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ORIGINAL RESEARCH

Association of Body Mass Index with Tumor-Infiltrating Lymphocytes and Treatment Response in Early HER2-Positive Breast Cancer

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Background: Immune-related biomarkers such as stromal tumor-infiltrating lymphocytes (TILs) are associated with response to neoadjuvant therapy in HER2+ breast cancer. Host-related factors, including BMI, may influence the tumor immune microenvironment and modify the predictive value of immune infiltration.

Methods: In this retrospective cohort study, we evaluated associations between BMI, stromal TIL density, selected immune checkpoint markers (PD-1 and TIM-3), leptin receptor (Ob-R) expression, and pathological complete response (pCR) in patients with early-stage HER2+ breast cancer treated with neoadjuvant HER2-targeted therapy.

Results: Of 101 patients analyzed, 48.0% had a BMI ≥25 kg/m^2. Patients with BMI ≥25 kg/m^2 exhibited higher stromal TIL density (p = 0.011), higher frequency of PD-1 positivity (p = 0.058), and higher Ob-R expression (p = 0.043). BMI was not directly associated with pCR. In multivariable analysis, hormone receptor positivity was inversely associated with pCR (odds ratio [OR] = 0.15; 95% confidence interval [CI], 0.04–0.51; p = 0.004), whereas higher stromal TIL density was independently associated with increased odds of pCR (OR = 1.37; 95% CI, 1.03–1.93; p = 0.048). A significant interaction observed between BMI and stromal TIL density (interaction OR = 0.99; 95% CI, 0.97–1.00; p = 0.044) indicated that the association between immune infiltration and treatment response differed according to BMI. In early-stage HER2+ breast cancer, stromal TIL density and PD-1 expression are associated with response to neoadjuvant therapy, while BMI appears to modify the relationship between immune infiltration and pCR.

Conclusion: Host-related factors, as captured by BMI, may influence the tumor immune microenvironment and predictive value of immune biomarkers in early-stage HER2-positive breast cancer. As the study was exploratory, these observations warrant further study.

Keywords: body mass index, human epidermal growth factor receptor 2-positive breast cancer, obesity, treatment response, tumor-infiltrating lymphocytes

Introduction

Human epidermal growth factor receptor 2-positive (HER2+) breast cancer accounts for approximately 15–20% of all breast cancers and is characterized by amplification or overexpression of the *ERBB2* gene.[1,2] Historically associated with an aggressive clinical course and a poor prognosis, the advent of HER2-targeted therapies—including monoclonal antibodies such as trastuzumab and pertuzumab, and antibody-drug conjugates—has

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significantly improved outcomes for patients with early-stage disease.[3–5] Nevertheless, a substantial proportion of patients fail to achieve a pathological complete response (pCR) following neoadjuvant therapy, and recurrence remains a concern, highlighting the need for additional predictive and prognostic biomarkers, both in the tumor and in the host.[6,7]

The tumor immune microenvironment has emerged as a key modulator of treatment response in HER2+ breast cancer. Stromal tumor-infiltrating lymphocytes (TILs), which represent a surrogate marker of host anti-tumor immune activity, have shown consistent associations with improved pCR rates and favorable long-term outcomes, particularly in early-stage HER2+ and triple-negative breast cancer (TNBC).[8–10] However, the immunological landscape of HER2+ tumors is heterogeneous, and the functional status of TILs—especially expression of immune checkpoints such as programmed cell death protein 1 (PD-1)—may influence the therapeutic efficacy of preoperative therapy.[11,12] PD-1 expression on TILs, along with its ligand PD-L1 on tumor and immune cells, plays a central role in immune evasion and is currently being explored as a target in combination with HER2-directed therapies.[13,14]

Host metabolic factors have also emerged as potential modulators of systemic and tumor-specific immune responses. Obesity has been associated with chronic low-grade inflammation, altered cytokine secretion, and immune dysfunction, all of which may influence the tumor immune microenvironment.[15,16] In clinical studies, body mass index (BMI) is commonly used as a pragmatic surrogate of body composition, although it represents an imperfect proxy for adiposity and does not capture fat distribution or metabolic heterogeneity. Although BMI is not consistently or directly associated with stromal TIL levels, it may modify their prognostic significance depending on breast cancer subtype. No statistically significant association was found between BMI category and stromal TIL levels in HR+/HER2-negative disease.[17] A large retrospective analysis in TNBC demonstrated that, while BMI categories were not directly associated with stromal TIL levels, BMI modified the predictive value of TILs for pCR, with lean patients deriving greater benefit from high immune infiltration than overweight or obese patients.[18] These findings suggest that host body composition may influence the functional relevance of immune infiltration, although the underlying biological mechanisms remain incompletely understood.

