MAEDICA – a Journal of Clinical Medicine
2023; 18(1): 27-34
https://doi.org/10.26574/maedica.2023.18.4.27

MÆDICA - a Journal of Clinical Medicine

ORIGINAL PAPER

Clinicopathological Study of Breast Carcinoma Patients with Equivocal HER2 Immunohistochemical Status: Five-Year Experience from a Tertiary Care Center

Yaqeen AL-ZADJALI[a], Afrah AL-RASHDI[a], Samya AL-HUSAINI[a], Suaad AL-BADI[a], Hajer Al-BADI[a], Mohammad ARAFA[a]

[a]Pathology Department, College of Medicine and Health Sciences, Sultan Qaboos University and Sultan Qaboos University hospital, Oman

ABSTRACT

Background and objectives: *Assessment of HER2 gene status has central role in the management of breast cancer patients. For determining HER2 status, immunohistochemistry (IHC) and fluorescence* in situ *hybridization (FISH) are the most commonly used tests. Immunohistochemistry scores of 3+ and 1+ were considered as HER2 positive and negative, respectively. On the other hand, HER2 equivocal cases need further confirmation by FISH test assessment. This study aimed to identify the clinicopathological characteristics of patients with HER2 equivocal tumors served by Sultan Qaboos University Hospital (SQUH) in Muscat, Oman, with emphasis on treatment plans and disease outcome.*

Methods: *This was a retrospective cross-sectional study, which involved all breast cancer female patients who were referred to SQUH from 2016 to 2020. It included a total of 108 patients who were diagnosed with HER2 (2+) breast cancer. Patients' data were analyzed in relation to the subsequent FISH status.*

Results: *During the study period, data from 108 females with HER2 2+ were collected; among them, 22 (20%) were FISH positive, 64 (59%) FISH negative, 17 (16%) FISH borderline and five (5%) with no results. Regarding patients' characteristics, 91.2% of all subjects had invasive ductal carcinoma, 93.2% expressed estrogen receptors and 77.6% progesterone receptor. Age, postmenopausal histopathology, tumor grade, TNM staging, ER, PR, Ki67, LVSI, NLR, treatment and follow-up did not show significant association with different FISH results.*

Conclusions: *The majority of HER2 equivocal breast cancer cases were FISH negative. In trastuzumab chemotherapy, an association between different FISH results was expected.*

Keywords: breast carcinoma, equivocal HER2, IHC, FISH.

Address for correspondence:
Mohammad Arafa, MD, PhD, Associate Professor
Department of Pathology, College of Medicine and Health Sciences, Sultan Qaboos University, Oman
Tel: (+968) 2414 1123; email: marafa@squ.edu.om

Article received on the 3rd of February 2023 and accepted for publication on the 13th of March 2023

INTRODUCTION

Breast cancer is one of the most commonly diagnosed types of cancer among women globally and it remains the main cause of cancer death among women worldwide (1). Breast cancer is the most frequently seen in Oman, ranking the third in cancer-related mortality in 2016 (2). Between 2015 and 2016, the median age at diagnosis was 46 years, predominantly in female patients, according to the Ministry of Health in Oman (2). In 2016, 269 new breast cancer cases were diagnosed in Oman, representing 27.36% and 10.29% of all cancer cases among woman and men, respectively. It was responsible for 21 deaths among females.

Human epidermal growth factor receptor 2 (HER 2) is a member of the growth factor receptor family. It is encoded by the gene ERBB2 or HER 2/neu, which is located on the long arm of chromosome 17 (17q12). HER2 protein overexpression or HER2 gene amplification may be present in 15-20% of cases (3). Determining HER2 status is crucial to define patients who can benefit from trastuzumab (herceptin) therapy (4). Trastuzumab, a humanized monoclonal antibody which binds to HER 2, improves the outcomes for patients with HER 2 positive breast cancer. Phase 3 randomized trials that involved more than 8,000 patients showed that trastuzumab improved overall survival and reduced the risk of cancer recurrence by approximately 50% (5).

