


RESEARCH

Open Access



Comparative analysis of metabolic characteristics and prognostic stratification of HER2-low and HER2-zero breast cancer using ^{18}F -FDG PET/CT imaging

Yuan Gao^{1,2†}, Lei Yin^{1†}, Linlin Ma¹, Caixia Wu¹, Xiaojuan Zhu³, Hongjin Liu², Li Liang³, Jinzhi Chen¹, Yulong Chen¹, Jingming Ye², Ling Xu² and Meng Liu^{1*} 

Abstract

Background Recent advancements in novel anti-human epidermal growth factor receptor 2 (HER2) antibody-drug conjugates (ADCs) have highlighted the emerging HER2-low breast cancer subtype with promising therapeutic efficacy. This study aimed to comparatively analyze the metabolic characteristics and prognostic stratification of HER2-low and HER2-zero breast cancer using baseline fluorine-18 fluorodeoxyglucose (^{18}F -FDG) positron emission tomography/computed tomography (PET/CT) imaging.

Methods Consecutive patients with newly diagnosed breast cancer who underwent ^{18}F -FDG PET/CT prior to therapy in our hospital were retrospectively reviewed. The relationship between metabolic parameters (maximum standardized uptake value (SUV_{max}), tumor-to-liver SUV ratio (TLR), total lesion glycolysis (TLG), and metabolic tumor volume (MTV)) in primary lesions and HER2 expression was analyzed. The survival analyses were performed to identify the prognostic factors for disease-free survival (DFS) in patients with HER2-negative (HER2-low versus -zero).

Results In total, 258 patients (mean age: 54 ± 12 years) were included. In hormone receptor (HR)-positive subgroup, SUV_{max} and TLR were significantly higher in HER2-low than in HER2-zero ($P = 0.045$ and 0.03 , respectively). But in HR-negative subgroup, there was no significant metabolic difference between HER2-low and HER2-zero (All $P > 0.05$). The four metabolic parameters were significant predictors of DFS in HER2-negative patients (All $P < 0.01$), but there was no significant difference in DFS between HER2-low and -zero, regardless of tumor metabolism. Moreover, in HER2-zero patients, the DFS of patients with high metabolism was significantly shorter than that of patients with low metabolism ($P_{\text{SUVmax}} = 0.002$, $P_{\text{MTV}} = 0.03$, $P_{\text{TLG}} = 0.005$, $P_{\text{TLR}} < 0.001$, respectively), but without a similar finding in HER2-low patients.

Conclusion Our study demonstrated the HR-positive HER2-low breast cancer exhibited a particularity in glucose metabolic profile. Additionally, HER2-zero patients with elevated metabolism were associated with inferior prognosis and warranted careful attention in clinical evaluations.

Keywords ^{18}F -FDG PET/CT, Breast cancer, HER2-low, Prognosis, Antibody-drug conjugates (ADCs)

[†]Yuan Gao and Lei Yin contributed equally to this work.

*Correspondence:

Meng Liu

loulisa_liu@bjmu.edu.cn

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Background

Given the encouraging results achieved by human epidermal growth factor receptor 2 (HER2)-targeted antibody-drug conjugates (ADCs) in breast cancer patients with HER2-low [1], it is important to fully understand the biological and clinical features of this subgroup [2, 3], as well as noninvasively stratify patients prior to treatment. So far, some studies have attempted to explore the differences in clinicopathological characteristics, biological behaviors, and prognosis between patients with HER2-low and -zero, but no consistent conclusions have been drawn to support HER2-low as an independent subtype [4–6].

The introduction of HER2-low changes the categorization of HER2 expression profiles, transforming from a dichotomous classification of HER2-negative versus -positive to a trichotomous classification of HER2-zero, -low, and -positive. Recently, multiparametric MRI radiomics has been performed to distinguish HER2-low from HER2-negative breast cancers [7, 8]. However, the characteristics regarding the glucose metabolic profiles in breast cancer with HER2-low, particularly compared to HER2-zero, has not been explored.

As a noninvasive technique to detect glucose metabolism in lesions, ^{18}F -FDG PET/CT is increasingly being applied in the systemic evaluation of breast cancer, especially in pretreatment staging and therapeutic monitoring of advanced breast cancer [9]. In the process of tumor progression, proliferative tumor cells undergo metabolic remodeling and depend on glucose metabolism heavily, resulting in increased FDG accumulation in primary or metastasis lesions [10]. Since the expression of HER2 is involved in regulating the proliferation, survival, and metastasis of tumor cells [11], it is speculated that various HER2-expressing populations may exhibit different glucose metabolic phenotypes.

Significantly, HER2 expression exhibits temporal and spatial heterogeneity, specifically reflected in HER2 expression changes throughout treatments (temporal heterogeneity), differences at various locations within the same tumoral lesion (spatial intralesional heterogeneity), and inconsistencies between primary and recurrent/metastatic lesions (spatial interlesional heterogeneity), which might impact treatment response and resistance [12, 13]. ^{18}F -FDG PET/CT, as a non-invasive, dynamic, and whole-body examination, has potential advantages in reflecting tumor heterogeneity.

To date, some studies have shown that breast cancer with HER2 overexpression is characterized by high glucose metabolism [14–16]. Other studies have indicated

that elevated baseline metabolism in primary tumors is associated with poor prognosis in patients with hormone receptor (HR)-positive HER2-negative breast cancer [17, 18]. To the best of our knowledge, the potential prognostic value of baseline ^{18}F -FDG PET/CT in HER2-low patients is still unclear.

In this study, we tried to comparatively analyze the metabolic characteristics of HER2-low and HER2-zero breast cancer using baseline ^{18}F -FDG PET/CT. Besides, the prognostic stratification of metabolic parameters in patients with HER2-low and -zero were analyzed, in order to explore the potential value of ^{18}F -FDG PET/CT in the hierarchical management of these patients.

Materials and methods

Patient characteristics

The data of consecutive breast cancer patients, who underwent ^{18}F -FDG PET/CT examination for evaluating of possible metastasis from January 2017 to October 2021 in our hospital, were retrospectively reviewed.

The inclusion criteria were as follows: (1) newly diagnosed breast cancer by primary tumor biopsy or surgical pathology; (2) a baseline ^{18}F -FDG PET/CT examination performed within two months prior to treatment. The exclusion criteria were as follows: (1) the data of immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) of HER2 missing; (2) Carcinoma in situ.

For further prognostic analysis, HER2-low and -zero patients in stage I-III who received operations were included. To remove confounding factors, the additional exclusion criteria were proposed as follows: (1) lost to follow-up; (2) presence of other synchronous primary malignancy or a history of malignancies; (3) bilateral synchronous breast cancer.

The documented clinicopathological parameters included age, gender, body mass index (BMI), menopausal status, tumor node metastasis stage [19], tumor histology, histologic grade, estrogen receptor (ER), progesterone receptor (PR), Ki-67, molecular classification, and therapeutic regimen. The metastatic lymph nodes were determined by baseline pathological results, including core needle biopsies and surgical specimens.

Patients' records were anonymized and de-identified before analysis. The retrospective data collection and analysis procedures were approved by the ethics committee of our hospital, waiving the need for written informed consent.

Imaging analysis of ^{18}F -FDG PET/CT

As described in previous studies [20], all patients fasted for at least 6 h before ^{18}F -FDG PET/CT examination, and

the images were acquired and independently reviewed by two experienced senior nuclear medicine physicians, who were blinded to all patients' information. If the results differed, they discussed the findings and then reached a consensus.

According to the ^{18}F -FDG PET/CT images, the metabolic parameters of primary tumor, including maximum standardized uptake value (SUVmax), mean standardized uptake value (SUVmean), and metabolic tumor volume (MTV), were measured by outlining a volume of interest, which was carefully put on the primary lesion to encompass the entire tumor. The parameters of SUVmean and MTV were calculated using an SUV threshold of 40% of SUVmax [21]. SUVmean of the liver was measured by drawing a volume of interest in the center of an area of non-diseased right hepatic lobe (diameter of 3 cm) [22]. Total lesion glycolysis (TLG) was automatically obtained as MTV multiplied by SUVmean, and tumor-to-liver SUV ratio (TLR) was defined as tumor SUVmax divided by liver SUVmean [23].