Among adipose tissue-derived factors, leptin and its receptor (Ob-R) have been implicated in breast cancer biology and immune regulation. Leptin exerts pleiotropic effects beyond energy homeostasis, including mitogenic and immunomodulatory functions.[19–21] Ob-R expression has been reported in a substantial proportion of breast cancer specimens and has been variably associated with tumor behavior and immune features.[22,23] However, the clinical relevance of Ob-R expression within the tumor microenvironment, particularly in relation to immune infiltration and treatment response in HER2+ breast cancer, remains uncertain.

In this study, we explored the relationship between BMI, stromal TIL density, selected immune checkpoint markers (PD-1 and TIM-3), Ob-R expression, and pCR in patients with early-stage HER2+ breast cancer treated with neoadjuvant HER2-targeted therapy. Given the retrospective design and use of BMI as an indirect measure of body composition, this analysis was conducted with a descriptive and hypothesis-generating intent, aiming to characterize immune-clinical interactions that may inform future mechanistic and prospective studies.

Materials and Methods

Study Design and Population

This retrospective cohort study included patients diagnosed with early-stage HER2-positive breast cancer who received standard neoadjuvant chemotherapy (anthracyclines and cyclophosphamide followed by paclitaxel in combination with trastuzumab and pertuzumab). Patients were treated at the MD Anderson Cancer Center Madrid and Hospital Ramón y Cajal (Madrid, Spain).

Eligibility criteria included availability of pre-treatment formalin-fixed paraffin-embedded tumor tissue and complete clinical and pathological data. Patients with metastatic disease at diagnosis were excluded. The study was approved by the Institutional Review Board of Hospital Ramón y Cajal (approval number 3–2022-0131) and conducted in accordance with the Declaration of Helsinki (2013 amendment). Due to the retrospective nature of the study, informed consent was waived. In respect of participants' privacy, all data are maintained with confidentiality.

Clinical and Pathological Variables

Demographic and clinical data, including age at diagnosis, weight, height, and menopausal status, were retrieved from electronic medical records. BMI was calculated as weight (kg) divided by height squared (m^2) and categorized according to World Health Organization criteria: <25 kg/m^2 (normal weight), 25–29.9 kg/m^2 (overweight), and ≥30 kg/m^2 (obesity).

For primary analyses, BMI was dichotomized as <25 kg/m^2 versus ≥25 kg/m^2 in order to capture patients with excess body weight and to preserve statistical power. Exploratory analyses using three BMI categories were limited by sample size and therefore considered descriptive.

Archived hematoxylin and eosin (H&E)-stained slides from diagnostic core biopsies obtained prior to neoadjuvant therapy were reviewed for histological confirmation. Estrogen receptor (ER) and progesterone receptor (PR) status were assessed by immunohistochemistry (IHC) using monoclonal antibodies (ER clone 6F11, dilution 1:200; PR clone 16, dilution 1:500; Leica Biosystems, Wetzlar, Germany). Nuclear staining in ≥1% of tumor cells was considered positive.[24] HER2 expression was evaluated by IHC (clone CB11; ready-to-use; Leica Biosystems) and interpreted according to the 2018 ASCO/CAP guidelines.[25] Equivocal cases (2+) were further assessed by fluorescence in situ hybridization.

Ki-67 proliferation index was assessed using monoclonal antibody MIB-1 (dilution 1:300; Dako/Agilent) and quantified as the percentage of positively stained tumor cells in hotspot areas using an automated image analysis system (i-Solution DT; IMT i-Solution Inc., Republic of Korea).