In clinical practice, immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) are used to determine HER2 status (6), with the following interpretation: a score of 3+ means the breast cancer is HER2 positive, a score of 0 and 1+ HER2 negative and a score of 2+ is equivocal. Besides IHC, HER2 status can be further assessed by FISH in the IHC HER2 equivocal cases, which need to be tested more thoroughly to clarify whether they belong to either the positive or negative side (7). Fluorescence in situ hybridization is conducted using either a single probe or dual probes to enumerate HER2 copies per a nucleus. Therefore, HER2 probe and chromosome 17 centromere probes were established; thus, chromosome enumeration probe 17 (CEP17) was used to determine the HER2/CEP17 ratio (8).

Many studies were conducted in order to better understand the features of patients with breast cancer and a HER2 dual equivocal status. A study conducted in China from 2009 January to December 2014 aimed to investigate whether HER2 (2+) could be managed as HER2 negative diseases. Among the 691 participants, 133 (19.25%) were HER2 positive, 533 (77.13%) HER2 negative and 25 (3.62%) HER2 dual equivocal. The authors concluded that HER2 dual equivocal showed more similarities with HER2 negative tumors, whereas the HER2 positive ones revealed different clinicopathological features from them. In 2018, another cohort study, which was conducted by the American Society of Clinical Oncology/College of American Pathologist (ASCO/CAP), showed no significant association between anti HER2 therapy and dual equivocal breast cancer patients, which reclassified as HER2 negative and overall survival (OS) and event free survival (EFS) on either primary disease cohort or recurrent metastatic disease cohort (9). In addition, another cohort study was carried out to explore the impact of alternative chromosome 17 probe (Ch17P) in resolving equivocal cases rule out any deleterious effect of dual equivocal HER2 (8).

The current study aimed to assess the clinicopathological characteristics, treatment and outcome of breast carcinoma patients with an equivocal HER 2 immunohistochemical status, who were treated in Sultan Qaboos University Hospital (SQUH) over a period of five years, with emphasis on treatment plans and disease outcome. ❑

MATERIALS AND METHODS

Study design and settings

This is a retrospective cross-sectional study conducted in SQUH, a tertiary referral center in the capital city of the Sultanate of Oman, Muscat. Before conducting the study, institutional ethical approval was obtained from the Medical Research Ethics Committee (MREC), College of Medicine and Health Science (COMHS) (MERC # 2489), Sultan Qaboos University. This study includes all female patients diagnosed with HER2 2+ breast cancer who were referred to the SQUH over a period of five years (from January 2016 to December 2020).

Data collection

All data were gathered from patient's medical record numbers in the SQUH. Data collection

started by choosing the medical record number "MRN" for 108 female patients who were diagnosed with HER2 2+ breast cancer. Data such as age, menopausal status, tumor grade, histopathology, TNM stage, estrogen receptor, progesterone receptor, Ki67, lymphovascular space invasion (LVSI), neutrophil to lymphocyte ratio (NLR), obtained by dividing the absolute number of neutrophils to the absolute number of lymphocytes, patients' follow-up and their treatment.

Statistical analysis

All data were analyzed using a statistical package for the social sciences (SPSS) software. Patients' data were manually inserted into the SPSS software. For numerical data, percentages were used and presented as tables, pie charts. For measuring the association between different pathological and clinical findings, the categorized variables were analyzed using the chi square test, and P-value of .05 or less were considered to be statistically significant. The end results were used to identify the clinicopathological characteristics, disease outcome and treatment pattern of patients with HER2 equivocal tumors admitted to the pathology lab of SQUH. All variables were addressed using SPSS and generated graphs to support and enhance the research findings. ❑

RESULTS

Demographic features

A total of 108 patients (out of 1197 breast cancer cases) had a HER2 2+ status. For further assessment, FISH analysis was conducted. All subjects were females with a median age of 49 years and a mean age of 51±12.4 years, of whom 22 (20%) were FISH positive, 64 (59%) FISH negative, 17 (16%) FISH borderline and five (5%) with no results (Figure 1).

Patient characteristics

The relation of the HER2 2+ status after FISH analysis, with different clinicopathological features, treatment and outcome, is shown in Table 1.

According to the menopausal status, (37.5%) of patients were in postmenopause and 62.5% in premenopause. The majority of premenopausal subjects were FISH negative (63.1%), 21.5% FISH positive and (15.4%) FISH borderline (p value 0.759).