In the case of unilateral multifocal tumors, the parameters of the highest metabolic tumor were used for prognostic analysis.

Immunohistochemical evaluation

The results of hematoxylin and eosin staining (HE) and IHC were independently reviewed by two pathologists who were unaware of the outcomes. Differences in diagnosis between the two pathologists were resolved by re-reviewing the biopsies to reach a consensus. ER and PR expression were classified as positive when clear cell membranous staining $\geq 1\%$ [24]. HR-positive means ER-positive, PR-positive, or both. HER2 expression was judged according to the HER2 detection standard [25]. IHC 0 was considered as HER2-zero, IHC 1+ and IHC 2+ with FISH (-) were HER2-low, while IHC 2+ with FISH (+) and IHC 3+ were HER2-positive. HER2-negative included both HER2-low and HER2-zero. Ki-67 $\geq 30\%$ was considered the threshold for the classification of high and low Ki-67. The histologic grades were evaluated based on the Nottingham grading system [26]. Based on the expression of ER, PR, HER2, and Ki-67, breast cancer was divided into four molecular types [27].

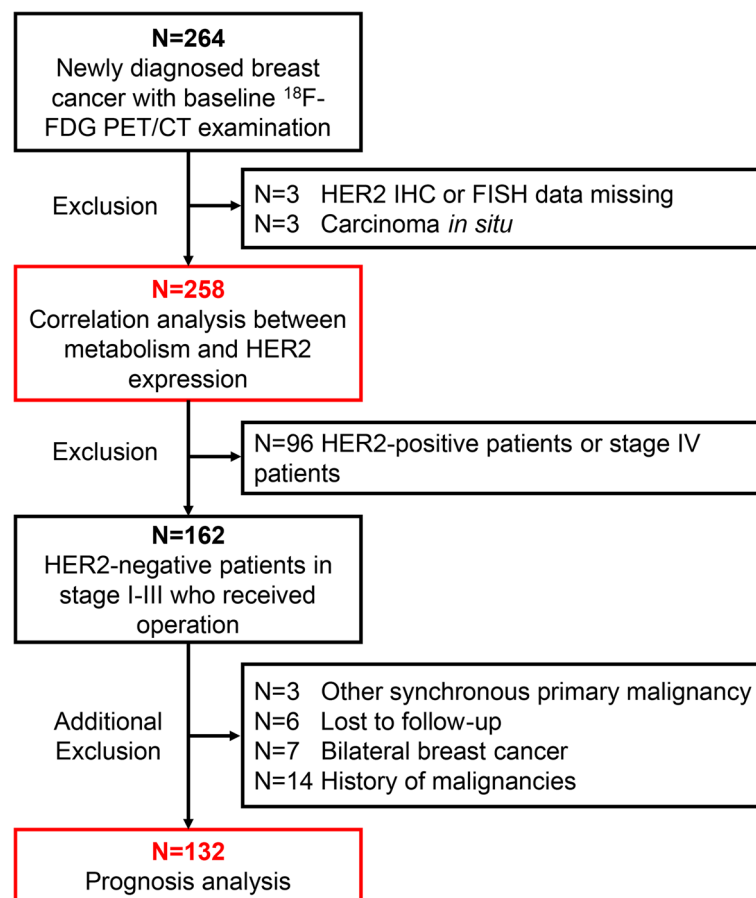


Fig. 1 Flowchart of patient inclusion and exclusion

Follow up and clinical endpoints

Follow-up surveillance after operation included breast and axillary lymph node ultrasound, breast MRI, abdominal ultrasound, abdominopelvic CT, chest X-ray, chest CT, and laboratory data. During the follow-up period, the information of every patient was first collected from 3 months to 6 months after operation, then regularly collected every 6 months for the first 3 years, and annually afterward. Gynecologic ultrasound was reviewed annually in patients receiving endocrine therapy.

Disease-free survival (DFS) was defined as the time from the date of primary surgery to the date of the radiological or histological evidence of recurrence for the first time, death of any cause, or censored at the last follow-up, whichever came first [6].

Statistical analysis

Continuous data were expressed as medians with interquartile range (IQR) in parentheses or mean ± standard deviation (SD), and categorical variables were shown as numbers (percentages). Analysis of variance (ANOVA), Kruskal-Wallis test, Chi-Squared test or Mann-Whitney U test was carried out to compare the variables between different groups, respectively. Bonferroni correction was applied in multiple comparisons. Jonckheere-Terpstra test (J-T test) was used for trend testing.

Receiver operating characteristic (ROC) curves were analyzed for the optimal cutoff value and area under the curve (AUC) for the continuous variables. Survival analysis was evaluated by Kaplan-Meier analysis, and the comparison of different Kaplan-Meier curves was performed using log-rank test and Bonferroni correction. Cox proportional hazard analyses were undertaken to identify the prognostic factors for DFS, and the hazard ratios (HR) and 95% confidence intervals (CI) of the predictors were acquired.

All statistical analyses were executed using the SPSS 26.0 software (SPSS Software Inc., Chicago, IL, USA) and R version 4.2.3 software (R Core Team 2023. R Foundation for Statistical Computing, Vienna, Austria.). $P < 0.05$ was considered statistically significant.

Results

General characteristics

A total of 258 patients with newly diagnosed breast cancer were included (Fig. 1), including 256 women (99.2%) and 2 men (0.8%), with a mean age of 54 ± 12 years (range: 26–90 years) (Table 1). Of the 258 cases, 245 cases had a single lesion, 6 cases had unilateral double lesions, and 7 cases had bilateral lesions. Thus, the histopathological results were obtained from a total of 271 primary tumors.

Table 1 Clinical characteristics of total patients

Characteristic	Distribution
Age, years (\pm SD)	54 ± 12
Gender	
Female	256 (99.2)
Male	2 (0.8)
BMI	$24.1 (22.0\text{--}26.4)$
Menopausal status^a	
Postmenopausal	152 (59.4)
Premenopausal	101 (39.5)
Unknown	3 (1.1)
Relatives with BC	
Present	24 (9.3)
Absent	234 (90.7)
Tumor focality	
Single focal BC	245 (95.0)
Unilateral double focal BC	6 (2.3)
Bilateral BC	7 (2.7)
Stage at BC diagnosis	
stage I-III	231(89.5)
Stage IV	27 (10.5)
Treatment for stage I-III BC	
Adjuvant	188 (72.9)
Neoadjuvant	135 (52.3)
Operation	231 (89.5)

Qualitative data are numbers followed by percentages in parentheses; continuous data are median followed by interquartile range (IQR) in parentheses, except for age

Abbreviations: BMI body mass index, BC breast cancer

^aTwo men were excluded

One hundred and thirty-two female patients with HER2-low and -zero in stage I-III who received operation were selected for prognostic analysis (Fig. 1), with a mean age of 54 ± 12 years (range 26–85 years). The median follow-up time was 23 months (range: 4–57 months). Sixteen patients experienced recurrence or death, totally accounting for 12.1%. The main recurrence sites are bone (7 sites), liver (6 sites), lung (5 sites), and regional lymph nodes (4 sites).

