HER2 status and pathological stage of the invasive breast carcinoma

Clinical Characteristic and Pathological Parameters		No of cases (%)
Histological Type (n= 710)	NST	667 (93.9%)
	Lobular	20 (2.8%)
	Others:	23 (3.3%)
	Mucinous (16)	
	Metaplastic (4)	
	Micropapillary (2)	
	Adenoid cystic carcinoma (1)	
Bloom and Richardson grade (n= 710)	1	85 (12.0%)
	2	341 (48.0%)
	3	284 (40.0%)
Hormone Receptor (HR) status (n=710)	HR negative	189 (26.6%)
	HR positive	521 (73.4%)
HER2 status (n= 710)	HER2-negative	177 (24.9%)
	HER2-low	342 (48.2%)
	HER2-positive	191(26.9%)
Pathological Stage (n= 403)	I	121 (30.0%)
	II	158 (39.2%)
	III	77 (19.1%)
	IV	47(11.7%)

from such therapy.

While more carcinomas (25.1%) occurring in patients less than 50-years of age were categorised as HER2-positive (in comparison with 22.0% HER2-negative and 16.4% HER2-low in this age group), the inverse was true in older patients 50-years-old or more. In this older group, HER2-low was predominant (83.6% compared with 78.0% in the HER2-negative and 74.9% in the HER2-positive groups, although the difference did not reach significance for the HER2-negative carcinomas). These findings are also consistent with those observed in other studies, supporting the thesis that HER2-low disease tends to occur in older patients.[20,33]

An overwhelming majority (93.9%) of the carcinomas in this study were classified as invasive carcinoma of no specific type (NST). Notably, a modest but statistically significant increase in the proportion of non-NST histological subtype was observed among the tumours with HER2-low compared with HER2-positive carcinoma. This finding suggests that HER2-low carcinomas may encompass a more heterogeneous histological spectrum than their HER2 positive counterparts. Supporting this

TABLE 4: HER2 status versus ethnicity, age, histological type, Bloom and Richardson grade, hormone receptor (HR) status and pathological stage of the invasive breast carcinomas

	N	HER2-negative (n=177)		HER2-low (n=342)		HER2-positive (n= 191)		P value
		No	%	No	%	No	%	
Ethnicity	710							
Chinese		87	49.1	186	54.4	97	50.8	0.315
Indian		26	14.7	58	17.0	24	12.6	
Malay		56	31.6	86	25.1	65	34.0	
Others		8	4.5	12	3.5	5	2.6	
Age	710	Range: 30-82 mean ±SD: 59.4 ± 23.3		Range: 33-92 mean±SD: 61.3± 12.2		Range: 28-87, mean±SD: 57.8 ± 5.7		
Age (years)	710							< 0.05
< 50		39	22.0	56	16.4	48	25.1	
≥ 50		138	78.0	286	83.6	143	74.9	
Histological Type	710							< 0.05
NST (n=667)		161	91.0	317	92.7	189	99.0	
Lobular (n=20		8	4.5	11	3.2	1	0.5	
Others (n=23)		8	4.5	14	4.1	1	0.5	
Bloom and Richardson grade 1 and 2	710	97	54.8	240	70.2	89	46.6	< 0.05
Grade 3		80	45.2	102	29.8	102	53.4	
HR Status	710							< 0.05
Positive		127	71.8	297	86.8	97	50.8	
Negative		50	28.2	45	13.2	94	49.2	
Pathological Stage	403							0.213
I		33	30.8	63	33.3	25	23.4	
II		38	35.5	74	39.2	46	43.0	
III		22	20.6	37	19.6	18	16.8	
IV		14	13.1	15	7.9	18	16.8	

TABLE 5: Ethnicity, age, histological type, Bloom and Richardson grade, hormone receptor (HR) status and pathological stage of the HER2-negative and HER2-low invasive breast carcinomas

	N	HER2-negative (n=177)		HER2-low (n=342)		P value
		No	%	No	%	
Ethnicity	519					0.379
Chinese		87	49.1	186	54.4	
Indian		26	14.7	58	17.0	
Malay		56	31.6	86	25.1	
Others		8	4.5	12	3.5	
Age (years)	519					0.11
<50		39	22.0	56	16.4	
≥50		138	78.0	286	83.6	
Histological Type	519					0.74
NST		161	91.0	317	92.7	
Lobular		8	4.5	11	3.2	
Others		8	4.5	14	4.1	
Bloom and Richardson Grade	519					< 0.05
1 & 2		97	54.8	240	70.2	
3		80	45.2	102	29.8	
HR Status	519					< 0.05
Positive		127	71.8	297	86.8	
Negative		50	28.2	45	13.2	
Pathological Stage	296					0.521
I		33	30.8	63	33.3	
II		38	35.5	74	39.2	
III		22	20.6	37	19.6	
IV		14	13.1	15	7.9	

observation, Bergeron *et al*.[34] reported that the lobular subtype was more frequently encountered in HER2-low carcinomas than in HER2-positive disease. These findings highlight potential biological distinctions between HER2-low and HER2-positive breast carcinomas, underscoring the importance of further investigation into their molecular characteristics and therapeutic implications.