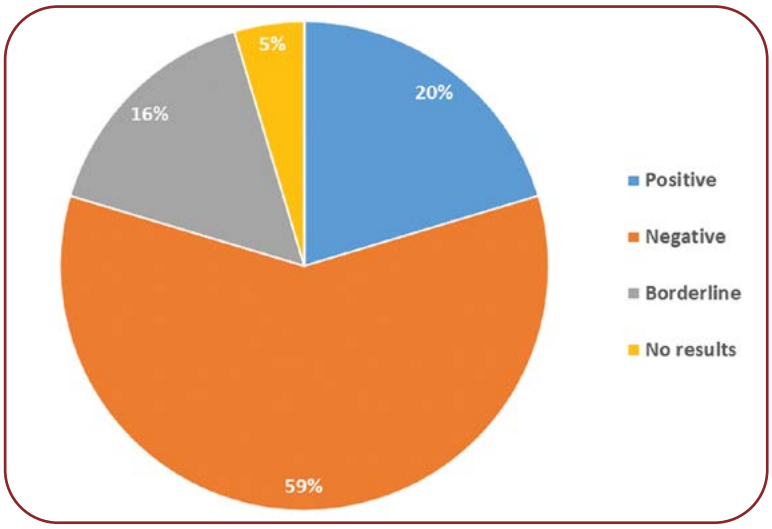

FIGURE 1. FISH result among female patients with HER2 2+ breast carcinoma

Overall, 94 out of 103 cases (five cases were missing) were diagnosed as invasive ductal carcinoma (IDC) (91.2%), 21.3% were FISH positive, 60,6% FISH negative and 18.1% FISH borderline, whereas nine (8.8%) patients were diagnosed as invasive lobular carcinoma (ILC), 22.2% were FISH positive, 77.8% FISH negative and none of all subjects had borderline FISH (p value 0.364).

In terms of tumor grading, there were 79 (76.7%) grade II tumors, with 20.3%, 63.3% and 16.5% of patients being FISH positive, negative and borderline, respectively (p value 0.800).

Regarding TNM staging, 36 (39.6%) cases had T2 stage, of which 25% were FISH positive, 61.1% FISH negative and 13.9% FISH borderline (p value 0.385). About one third of cases (30.0%) without metastases in axillary lymph node (N0) were FISH positive (18.5%), FISH negative (63.0%) and FISH borderline (18.5%) (p value 0.792). About half of cases (49.5%) with no metastasis in other areas of the body (M0) were FISH positive (22.7%), FISH negative (59.1%) and FISH borderline (18.2%) (p value 0.847).

Lymphovascular space invasion was found in 19 (18.6%) of all patients and absent in the majority of them (83; 81.4%).

Estrogen and progesterone receptors were differently distributed among FISH positive, FISH negative and FISH borderline explanation in Table 1. The chi square test showed a significant difference in estrogen receptors (ER) between