Correlations of metabolic parameters and clinicopathological characteristics with HER2 expression

A total of 271 primary tumors were analyzed, including 62 lesions in HER2-zero (22.9%), 120 lesions in HER2-low (44.3%), and 89 lesions in HER2-positive (32.8%) group, respectively. As shown in Table 2, the parameters of primary tumor SUVmax, TLR, histologic grades, ER,

Table 2 Correlations of metabolic parameters and clinicopathological characteristics with HER2 expression

	Total tumor numbers (n = 271)	HER2-zero (n = 62)	HER2-low (n = 120)	HER2-positive (n = 89)	P value
Age, years (\pm SD)		55 \pm 12	55 \pm 13	52 \pm 12	0.17 ^a
BMI		24.8 (22.7–27.7)	23.9 (22.1–26.1)	23.5 (21.0–26.5)	0.06 ^b
T stage					0.39 ^c
1/2	235 (86.7)	57 (91.9)	102 (85.0)	76 (85.4)	
3/4	36 (13.3)	5 (8.1)	18 (15.0)	13 (14.6)	
Lymphatic metastasis					0.16 ^c
Absent	87 (32.1)	26 (41.9)	34 (28.3)	27 (30.3)	
Present	184 (67.9)	36 (58.1)	86 (71.7)	62 (69.7)	
Distant metastasis					0.02 ^{*c}
Absent	244 (90.0)	61 (98.4)	108 (90.0)	75 (84.3)	
Present	27 (10.0)	1 (1.6)	12 (10.0)	14 (15.7)	
Histologic grade					0.04 ^{*b}
Grade I	15 (5.6)	5 (8.1)	10 (8.3)	0 (0.0)	
Grade II	121 (44.6)	31 (50.0)	53 (44.2)	37 (41.6)	
Grade III	135 (49.8)	26 (41.9)	57 (47.5)	52 (58.4)	
Tumor histology					0.96 ^c
Ductal	223 (82.3)	51 (82.3)	98 (81.7)	74 (83.1)	
Non-ductal	48 (17.7)	11 (17.7)	22 (18.3)	15 (16.9)	
ER					0.002 ^{*c}
Negative	74 (27.3)	23 (37.1)	20 (16.7)	31 (34.8)	
Positive	197 (72.7)	39 (62.9)	100 (83.3)	58 (65.2)	
PR					0.002 ^{*c}
Negative	116 (42.8)	28 (45.2)	38 (31.7)	50 (56.2)	
Positive	155 (57.2)	34 (54.8)	82 (68.3)	39 (43.8)	
Ki-67					0.01 ^{*c}
< 30%	84 (31.0)	19 (30.6)	47 (39.2)	18 (20.2)	
\geq 30%	187 (69.0)	43 (69.4)	73 (60.8)	71 (79.8)	
SUVmax		4.81 (3.23–9.57)	6.07 (3.90–9.15)	7.15 (4.68–10.75)	0.049 ^{*b}
MTV		7.17 (4.10–15.23)	6.59 (3.50–11.82)	6.21 (3.36–13.50)	0.55 ^b
TLG		27.26 (10.08–54.56)	21.93 (9.94–48.66)	27.27 (12.54–60.68)	0.50 ^b
TLR		1.90 (1.34–3.78)	2.46 (1.50–3.68)	2.96 (1.84–4.05)	0.03 ^{*b}

Qualitative data are numbers followed by percentages in parentheses; continuous data are median followed by interquartile range (IQR) in parentheses, except for age. Hormone receptor (HR) has the same distribution as ER

Abbreviations: *HER2* human epidermal growth factor receptor 2, *BMI* body mass index, *ER* estrogen receptor, *PR* progesterone receptor, *SUVmax* maximum standardized uptake value, *MTV* metabolic tumor volume, *TLG* total lesion glycolysis, *TLR* tumor-to-liver SUV ratio

* P value < 0.05. ^aanalysis of variance (ANOVA); ^bKruskal-Wallis test; ^cChi-Squared test

PR, Ki-67 and distant metastasis showed significant differences among these three groups, respectively.

In multiple comparisons, TLR of HER2-positive group was significantly higher than that of HER2-zero group ($P=0.04$), and SUVmax had the same tendency but was not significant after Bonferroni correction ($P=0.06$) (Fig. 2a and b). Trends in metabolic parameters with HER2 expression (HER2-zero, -low and -positive) were analyzed by the Jonckheere-Terpstra test, which showed that P values for the four metabolic parameters were 0.01

(SUVmax), 0.43 (MTV), 0.43 (TLG), and 0.009 (TLR), respectively.

Additionally, the HR-positive rate of HER2-low group (83.3%) was significantly higher than both of HER2-zero group (62.9%, $P=0.006$) and HER2-positive group (65.2%, $P=0.009$), respectively (Fig. 2c). And the high Ki-67 rate of HER2-positive group (79.8%) was significantly higher than that of HER2-low group (60.8%, $P=0.009$) (Fig. 2d).

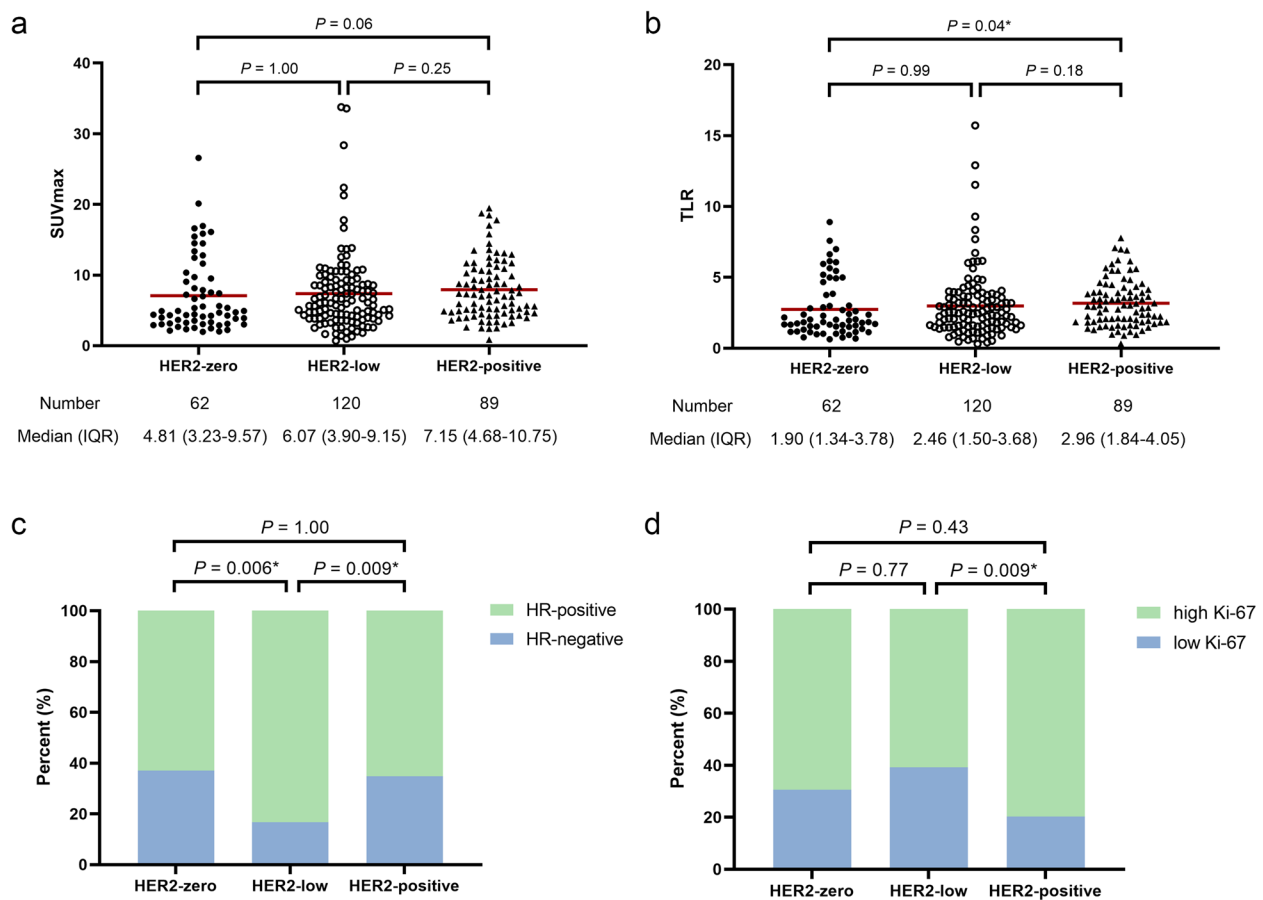


Fig. 2 Multiple comparisons of maximum standardized uptake value (SUVmax) (a), tumor-to-liver SUV ratio (TLR) (b), hormone receptor (HR) (c), and Ki-67 (d) in different HER2 expression statuses. Ki-67 $\geq 30\%$ was considered the threshold for the classification of high and low Ki-67. All the P values were corrected by Bonferroni correction. * Significant after Bonferroni correction

Subgroup analysis of metabolic characteristics between HER2-low and -zero under different HR statuses

As shown in Table 3, under the HR-positive status, both SUVmax and TLR exhibited significant differences between HER2-low and -zero ($P = 0.045$ and 0.03 , respectively), indicating that the HR-positive HER2-low

subgroup corresponded to relatively higher glucose metabolism compared to HR-positive HER2-zero subgroup. But, in the HR-negative subgroup (TNBC), no metabolic parameters indicated significant differences between HER2-low and -zero.