The majority of the HER2-low carcinomas were categorised as Bloom and Richardson grade 1 and 2 (70.2%) tumours, compared to HER2-negative (54.8%) and HER2-positive (46.6%) carcinomas. For all the lower grade tumours i.e. Bloom and Richardson grade 1 and 2 in this study (n=426), 56.3% (240/426) were HER2-low, 22.8% HER2-negative (97/426) and 20.9% (89/426) HER2-positive. These findings suggest an association of HER2-low with lower histological grade of invasive breast carcinomas, as also noted by others.[19,35,36]

As observed before, this study also demonstrated a significant proclivity for HR expression in the HER2-low carcinomas, where 86.8% of HER2-low breast carcinomas were hormone receptor positive as compared with 50.8% of HER2-positive, and 71.8% of HER-negative carcinomas.[30,37,38]

In this study, the HER2 status was not associated with pathological stage; hence, HER2-low breast carcinomas were not shown to be particularly predominant in any stage. Although

TABLE 6: Ethnicity, age, histological type, Bloom and Richardson grade, hormone receptor (HR) status and pathological stage of the HER2-positive and HER2-low breast carcinomas

	N	HER2-positive (n= 191)		HER2-low (n=342)		P value
		No	%	No	%	
Ethnicity	533					0.475
Chinese		97	50.8	186	54.4	
Indian		24	12.6	58	17.0	
Malay		65	34.0	86	25.1	
Others		5	2.6	12	3.5	
Age (years)	533					<0.05
< 50		48	25.1	56	16.4	
≥ 50		143	74.9	286	83.6	
Histological Type	533					<0.05
NST		189	99.0	317	92.7	
Lobular		1	0.5	11	3.2	
Others		1	0.5	14	4.1	
Bloom and Richardson Grade	533					<0.05
1 & 2		89	46.6	240	70.2	
3		102	53.4	102	29.8	
HR Status	533					<0.05
Positive		97	50.8	297	86.8	
Negative		94	49.2	45	13.2	
Pathological Stage	296					0.08
I		25	23.4	63	33.3	
II		46	43.0	74	39.2	
III		18	16.8	37	19.6	
IV		18	16.8	15	7.9	

NST, no specific type

Gamrani *et al*.[39] reported stage III and Tang *et al*.[40] and Li *et al*.[41] stage IV as common stages for HER2-low breast carcinoma, correlation of HER2-low with stage remains unresolved at this point and will need further studies to provide more insight.

Based on the above, the HER2-low category of invasive breast carcinoma appears to be a unique subset, demonstrating lower histological grades with predilection for HR expression. There may be a propensity for older individuals and a lower tendency to be of the common NST histological subtype. Recognising this new subtype should be emphasised in the face of new treatment options: very importantly, 75% of the breast carcinomas in this study would be potentially amenable to anti-HER2 manipulation instead of the previous 27%.

Having said the above, the definition of this new subtype is predicated on the robust establishment of *HER2* gene amplification, wherein lie some challenges, especially in low-middle income countries with limited funding, technological know-how and infrastructure.[17] In this study alone, 52 of 762 cases (6.8%) of the carcinomas deemed to be HER2 (2+), by IHC

testing and thus requiring gene amplification study for accurate categorisation did not undergo the necessary follow-through examination. While the reasons for failure of these cases to be tested and the possible solutions are beyond the scope of this work, out-of-pocket affordability remains a real constraint in many cases.

As a final cautionary footnote, it is important to acknowledge that the recognition of the HER2- low category, according to the updated ASCO/CAP guideline, is very much dependent on the initial IHC testing, and this should be given its due consideration.[16] Apart from possible technical flaws in the staining, pathologists may still occasionally encounter challenges in the interpretation of HER2 IHC due to tumour heterogeneity, unusual staining patterns or sometimes even deciphering the slightly subjective eyeball cut-offs between the different scores.[42-44] Recent studies suggest that image-based artificial intelligence may improve objectivity with some of the aforesaid issues.[45-48]

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Author contributions: PLC, SFC- Conceptualisation. SFC - Review and re-evaluation of the tumour pathology profiles and classification. SFC – Writing. SFC, PLC, LML- Review and editing. SWC - Review and confirmation of the statistical analysis. All authors contributed to interpretation of findings, critically reviewed and edited the manuscript for intellectual content. All authors read and approved the manuscript.

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