TABLE 1. Patient characteristics by different FISH results

Characteristics	Total n=108 (%)	FISH positive n=22 (20%)	FISH negative n=64 (59%)	FISH borderline n=17 (16%)	P value
Postmenopause					0.759
Yes	39 (37.5)	9 (23.1)	26 (66.7)	4 (10.3)	
No	65 (62.5)	14 (21.5)	41 (63.1)	10 (15.4)	
Site of cancer					0.527
Right breast	51 (49.5)	9 (17.6)	34 (66.7)	8 (15.7)	
Left breast	52 (50.5)	14 (26.9)	31 (59.6)	7 (13.5)	
Histopathology					0.364
IDC (invasive ductal carcinoma)	94 (91.2)	20 (21.3)	57 (60.6)	17 (18.1)	
ILC (invasive lobular carcinoma)	9 (8.8)	2 (22.2)	7 (77.8)	0	
Tumor grade					0.800
I	7 (6.8)	1 (14.3)	4 (57.1)	2 (28.6)	
II	79 (76.7)	16 (20.3)	50 (63.3)	13 (16.5)	
III	17 (16.5)	5 (29.4)	10 (58.8)	2 (11.8)	
T (of TNM)					0.385
T1	21 (23.0)	5 (23.8)	12 (57.1)	4 (19.0)	
T2	36 (39.6)	9 (25.0)	22 (61.1)	5 (13.9)	
T3	15 (16.5)	0	13 (86.7)	2 (13.3)	
T4	19 (20.9)	6 (31.6)	10 (52.6)	3 (15.8)	
N (of TNM)					0.792
Nx	2 (2.2)	1 (50.0)	1 (50.0)	0	
N0	27 (30.0)	5 (18.5)	17 (63.0)	5 (18.5)	
N1	32 (35.6)	5 (15.6)	22 (68.8)	5 (15.6)	
N2	9 (10.0)	3 (33.3)	6 (66.7)	0	
N3	20 (22.2)	5 (25.5)	11 (55.0)	4 (20.0)	
M (of TNM)					0.847
Mx	22 (24.7)	4 (18.2)	14 (63.6)	4 (18.2)	
M0	44 (49.5)	10 (22.7)	26 (59.1)	8 (18.2)	
M1	23 (25.8)	5 (21.7)	16 (69.6)	2 (8.7)	
Ki67 labelling index					0.662
<20	31 (30.7)	6 (19.4)	21 (67.7)	4 (12.9)	
>=20	70 (69.3)	16 (22.9)	41 (58.6)	13 (18.6)	
LVSI (lymphovascular space invasion)					0.834
Present	19 (18.6)	4 (21.1)	11 (57.9)	4 (21.1)	
Absent	83 (81.4)	17 (20.5)	53 (63.9)	13 (15.7)	
NLR (neutrophils to lymphocytes ratio)					0.147
<1.26	35 (34.7)	11 (31.4)	22 (62.9)	2 (5.7)	
1.26-1.85	22 (21.8)	3 (13.6)	13 (59.1)	6 (27.3)	
>1.85	44 (43.5)	8 (18.2)	27 (61.4)	9 (20.5)	
ER (estrogen receptors)					0.053
Positive	96 (93.2)	18 (18.8)	62 (64.6)	16 (16.7)	
Negative	7 (6.8)	4 (57.1)	2 (28.6)	1 (14.3)	
PR (progesterone receptors)					0.203
Positive	80 (77.7)	14 (17.5)	52 (65.0)	14 (17.5)	
Negative	23 (22.3)	8 (34.8)	12 (52.2)	3 (13.0)	
Treatment					0.680
Chemotherapy	13 (13.0)	4 (30.8)	6 (46.2)	3 (23.1)	
Hormonotherapy	1 (1.0)	0	1 (100.0)	0	
Chemo-radio	17 (17.0)	5 (29.4)	10 (58.8)	2 (11.8)	
Radio-hormone	9 (9.0)	1 (11.1)	7 (77.8)	1 (11.1)	
Chemo-hormone	16 (16.0)	3 (18.8)	13 (81.3)	0	
Chemo-radio-hormone	44 (44.0)	10 (22.7)	26 (59.1)	8 (18.2)	
Trastuzumab					5.43×10^{-14}
Yes	24 (24.2)	19 (79.2)	2 (8.3)	3 (12.5)	
No	75 (75.8)	3 (4.0)	59 (78.7)	13 (17.3)	
Follow-up					0.618
Cure	62 (62.6)	15 (24.2)	38 (61.3)	9 (14.5)	
Recurrence	24 (24.3)	5 (20.8)	16 (66.7)	3 (12.5)	
Death	13 (13.1)	2 (15.4)	7 (53.8)	4 (30.8)	

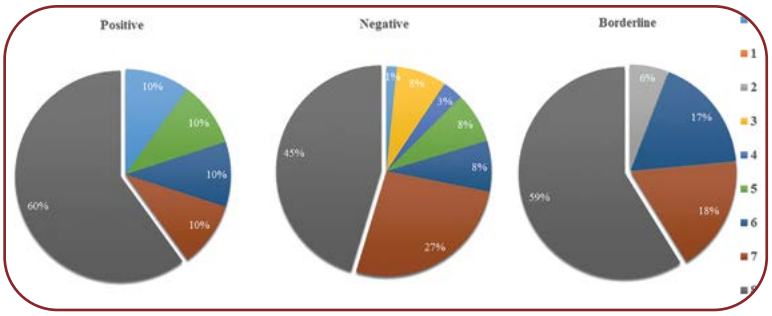

FIGURE 2. Estrogen receptor (ER) scores distribution among the different FISH results. The scores (1 to 8) correspond to Allred score, which is routinely used for the evaluation of ER immunohistochemical staining, considering the staining intensity and the percentage of positive cells