Evaluation of Tumor-Infiltrating Lymphocytes and Immune Markers

Stromal TILs were assessed on H&E-stained sections from baseline biopsies according to recommendations of the International TILs Working Group.[26,27] TILs were independently evaluated by two experienced breast pathologists who were blinded to clinical outcomes. For each case, 3–5 representative fields at 200× magnification located at the invasive tumor front were analyzed, and the percentage of mononuclear inflammatory cells within the stromal compartment was recorded. A consensus score was reached for each case and used for analysis.

Stromal TILs were analyzed both as a continuous variable and as a categorical variable using a predefined cut-off of 30%, in accordance with international guidelines.

PD-1 expression was evaluated by IHC using the PD-1 (CAL20) ready-to-use antibody (Leica Biosystems). PD-1 positivity was quantified as the percentage of immunostained immune cells within intratumoral and peritumoral areas. PD-1 expression was analyzed both as a continuous variable and dichotomized using a 1% threshold, based on prior literature.[28,29]

TIM-3 expression was assessed using a human TIM-3 affinity-purified antibody (AF2365; Bio-Techne R&D Systems). TIM-3 staining was evaluated on immune cells and classified dichotomously, with high expression defined as ≥50% of immune cells showing positive staining. Tonsil tissue was used as an external positive control. Due to tissue availability constraints, TIM-3 assessment was feasible in a subset of cases.

Ob-R expression was evaluated by IHC using a previously described protocol.[30] Tumor cell membrane and cytoplasmic staining intensity was scored semi-quantitatively on a scale from 0 to 3 by trained pathologists. For analysis, Ob-R expression was categorized as low (scores 0–1) or high (scores 2–3).

Statistical Analysis

Descriptive statistics were used to summarize patient characteristics. Continuous variables are presented as mean ± standard deviation or median with interquartile range (IQR), as appropriate, and categorical variables as absolute and relative frequencies.

Comparisons between groups were performed using Student's *t* test or Mann–Whitney-*U* test for continuous variables and Chi-square or Fisher's exact test for categorical variables. Associations between clinical, pathological, and immune variables and pCR were evaluated using univariate logistic regression.

Multivariable logistic regression models were constructed to identify independent predictors of pCR, including variables selected based on clinical relevance and univariate results. To explore the potential modifying effect of BMI on immune parameters, interaction terms were tested where appropriate. Model refinement was performed using backward stepwise selection based on the Akaike Information Criterion. A two-sided p value <0.05 was considered statistically significant. All analyses were conducted using R software (version 4.4).

Results

Patient Characteristics

A total of 101 patients with early-stage HER2-positive breast cancer were included in the analysis. The median age at diagnosis was 49 years (IQR, 45–60), and the mean BMI was 24.9 ± 4.8 kg/m^2. BMI data were available for 100 patients; among them, 48 patients (48.0%) had a BMI ≥25 kg/m^2, including 14 patients (14.0%) with BMI ≥30 kg/m^2.

Patients with BMI ≥25 kg/m^2 were significantly older than those with BMI <25 kg/m^2 (mean age 56.9 ± 14.7 vs 50.0 ± 8.6 years; p = 0.022) and were more frequently postmenopausal (Table 1).

All patients received neoadjuvant chemotherapy consisting of an anthracycline-based regimen followed by taxanes in combination with trastuzumab and pertuzumab. pCR (ypT0/is ypN0) was achieved in 56 of 99 evaluable patients (56.6%).

Table 1 Clinical and Pathological Variables of the Total Population and Stratified by Body Mass Index