Table 3 Subgroup analysis of metabolic characteristics between HER2-low and HER2-zero under different HR statuses

	HR-positive ($n = 139$)			HR-negative (Triple-negative) ($n = 43$)		
	HER2-zero ($n = 39$)	HER2-low ($n = 100$)	P value	HER2-zero ($n = 23$)	HER2-low ($n = 20$)	P value
SUVmax	4.27 (3.07–5.62)	5.64 (3.71–8.79)	0.045*	7.87 (4.34–14.51)	7.36 (5.98–10.42)	0.85
MTV	6.02 (4.10–13.89)	7.14 (3.66–12.27)	0.89	9.09 (4.10–17.66)	4.22 (2.35–8.30)	0.06
TLG	18.60 (7.88–34.67)	21.41 (10.28–48.66)	0.44	31.18 (14.22–102.95)	23.42 (8.75–66.53)	0.18
TLR	1.66 (1.15–2.37)	2.32 (1.46–3.68)	0.03*	2.88 (1.96–5.45)	3.21 (2.26–4.20)	0.90

* P value < 0.05

Data are median followed by interquartile range (IQR) in parentheses. All using Mann-Whitney U test

Abbreviations: HER2 human epidermal growth factor receptor 2, HR hormone receptor, SUVmax maximum standardized uptake value, MTV metabolic tumor volume, TLG total lesion glycolysis, TLR tumor-to-liver SUV ratio

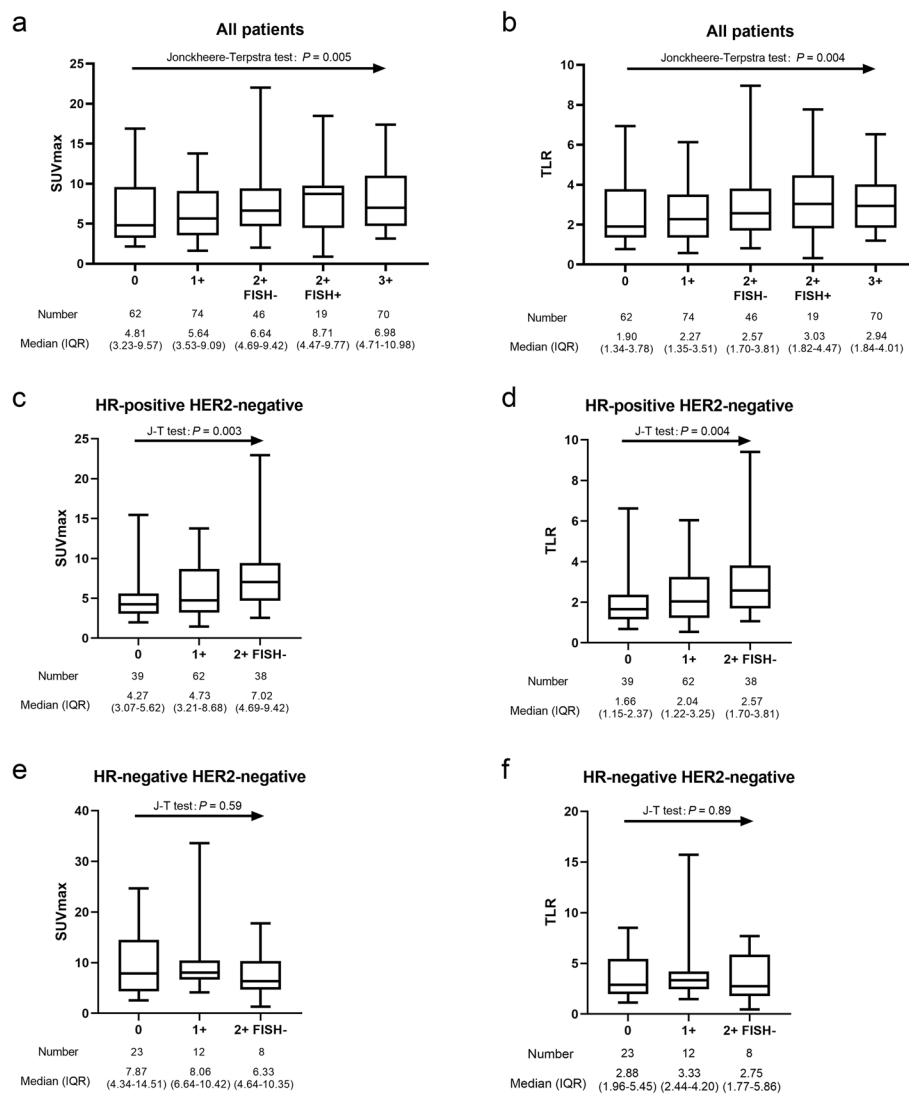


Fig. 3 Analysis of maximum standardized uptake value (SUVmax) and tumor-to-liver SUV ratio (TLR) under detailed HER2 expression statuses in different groups. **(a and b)** all patients, **(c and d)** hormone receptor (HR)-positive HER2-negative group, **(e and f)** HR-negative HER2-negative group. Jonckheere-Terpstra test (J-T test) was used for trend testing

Metabolic characteristics of detailed HER2 expression

We further explored the correlation of metabolic parameters (SUVmax and TLR) with different HER2 expression statuses. In all patients and in the HR-positive HER2-negative subgroup, SUVmax and TLR tended to increase with increasing HER2 expression (All the P values were less than 0.01 in Jonckheere-Terpstra test, Fig. 3a and b). However, this tendency was not shown in the HR-negative HER2-negative subgroup (i.e. triple-negative breast cancer, TNBC) (All the P values were more than 0.05 in Jonckheere-Terpstra test, Fig. 3c). Representative cases were displayed in Fig. 4.

Prognostic analysis

General characteristics of patients with HER2-low and -zero breast cancer in prognostic analysis cohort were given in Table 4. In univariate Cox proportional hazards analysis, all of the four metabolic parameters were significant factors for predicting DFS ($P < 0.01$), and the remaining variables were not statistically significant, except for ER and molecular types ($P = 0.02$ for both) (Fig. 5).

The prognostic cohort was further grouped according to metabolism and HER2 status for Kaplan-Meier analysis (Fig. 6). The results showed that there was no significant difference in DFS between HER2-low and