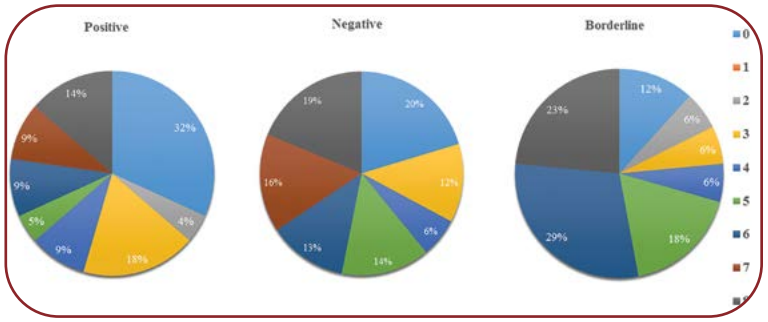

FIGURE 3. Progesterone receptor scores distribution among the different FISH results. The scores (1 to 8) correspond to Allred score, which is routinely used for the evaluation of ER immunohistochemical staining, considering the staining intensity and the percentage of positive cells

different FISH results (FISH positive, FISH negative and FISH borderline). Ninety-six (93.2%) of all patients had estrogen receptor expression, of which 18 (18.8%) were FISH positive, 64.6% FISH negative and 16.7% FISH borderline. On the other hand, 77.7% of patients had progesterone receptor expression (PR), of which 17.5% were FISH positive, 65.0% FISH negative and 17.5% FISH borderline. By using immunohistochemical assessment, estrogen and progesterone receptor expression scored from 0 to 8. Estrogen receptor score 8 was highly dominant among other scores, with 60%, 45% and 59% of cases being FISH positive, negative and borderline, respectively (Figure 2). There was a fluctuation in progesterone score among different FISH results, as score 0 was highly dominant among FISH positive (32%) and FISH negative (20%) cases, but score 6 was highly dominant in FISH borderline (29%) cases (Figure 3).

Regarding the proliferation index (Ki67), 20 out of 101 cases (seven cases were missed) had a

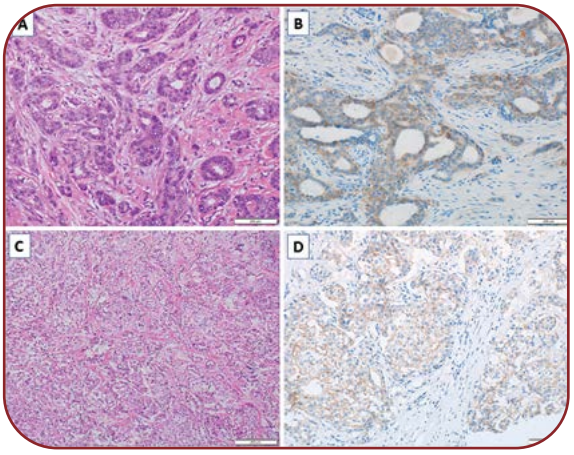

FIGURE 4. Examples of two cases of invasive ductal carcinomas (NST) grades 1 (A, H&E 200x) and 2 (C, H&E 200x), with both being HER2 equivocal (2+) by IHC (B, immunoperoxidase 200x) and (D, immunoperoxidase 200x), as shown by the complete week to moderate membranous staining in more than 10% of invasive tumor cells, but turning to be negative by FISH

proliferating index more than equal 20, with 22.9% of cases being FISH positive, 58.6% FISH negative and 18.6% FISH borderline, while 30.7% of patients had a proliferating index less than 20. There was no significant difference in Ki67 between different FISH results since (p value > 0.05) (Table 1).

Regarding the neutrophil to lymphocyte ratio (NLR), 35 out of 101 cases (seven cases were missed) had NLR less than 1.26, with 22 patients having an NLR 1.26–1.85 and 44 of them an increased NLR (> 1.85) (Table 1).

Patients' management and outcome

Out of 100 patients (eight were missed), 13.0% received chemotherapy, 1.0% hormone therapy, 17.0% both chemotherapy and radiation, 9.0% both radiation and hormone therapy, 16.0% both chemotherapy and hormone therapy and 44.0% all three types of treatment (chemotherapy, radiation and hormone therapy). Chi square test showed no association in treatment between different FISH results (p value 0.680). The majority of patients (62.6%) were cured from HER2 2+ breast cancer, with 24.2% of them having been FISH positive, 61.3% FISH negative and 14.5% FISH borderline. Twenty-four patients had breast cancer recurrence and 13 of them unfortunately dead (Table 1). Nineteen (79.2%) patients had a FISH positive diagnosis and were given trastu-

zumab (herceptin) chemotherapy, while three of them (4.0%) did not receive it. Also, 59 (78.7%) patients had a FISH negative diagnosis and did not receive trastuzumab, whereas two of them (8.3%) did. The chi square test showed significance difference in trastuzumab chemotherapy between a different FISH status (p value < 0.05) (Table 1). A representative example from one case is illustrated in Figure 4. ❑

DISCUSSION

This is a retrospective cross-sectional study aimed to identify the clinicopathological characteristics of patients with HER2 equivocal tumors served by SQUH over a period of five years.

There was a small difference in comparison to prior published data regarding patients' demographics. Older age at presentation in SQUH is a frequent finding, the median age in our study is about 49 years old (age range 25–85 years), similarly to findings from a prior study conducted in India (subjects' median age at presentation 50 years, with an age range between 25 and 81 years) (10).

In our study, out of 108 cases, 22 (20%) patients were FISH positive, 64 (59%) FISH negative, 17 (16%) FISH borderline and five (5%) with no results, which was in contrast with the above-mentioned study (10), that found HER2 amplification by FISH in 72 (54%) cases, while there was no HER2 amplification in 52 (39%) patients and ten subjects were identified as borderline FISH (10).

According to the type of breast cancer, IDC was dominant in our study, with 94 (21.3%) patients being FISH positive, 60.6% FISH negative and 18.1% FISH borderline, which was different from a previous study done in the US showing that 80% of all subjects had known or unknown HER2 assays, 12% were FISH positive, 7% FISH negative and 1% FISH borderline (11).

Regarding the tumor grading, we found no significant difference in grade between different FISH results, which was in agreement with the findings of a previous study (4).

Regarding TNM staging, there was no significant association between TNM stage and the FISH results, which was not consistent with a study conducted in 2014 that found a significant difference in TNM staging between different FISH results (12).

The hormone receptor status for estrogen receptor (ER) and progesterone receptor (PR) was also analyzed for all cases. The immunohistochemical expression of ER and PR determines whether a patient can be treated with hormonal therapy that will block these receptors leading to slow the tumor growth (4). We found no significant difference in the immunohistochemical ER and PR expression between the various FISH results. A previous study done in Shanghai showed significant differences between ER and different FISH results, which was in disagreement with our results, but found no difference between PR and different FISH results, which was in agreement with our results (4).

The proliferation index, as assessed by the immunohistochemical expression of Ki67, is strongly associated with tumor cell growth and proliferation, and is therefore used as a proliferation marker in routine pathological investigation (13). In our study, there was no significant difference in Ki67 labeling indices between the various FISH results. In comparison, a Chinese retrospective study found that Ki67 statuses were differently distributed among FISH positive, FISH negative and FISH borderline (4); it also revealed that patients with higher Ki67 had more advanced breast cancer compared to those with lower Ki67 due to the association with rapidly growing and dividing cells.