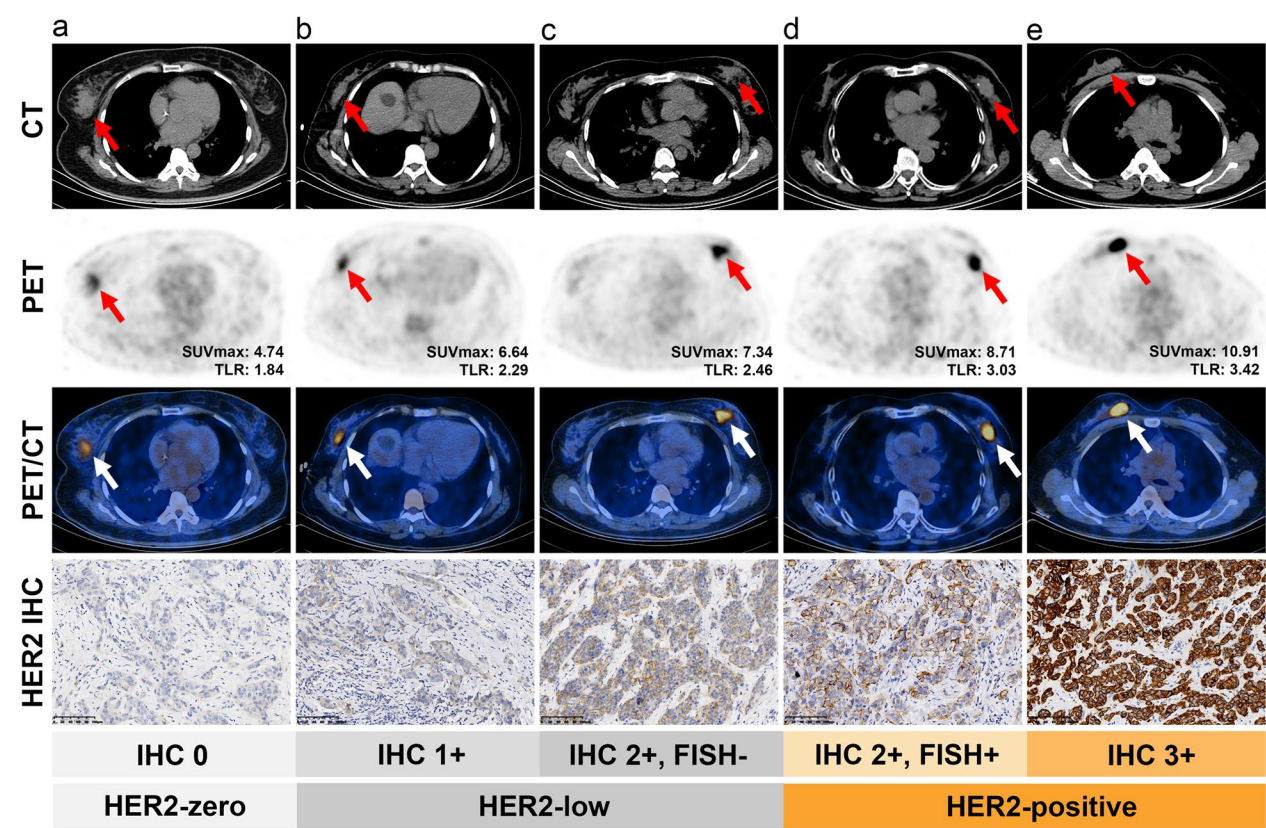


Fig. 4 The typical images of glucose metabolism in primary lesions and their human epidermal growth factor receptor 2 (HER2) expression by immunohistochemistry (IHC). **a** A primary tumor with HER2-zero (IHC 0). **b** A primary tumor with HER2-low (IHC 1+). **c** A primary tumor with HER2-low (IHC 2+ with fluorescence in situ hybridization (FISH) (-)). **d** A primary tumor with HER2-positive (IHC 2+ with FISH (+)). **e** A primary tumor with HER2-positive (IHC 3+). The arrows indicated primary tumors. The scale of pathology images is 100 μ m

-zero, regardless of high or low metabolism in the primary lesion. Furthermore, among HER2-zero patients, DFS was significantly shorter in those with high metabolism than in those with low metabolism, whereas there was no similar significant difference in HER2-low patients.

Discussion

At present, anti-HER2 ADCs have opened up new therapeutic options for breast cancer patients with HER2-low, and there is increasing interest in the biological and clinical characteristics of this subgroup. However, the glucose metabolism characteristics and prognostic features of HER2-low based on pretreatment ¹⁸F-FDG PET/CT have not been well clarified. Our research attempted to comprehensively analyze whether baseline ¹⁸F-FDG PET/CT could reflect the metabolic features and disease outcomes in patients with HER2-low, especially when compared to patients with HER2-zero, in order to provide possible image-guided therapeutic strategies.

In our study, TLR showed significantly higher in the HER2-positive group than in the HER2-zero group. Additionally, HER2-low tumors were more frequent in HR-positive than HR-negative and were associated with lower Ki-67 statuses, which were consistent with prior reports [6, 28, 29]. In further exploratory analyses, we found that in both all patients and the HR-positive HER2-negative subgroup, SUVmax and TLR of the primary lesion tended to increase with the increase of HER2 expression (All the *P* values were less than 0.01), which suggested that the glucose metabolism of the different spectrum of HER2 expression might be a process of continuous evolution.

Specifically, SUVmax and TLR were significantly higher in HER2-low than in HER2-zero in the HR-positive subgroup (*P*=0.045, *P*=0.03, respectively), while there was no significant difference in HR-negative breast cancer (TNBC). Schettini et al. proposed that compared with HR-positive HER2-zero, HR-positive HER2-low tumors showed relatively lower expression of

Table 4 General characteristics of patients with HER2-negative breast cancer in prognostic analysis cohort

	Total numbers (n = 132)	Disease-free numbers (n = 116)	Disease-progressive numbers (n = 16)
Age, years (\pm SD)			
< 50	51 (38.6)	43 (37.1)	8 (50.0)
\geq 50	81 (61.4)	73 (62.9)	8 (50.0)
BMI[‡]			
< 25	85 (64.4)	76 (65.5)	9 (56.3)
\geq 25	47 (35.6)	40 (34.5)	7 (43.7)
T stage			
1/2	117 (88.6)	104 (89.7)	13 (81.3)
3/4	15 (11.4)	12 (10.3)	3 (18.7)
Lymphatic metastasis			
Absent	42 (31.8)	39 (33.6)	3 (18.8)
Present	90 (68.2)	77 (66.4)	13 (81.2)
Histologic grade			
Grade I/ II	67 (50.8)	60 (51.7)	7 (43.8)
Grade III	65 (49.2)	56 (48.3)	9 (56.2)
Tumor histology			
Ductal	111 (84.1)	97 (83.6)	14 (87.5)
Non-ductal	21 (15.9)	19 (16.4)	2 (12.5)
ER			
Negative	39 (29.5)	31 (26.7)	8 (50.0)
Positive	93 (70.5)	85 (73.3)	8 (50.0)
PR			
Negative	51 (38.6)	42 (36.2)	9 (56.2)
Positive	81 (61.4)	74 (63.8)	7 (43.8)
HER2			
Zero	50 (37.9)	42 (36.2)	8 (50.0)
Low	82 (62.1)	74 (63.8)	8 (50.0)
Ki-67			
< 30%	42 (31.8)	38 (32.8)	4 (25.0)
\geq 30%	90 (68.2)	78 (67.2)	12 (75.0)
Molecular types			
Luminal	93 (70.5)	85 (73.3)	8 (50.0)
Triple-negative	39 (29.5)	31 (26.7)	8 (50.0)
Treatment			
NAT	72 (54.5)	62 (53.4)	10 (62.5)
Non-NAT	60 (45.5)	54 (46.6)	6 (37.5)
SUVmax		5.69 (3.58–9.04)	7.97 (4.16–16.05)
MTV		7.01 (4.10–12.38)	13.82 (4.40–22.05)
TLG		24.62 (10.56–45.14)	35.87 (16.32–209.96)
TLR		2.36 (1.46–3.68)	2.74 (1.63–5.97)

Qualitative data are numbers followed by percentages in parentheses; continuous data are median followed by interquartile range (IQR) in parentheses, except for age. Hormone receptor (HR) has the same distribution as ER

Abbreviations: *HER2* human epidermal growth factor receptor 2, *BMI* body mass index, *ER* estrogen receptor, *PR* progesterone receptor, *NAT* neoadjuvant therapy, *SUVmax* maximum standardized uptake value, *MTV* metabolic tumor volume, *TLG* total lesion glycolysis, *TLR* tumor-to-liver SUV ratio

proliferation-related genes and three PAM50 (Prediction Analysis of Microarray 50) signatures, and higher expression of luminal-related genes and other two PAM50 signatures. But no individual gene or PAM50 signature was found differentially expressed between HER2-low

and -zero in HR-negative breast cancer (TNBC) [30]. In addition, Shao et al. found that the pathologic complete response rates of HER2-low and -zero were significantly different in the HR-positive subgroup but not in HR-negative breast cancer (TNBC) [4]. Overall, the