Neutrophil to lymphocyte ratio is not among the routinely assessed variables of patients with breast cancer. However, we chose to explore this parameter because NLR has been now considered as an inflammatory marker that could be raised in some tumors, including breast cancer (14). In our study there was no significant difference between NLR and various FISH results, which was inconsistent with the findings of a previously conducted study in Asian women (15), reporting that NLR was significantly higher in breast cancer patients, with NLR >1.67 being related to an increased risk of breast cancer. Multiple studies conducted in US, Scotland and UK showed that higher NLR was associated not only with more aggressive and advanced breast cancer but also with adverse survival (14, 16, 17).

Trastuzumab is an anti HER2 therapy proven to be effective in blocking HER2 in FISH positive patients (18). We found an expected significant

difference in trastuzumab chemotherapy between different FISH results, which was consistent with a retrospective study from China (4). In our study, 19 FISH positive patients received trastuzumab, less than the 25 subjects from the Chinese study (4). There were three HER2 2+ FISH positive patients who did not receive trastuzumab because of certain drug contraindications and intolerance. Also, 59 of our patients did not receive trastuzumab because their HER2 was not amplified or overexpressed, so they would not benefit from trastuzumab, and two FISH negative patients received trastuzumab. A study carried out in the University of Michigan, US, revealed that HER2 negative patients could benefit from trastuzumab. The explanation is that HER2 is selectively expressed in cancer stem cells of many HER2 negative breast tumors. Moreover, given that stem cells are not at all numerous in the tumor, the amount of HER2 is not sufficiently high as to meet the threshold for a HER2 positive cancer (19).

There was no significant association in recurrence of breast cancer between different FISH results, which was consistent with a previous US study showing that patients with HER2 equivocal status did not have a significantly higher risk of recurrence (7).

Our study has some limitations. First, it included only one tertiary health center (SQUH), albeit a large referral unit, so the results may not be representative for the entire population of Oman. A small sample size of only 108 HER2 2+ patients was not enough to show the significance of some variables. Moreover, there was some missing data in patients' record system, including TNM staging, ER and PR. Also, the present study was restricted to HER2 2+ female patients only.

Further studies concerning the clinicopathological characteristics of patients with HER2 equivocal tumors needed to be conducted. We aim to continue this study by including a larger sample size and multiple health centers in Oman. We also plan to include both male and female patients in order to compare the results between both sexes. In addition, we aim to study some additional characteristics of HER2 2+ patients in comparison with HER2 positive and HER2 negative patients using the same variables in our study. ❑

CONCLUSIONS

The majority of HER2 equivocal breast cancer cases had a FISH negative status, showing a negative HER2 expression. An expected association in anti HER2 therapy (trastuzumab) between different FISH results was observed. HER2 equivocal cases seemed to have a clinical behavior similar to that of HER2 negative breast cancers. We recommend, future expansion of study to compare with HER2 positive and HER2 negative cases. Also, we recommend to expand the study at the national level. ❑

Ethics statement: The study was approved by the ethical committee of Sultan Qaboos University "Medical Research Ethics Committee (MREC), College of Medicine and Health Science (COMHS) (MERC # 2489)". Formal written informed consent was not required with a waiver by the IRB.

Authors' contributions: MA – conceptualization; YZ, AR, SH, HB and SB – data collection; YZ, AR, SH, HB and SB – formal analysis; YZ and MA – writing, review & editing.

Conflicts of interest: none declared.

Financial support: none declared.

References

1. **Bray F, Ferlay J, Soerjomataram I, et al.** Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424. doi: 10.3322/caac.21492.
2. **Al-Bahrani DB, Al-Lawati DNA, Al-Siyabi N, et al.** Cancer Incidence in Oman. 2016, Ministry of health. Accessed: 24 April 2022. https://www.moh.gov.om/documents/272928/1232802/Cancer+Incidence+In+Oman+2016/3ff52524-8983-6088-147b-fd09f7f262b4.
3. **Loibl S, Gianni L.** HER2-positive breast cancer. *Lancet* 2017;389:2415-2429. doi: 10.1016/S0140-6736(16)32417-5.
4. **Tong Y, Chen X, Fei X, et al.** Can breast cancer patients with HER2 dual-equivocal tumours be managed as HER2-negative disease?. *Eur J Cancer* 2018;89:9-18. doi: 10.1016/j.ejca.2017.10.033
5. **Petroni S, Caldarola L, Scamarcio R, et al.** FISH testing of HER2 immunohistochemistry 1+ invasive breast cancer with unfavorable characteristics. *Oncol Lett* 2016;12:3115-3122. doi: 10.3892/ol.2016.5125.
6. **Hanna WM, Rüschoff J, Bilous M, et al.** HER2 in situ hybridization in breast

cancer: clinical implications of polysomy 17 and genetic heterogeneity. *Mod Pathol* 2014;27:4-18. doi: 10.1038/modpathol.2013.103