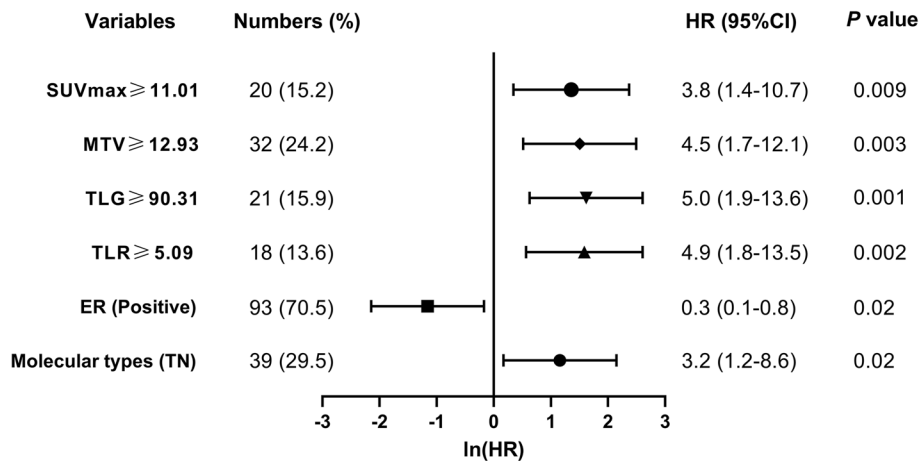


Fig. 5 Forest plot of the prognostic predictors for disease-free survival (DFS) in HER2-negative patients using the univariate Cox hazards analysis. Receiver operating characteristic (ROC) curves were analyzed for the optimal cutoff value

HR-positive HER2-low subtype may have a particularity in glucose metabolic profile, gene expression, and therapeutic response. Thus, it has the potential to be an independent subtype that deserves further exploration. As for HR-negative breast cancer (TNBC), we guessed that its highly invasive characteristics might mask metabolic differences between the HER2-low and -zero subgroups.

Most of the available studies aimed at comparing the differences in clinicopathological features and prognostic characteristics between HER2-low and -zero patients have yielded inconsistent results [4, 6, 31], with the majority of them showing no difference in prognosis between these two groups. However, there is a lack of research focusing on the prognostic role of metabolic parameters. Our study showed that the four included metabolic parameters were significant predictors of DFS in patients with HER2-negative (including HER2-low and -zero), but there was no significant difference in DFS between HER2-low and -zero, even under stratification of different metabolic levels.

In further stratified analysis, we found that the prognosis of HER2-zero patients with high metabolism was significantly worse than that of those with low metabolism, but no similar difference had been concluded in HER2-low patients. Recently, the concept of ultra-low HER2 expression (belong to the HER2-zero group) has received widespread attention from researchers [32], which is defined as having $\leq 10\%$ of tumor cells with incomplete and weak staining despite an IHC score of zero [33]. In fact, the DESTINY-Breast06 trial has taken into account the potential benefits of ADC treatment for ultra-low HER2 patients and recently revealed the positive conclusion [34, 35].

On the other hand, more and more evidences suggest that the current definition of HER2-low by pathological examination does not seem to perfectly distinguish HER2 expression from non-HER2 expression, and its diagnostic accuracy in differentiating HER2-zero and HER2-low is also unsatisfactory [36, 37]. Our findings may provide complementary imaging indicators to help screen for patients with ultra-low HER2 who may benefit from ADC therapy. Of course, further investigations needed to determine whether ultra-low HER2 expression exists in HER2-zero patients with high metabolism, as well as to explore whether they may benefit from HER2-targeted ADC treatment.

Certainly, our study has several limitations that should be considered. First, our sample size was insufficient to thoroughly analyze the metabolic characteristics of various subgroups with different HER2 as well as HR status. The research on metastatic stage patients was also limited by a small sample size. At the same time, retrospective case collation may be prone to inadvertent bias. Second, because of the relatively good prognosis of non-metastatic breast cancer (5-year survival rate of approximately 90%) [38], we need to continue follow-up to further refine our prognostic findings, especially the subgroup analysis of different HR status. Third, radiomics based on ^{18}F -FDG PET/CT imaging can provide more comprehensive imaging information and is expected to serve as an alternative method for detecting HER2-low expression. Last but not least, it is necessary to explore other more precise methods capable of evaluating HER2 in detail. HER2-targeted PET imaging is a possible option for determining the HER2 status [39].

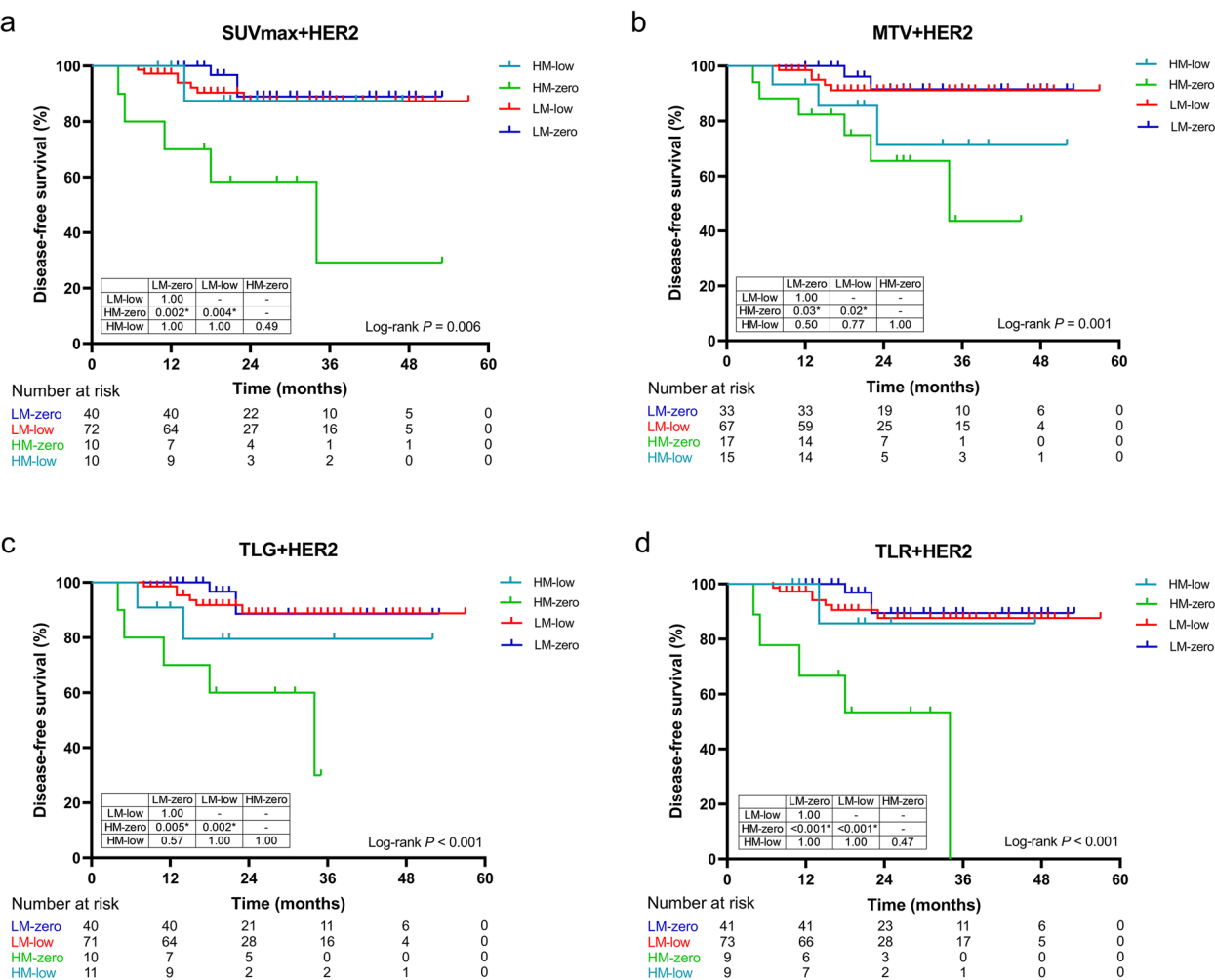


Fig. 6 Kaplan-Meier survival curve analyses for disease-free survival (DFS) in HER2-negative patients. The cutoff values for distinguishing between high metabolism (HM) and low metabolism (LM) were 11.01 (**a**, maximum standardized uptake value (SUVmax)), 12.93 (**b**, metabolic tumor volume (MTV)), 90.31 (**c**, total lesion glycolysis (TLG)), and 5.09 (**d**, tumor-to-liver SUV ratio (TLR)), respectively. The bottom tables showed absolute numbers at risk. Tables below the survival curves showed the results of multiple comparisons, and P values have been corrected by Bonferroni correction. * Significant after Bonferroni correction