7. **Criscitiello C, Bagnardi V, Viale G, et al.** HER2 Equivocal Status in Early Breast Cancer Is Not Associated with Higher Risk of Recurrence. *Anticancer Res* 2016;36:3537-3540.
8. **Sneige N, Hess KR, Multani AS, et al.** Prognostic significance of equivocal human epidermal growth factor receptor 2 results and clinical utility of alternative chromosome 17 genes in patients with invasive breast cancer: A cohort study. *Cancer* 2017;123:1115-1123. doi: 10.1002/cncr.30460.
9. **Crespo J, Sun H, Wu J, et al.** Rate of reclassification of HER2-equivocal breast cancer cases to HER2-negative per the 2018 ASCO/CAP guidelines and response of HER2-equivocal cases to anti-HER2 therapy. *PLoS One* 2020;15:e0241775. doi: 10.1371/journal.pone.0241775.
10. **Patil Okaly GV, Panwar D, Lingappa KB, et al.** FISH and HER2/neu equivocal immunohistochemistry in breast carcinoma. *Indian J Cancer* 2019;56:119-123. doi: 10.4103/ijc.IJC_333_18.
11. **Zujewski JA, Harlan LC, Morrell DM, Stevens JL.** Ductal carcinoma in situ: trends in treatment over time in the US. *Breast Cancer Res Treat* 2011;127:251-257. doi: 10.1007/s10549-010-1198-z.
12. **Bagaria SP, Ray PS, Sim MS, et al.** Personalizing breast cancer staging by the inclusion of ER, PR, and HER2. *JAMA Surg* 2014;149:125-129. doi: 10.1001/jamasurg.2013.3181.
13. **Li LT, Jiang G, Chen Q, Zheng JN.** Ki67 is a promising molecular target in the diagnosis of cancer (review). *Mol Med Rep* 2015;11:1566-1572. doi: 10.3892/mmr.2014.2914.
14. **Koh CH, Bhoo-Pathy N, Ng KL, et al.** Utility of pre-treatment neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as prognostic factors in breast cancer. *Br J Cancer* 2015;113:150-158. doi: 10.1038/bjc.2015.183.
15. **Gago-Dominguez M, Matabuena M, Redondo CM, et al.** Neutrophil to lymphocyte ratio and breast cancer risk: analysis by subtype and potential interactions [published correction appears in *Sci Rep*. 2020 Nov 20;10(1):20641]. *Sci Rep* 2020;10:13203. Published 2020 Aug 6. doi: 10.1038/s41598-020-70077-z.
16. **Azab B, Bhatt VR, Phookan J, et al.** Usefulness of the neutrophil-to-lymphocyte ratio in predicting short- and long-term mortality in breast cancer patients. *Ann Surg Oncol* 2012;19:217-224. doi: 10.1245/s10434-011-1814-0.
17. **Proctor MJ, McMillan DC, Morrison DS, et al.** A derived neutrophil to lymphocyte ratio predicts survival in patients with cancer. *Br J Cancer* 2012;107:695-699. doi: 10.1038/bjc.2012.292
18. **Lee HJ, Seo AN, Kim EJ, et al.** HER2 heterogeneity affects trastuzumab responses and survival in patients with HER2-positive metastatic breast cancer. *Am J Clin Pathol* 2014;142:755-766. doi: 10.1309/AJCPIRL4GUVGK3YX.
19. **Boltz, K.** (2013) Trastuzumab may also be effective for women with HER2-negative breast cancer - Oncology Nurse Advisor. Accessed: 24 April 2022. https://www.oncologynurseadvisor.com/home/headlines/web-exclusives/trastuzumab-may-also-be-effective-for-women-with-her2-negative-breast-cancer/.