Conclusion

Our study revealed that the HR-positive HER2-low subgroup exhibited a particularity in glucose metabolic profile, which showed higher glucose metabolism compared with HR-positive HER2-zero. In addition, we found that HER2-zero breast cancer patients with elevated metabolic parameters had a poorer prognosis and warranted careful attention in clinical evaluations.

Abbreviations

ADCs	Antibody drug conjugates
AUC	Area under the curve
CI	Confidence intervals
DFS	Disease-free survival
ER	Estrogen receptor
FDG	Fluorodeoxyglucose
FISH	Fluorescence in situ hybridization
HE	Hematoxylin and eosin staining

HER2	Human epidermal growth factor receptor 2
HR	Hormone receptor
HR	Hazard ratios
IHC	Immunohistochemistry
MRI	Magnetic resonance imaging
MTV	Metabolic tumor volume
PAM50	Prediction Analysis of Microarray 50
PET/CT	Positron emission tomography/computed tomography
PR	Progesterone receptor
ROC	Receiver operating characteristic
SUVmax	Maximum standardized uptake value
TLG	Total lesion glycolysis
TLR	Tumor-to-liver SUV ratio

Acknowledgements

None.

Authors' contributions

Conceptualization: [M.L.]; Methodology: [M.L.], [Y.G.], [L.Y.]; Material preparation: [Y. G.], [L.Y.], [L.M.], [X.Z.], [H.L.], [J.C.], [Y.C.]; Data analysis: [Y.G.], [L.Y.], [C.W.], [L.L.], [J.Y.], [L.X.]; Writing-original draft preparation: [Y.G.], [L.Y.], [C.W.];

Writing-review and editing: [M.L.]; Funding acquisition: [M.L.], [L.Y.]; Supervision: [M.L.]. All authors have read and approved the final manuscript.

Funding

This study was supported by grants from the National Natural Science Foundation of China (82172052), Beijing Natural Science Foundation (Z210007), National High-Level Hospital Clinical Research Funding (Interdepartmental Clinical Research Project of Peking University First Hospital) (2022CR34), and Youth Clinical Research Special Fund Project of Peking University First Hospital (2023YC26). No other potential conflict of interest relevant to this article was reported.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Patients' records were anonymized and de-identified before analysis. The retrospective data collection and analysis procedures were approved by the Ethics Committee of Peking University First Hospital, waiving the need for written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Department of Nuclear Medicine, Peking University First Hospital, No.8, Xishiku Street, West District, Beijing 100034, China. ²Thyroid and Breast Surgery, Peking University First Hospital, Beijing, China. ³Department of Pathology, Peking University First Hospital, Beijing, China.

Received: 28 July 2024 Accepted: 5 December 2024

Published online: 18 December 2024

References

- Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab Deruxtecan in previously treated HER2-Low advanced breast Cancer. *N Engl J Med*. 2022;387:9–20. <https://doi.org/10.1056/NEJMoa2203690>.
- Tarantino P, Hamilton E, Tolane SM, Cortes J, Morganti S, Ferraro E, et al. HER2-Low breast Cancer: pathological and clinical Landscape. *J Clin Oncol*. 2020;38:1951–62. <https://doi.org/10.1200/jco.19.02488>.
- Zhang G, Ren C, Li C, Wang Y, Chen B, Wen L, et al. Distinct clinical and somatic mutational features of breast tumors with high-, low-, or non-expressing human epidermal growth factor receptor 2 status. *BMC Med*. 2022;20:142. <https://doi.org/10.1186/s12916-022-02346-9>.
- Shao Y, Yu Y, Luo Z, Guan H, Zhu F, He Y, et al. Clinical, Pathological Complete Response, and prognosis characteristics of HER2-Low breast Cancer in the Neoadjuvant Chemotherapy setting: a retrospective analysis. *Ann Surg Oncol*. 2022;29:8026–34. <https://doi.org/10.1245/s10434-022-12369-4>.
- Agostinetto E, Rediti M, Fimereli D, Debien V, Piccart M, Aftimos P, et al. HER2-Low breast Cancer: molecular characteristics and prognosis. *Cancers (Basel)*. 2021;13:2824. <https://doi.org/10.3390/cancers13112824>.
- Tarantino P, Jin Q, Tayob N, Jeselsohn RM, Schnitt SJ, Vinciguilla J, et al. Prognostic and biologic significance of ERBB2-Low expression in early-stage breast Cancer. *JAMA Oncol*. 2022;8:1177–83. <https://doi.org/10.1001/jamaoncol.2022.2286>.
- Ramtohul T, Djerroudi L, Lissavalid E, Nhy C, Redon L, Ikni L, et al. Multiparametric MRI and Radiomics for the prediction of HER2-Zero, -Low, and -positive breast cancers. *Radiology*. 2023;308:e222646. <https://doi.org/10.1148/radiol.222646>.
- Bian X, Du S, Yue Z, Gao S, Zhao R, Huang G, et al. Potential antihuman epidermal growth factor receptor 2 target therapy beneficiaries: the role of MRI-Based Radiomics in distinguishing human epidermal growth factor receptor 2-Low status of breast Cancer. *J Magn Reson Imaging*. 2023;58:1603–14. <https://doi.org/10.1002/jmri.28628>.
- Groheux D, Cochet A, Humbert O, Alberini JL, Hindié E, Mankoff D. ¹⁸F-FDG PET/CT for staging and restaging of breast Cancer. *J Nucl Med*. 2016;57(Suppl 1):s17–26. <https://doi.org/10.2967/jnumed.115.157859>.
- Elia I, Haigis MC. Metabolites and the tumour microenvironment: from cellular mechanisms to systemic metabolism. *Nat Metab*. 2021;3:21–32. <https://doi.org/10.1038/s42255-020-00317-z>.
- Gutierrez C, Schiff R. HER2: biology, detection, and clinical implications. *Arch Pathol Lab Med*. 2011;135:55–62. <https://doi.org/10.5858/2010-0454-rar.1>.
- Marchiò C, Annaratone L, Marques A, Casorzo L, Berrino E, Sapino A. Evolving concepts in HER2 evaluation in breast cancer: heterogeneity, HER2-low carcinomas and beyond. *Semin Cancer Biol*. 2021;72:123–35. <https://doi.org/10.1016/j.semcancer.2020.02.016>.
- Valenza C, Guidi L, Battaiotto E, Trapani D, Sartore Bianchi A, Siena S, et al. Targeting HER2 heterogeneity in breast and gastrointestinal cancers. *Trends Cancer*. 2024;10:113–23. <https://doi.org/10.1016/j.trecan.2023.11.001>.
- Zhang J, Liu Y, Fan H, Wang W, Shao W, Cao G, et al. Prediction of clinical molecular typing of breast invasive Ductal Carcinoma using (18)F-FDG PET/CT dual-phase imaging. *Acad Radiol*. 2023. <https://doi.org/10.1016/j.acra.2022.12.036>.
- Sasada S, Masumoto N, Suzuki E, Sueoka S, Goda N, Kajitani K, et al. Prediction of biological characteristics of breast cancer using dual-phase FDG PET/CT. *Eur J Nucl Med Mol Imaging*. 2019;46:831–7. <https://doi.org/10.1007/s00259-019-4259-5>.
- Groheux D, Mankoff D, Espié M, Hindié E. ¹⁸F-FDG PET/CT in the early prediction of pathological response in aggressive subtypes of breast cancer: review of the literature and recommendations for use in clinical trials. *Eur J Nucl Med Mol Imaging*. 2016;43:983–93. <https://doi.org/10.1007/s00259-015-3295-z>.
- Chae SY, Park SH, Lee HS, Ahn JH, Kim SB, Jung KH, et al. Association between tumor (18)F-fluorodeoxyglucose metabolism and survival in women with estrogen receptor-positive, HER2-negative breast cancer. *Sci Rep*. 2022;12:7858. <https://doi.org/10.1038/s41598-022-11603-z>.
- Groheux D, Sanna A, Majdoub M, de Cremoux P, Giacchetti S, Teixeira L, et al. Baseline tumor 18F-FDG uptake and modifications after 2 cycles of Neoadjuvant Chemotherapy are Prognostic of Outcome in ER+/HER2-breast Cancer. *J Nucl Med*. 2015;56:824–31. <https://doi.org/10.2967/jnumed.115.154138>.
- Giuliano AE, Edge SB, Hortobagyi GN. Eighth Edition of the AJCC Cancer staging Manual: breast Cancer. *Ann Surg Oncol*. 2018;25:1783–5. <https://doi.org/10.1245/s10434-018-6486-6>.
- Wu C, Cui Y, Liu J, Ma L, Xiong Y, Gong Y, et al. Noninvasive evaluation of tumor immune microenvironment in patients with clear cell renal cell carcinoma using metabolic parameter from preoperative 2-[(18)F]FDG PET/CT. *Eur J Nucl Med Mol Imaging*. 2021;48:4054–66. <https://doi.org/10.1007/s00259-021-05399-9>.
- Urso L, Evangelista L, Alongi P, Quartuccio N, Cittanti C, Rambaldi I, et al. The value of semiquantitative parameters derived from (18)F-FDG PET/CT for Predicting Response to Neoadjuvant Chemotherapy in a cohort of patients with different molecular subtypes of breast Cancer. *Cancers (Basel)*. 2022;14:5869. <https://doi.org/10.3390/cancers14235869>.
- Nakamoto R, Zaba LC, Liang T, Reddy SA, Davidzon G, Aparici CM, et al. Prognostic value of bone marrow metabolism on pretreatment (18) F-FDG PET/CT in patients with metastatic melanoma treated with Anti-PD-1 therapy. *J Nucl Med*. 2021;62:1380–3. <https://doi.org/10.2967/jnumed.120.254482>.
- Zhao Y, Wu C, Li W, Chen X, Li Z, Liao X, et al. 2-[18F]FDG PET/CT parameters associated with WHO/ISUP grade in clear cell renal cell carcinoma. *Eur J Nucl Med Mol Imaging*. 2021;48:570–9. <https://doi.org/10.1007/s00259-020-04996-4>.

24. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol*. 2010;28:2784–95. <https://doi.org/10.1200/jco.2009.25.6529>.
25. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human epidermal growth factor receptor 2 testing in breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*. 2018;36:2105–22. <https://doi.org/10.1200/jco.2018.77.8738>.
26. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology*. 1991;19:403–10. <https://doi.org/10.1111/j.1365-2559.1991.tb00229.x>.
27. Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E, et al. Primary breast cancer: ESMO Clinical Practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2015;26(Suppl 5):v8–30. <https://doi.org/10.1093/annonc/mdv298>.
28. Peiffer DS, Zhao F, Chen N, Hahn OM, Nanda R, Olopade OI, et al. Clinicopathologic characteristics and prognosis of ERBB2-Low breast Cancer among patients in the National Cancer Database. *JAMA Oncol*. 2023;9:500–10. <https://doi.org/10.1001/jamaoncol.2022.7476>.
29. Denkert C, Seither F, Schneeweiss A, Link T, Blohmer JU, Just M, et al. Clinical and molecular characteristics of HER2-low-positive breast cancer: pooled analysis of individual patient data from four prospective, neoadjuvant clinical trials. *Lancet Oncol*. 2021;22:1151–61. [https://doi.org/10.1016/s1470-2045\(21\)00301-6](https://doi.org/10.1016/s1470-2045(21)00301-6).
30. Schettini F, Chic N, Brasó-Maristany F, Paré L, Pascual T, Conte B, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. *npj Breast Cancer*. 2021;7:1. <https://doi.org/10.1038/s41523-020-00208-2>.
31. Almstedt K, Heimes AS, Kappenberg F, Battista MJ, Lehr HA, Krajnak S, et al. Long-term prognostic significance of HER2-low and HER2-zero in node-negative breast cancer. *Eur J Cancer*. 2022;173:10–9. <https://doi.org/10.1016/j.ejca.2022.06.012>.
32. Franchina M, Pizzimenti C, Fiorentino V, Martini M, Ricciardi GRR, Silvestris N, et al. Low and ultra-low HER2 in human breast Cancer: an effort to define New Neoplastic subtypes. *Int J Mol Sci*. 2023;24:12795. <https://doi.org/10.3390/ijms241612795>.
33. Venetis K, Crimini E, Sajjadi E, Corti C, Guerini-Rocco E, Viale G, et al. HER2 Low, ultra-low, and Novel complementary biomarkers: expanding the spectrum of HER2 positivity in breast Cancer. *Front Mol Biosci*. 2022;9:834651. <https://doi.org/10.3389/fmolb.2022.834651>.
34. Bardia A, Barrios C, Dent R, Hu X, O'Shaughnessy J, Yonemori K et al. Abstract OT-03-09: Trastuzumab deruxtecan (T-DXd; DS-8201) vs investigator's choice of chemotherapy in patients with hormone receptor-positive (HR+), HER2 low metastatic breast cancer whose disease has progressed on endocrine therapy in the metastatic setting: A randomized, global phase 3 trial (DESTINY-Breast06). *Cancer Research*. 2021;81:OT-03-9-OT-9. <https://doi.org/10.1158/1538-7445.Sabcs20-ot-03-09>
35. Curigliano G, Hu X, Dent RA, Yonemori K, Carlos H, Barrios S, O'Shaughnessy J, et al. Trastuzumab deruxtecan (T-DXd) vs physician's choice of chemotherapy (TPC) in patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor 2 (HER2)-low or HER2-ultralow metastatic breast cancer (mBC) with prior endocrine therapy (ET): primary results from DESTINY-Breast06 (DB-06). *J Clin Oncol*. 2024;42:LBA1000–LBA. https://doi.org/10.1200/JCO.2024.42.17_suppl.LBA1000.
36. Fernandez AI, Liu M, Bellizzi A, Brock J, Fadare O, Hanley K, et al. Examination of low ERBB2 protein expression in breast Cancer tissue. *JAMA Oncol*. 2022;8:1–4. <https://doi.org/10.1001/jamaoncol.2021.7239>.
37. Moutafi M, Robbins CJ, Yaghoobi V, Fernandez AI, Martinez-Morilla S, Xirou V, et al. Quantitative measurement of HER2 expression to subclassify ERBB2 unamplified breast cancer. *Lab Invest*. 2022;102:1101–8. <https://doi.org/10.1038/s41374-022-00804-9>.
38. Hassaine Y, Jacquet E, Seigneurin A, Delafosse P. Evolution of breast cancer incidence in young women in a French registry from 1990 to 2018: towards a change in screening strategy? *Breast Cancer Res*. 2022;24:87. <https://doi.org/10.1186/s13058-022-01581-5>.
39. Eisses B, van Geel JJJ, Brouwers AH, Bensch F, Elias SG, Kuip EJM, et al. Whole-body HER2 heterogeneity identified on HER2 PET in HER2-Negative, -Low, and -positive metastatic breast Cancer. *J Nucl Med*. 2024;65:1540–7. <https://doi.org/10.2967/jnumed.124.267636>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.