Variable	All Patients (n = 101)	BMI		p value[a]
		<25 mg/m^2 (n = 52)	≥25 mg/m^2 (n = 48)	
Age, years				0.022
Mean ± SD	53.2 ± 12.3	50.0 ± 8.6	56.9 ± 14.7	
Median [IQR]	49.0 [45.0–60.0]	48.5 [45.0–54.0]	56.0 [46.0–66.5]	
Weight, kg				<0.001
Mean ± SD	65.5 ± 12.3	57.0 ± 6.5	74.6 ± 10.4	
Median [IQR]	64.0 [56.0–73.5]	58.0 [51.5–63.0]	74.0 [67.0–81.5]	
Height, cm				0.063
Mean ± SD	162.5 ± 7.0	164.0 ± 7.1	160.9 ± 6.6	
Median [IQR]	161.0 [157.0–168.0]	163.0 [158.0–168.5]	160.0 [157.0–165.5]	
Menopausal status, n (%)				0.01
No	56 (55.4)	35 (67.3)	20 (41.7)	
Yes	45 (44.6)	17 (32.7)	28 (58.3)	
ER expression, %				0.407
Mean ± SD	49.6 ± 40.8	52.1 ± 41.3	46.4 ± 40.5	
Median [IQR]	70.0 [0.0–85.0]	70.0 [0.0–90.0]	67.5 [0.0–80.0]	
PR expression, %				0.262
Mean ± SD	22.5 ± 32.7	25.8 ± 35.5	17.8 ± 28.4	
Median [IQR]	0.0 [0.0–45.0]	0.0 [0.0–55.0]	0.0 [0.0–35.0]	
Hormonal receptors, n (%)				0.621
No	33 (32.7)	16 (30.8)	17 (35.4)	
Yes	68 (67.3)	36 (69.2)	31 (64.6)	

(*Continued*)

Table 1 (Continued).

Variable	All Patients (n = 101)	BMI		*p* value[a]
		<25 mg/m^2 (n = 52)	≥25 mg/m^2 (n = 48)	
Ki67 proliferative index, %				0.98
Mean ± SD	32.5 ± 18.7	32.8 ± 19.2	32.1 ± 18.6	
Median [IQR]	28.0 [20.0–44.0]	25.0 [20.0–50.0]	30.0 [18.0–40.0]	
TILs, %				0.011
Mean ± SD	17.7 ± 17.6	14.6 ± 15.3	21.3 ± 19.5	
Median [IQR]	13.5 [5.0–20.0]	10.0 [5.0–20.0]	15.0 [10.0–30.0]	
TILs categories, n (%)				0.209
<30%	80 (80.0)	44 (84.6)	35 (74.5)	
≥30%	20 (20.0)	8 (15.4)	12 (25.5)	
PD-1 expression, %				0.117
Mean ± SD	6.4 ± 8.4	5.5 ± 7.6	7.2 ± 9.1	
Median [IQR]	3.0 [0.5–10.0]	1.0 [0.0–10.0]	3.5 [1.0–10.0]	
PD-1 categories, n (%)				0.058
≤1%	35 (40.7)	22 (51.2)	13 (31.0)	
>1%	51 (59.3)	21 (48.8)	29 (69.0)	
TIM-3, n (%)				0.522
Low	26 (46.4)	13 (52.0)	13 (43.3)	
High	30 (53.6)	12 (48.0)	17 (56.7)	
Ob-R expression, n (%)				0.043
Score 0–1	32 (33.0)	21 (42.9)	11 (23.4)	
Score 2–3	65 (67.0)	28 (57.1)	36 (76.6)	
Pathological complete response, n (%)				0.373
No	43 (43.4)	25 (48.1)	18 (39.1)	
Yes	56 (56.6)	27 (51.9)	28 (60.9)	

Note: [a]Wilcoxon rank sum test; Pearson's Chi-squared test.

Abbreviations: BMI, body mass index; ER, estrogen receptor, IQR, interquartile range; Ki67, antigen kiel 67; Ob-R, leptin receptor; PD-1, programmed cell death protein 1; PR, progesterone receptor; SD, standard deviation; TILs, tumor-infiltrating lymphocytes; TIM-3, T-cell immunoglobulin and mucin-domain containing-3 protein.

Immune and Pathological Characteristics

Hormone receptor-positive tumors were observed in 67.3% of patients (68/101). The median Ki-67 index was 28.0% (IQR, 20.0–44.0). Median stromal TIL density was 13.5% (IQR, 5.0–20.0), with 20.0% of tumors meeting the ≥30% TIL threshold.

PD-1 expression data were available for 86 patients, among whom 51 (59.3%) were PD-1 positive using the >1% cut-off. TIM-3 expression could be evaluated in 56 cases due to tissue availability; high TIM-3 expression (≥50% positive immune cells) was observed in 30 tumors (53.6%). Ob-R expression data were available for 97 patients, with high expression (score 2–3) observed in 65 cases (67.0%) (Table 1 and Figure 1).

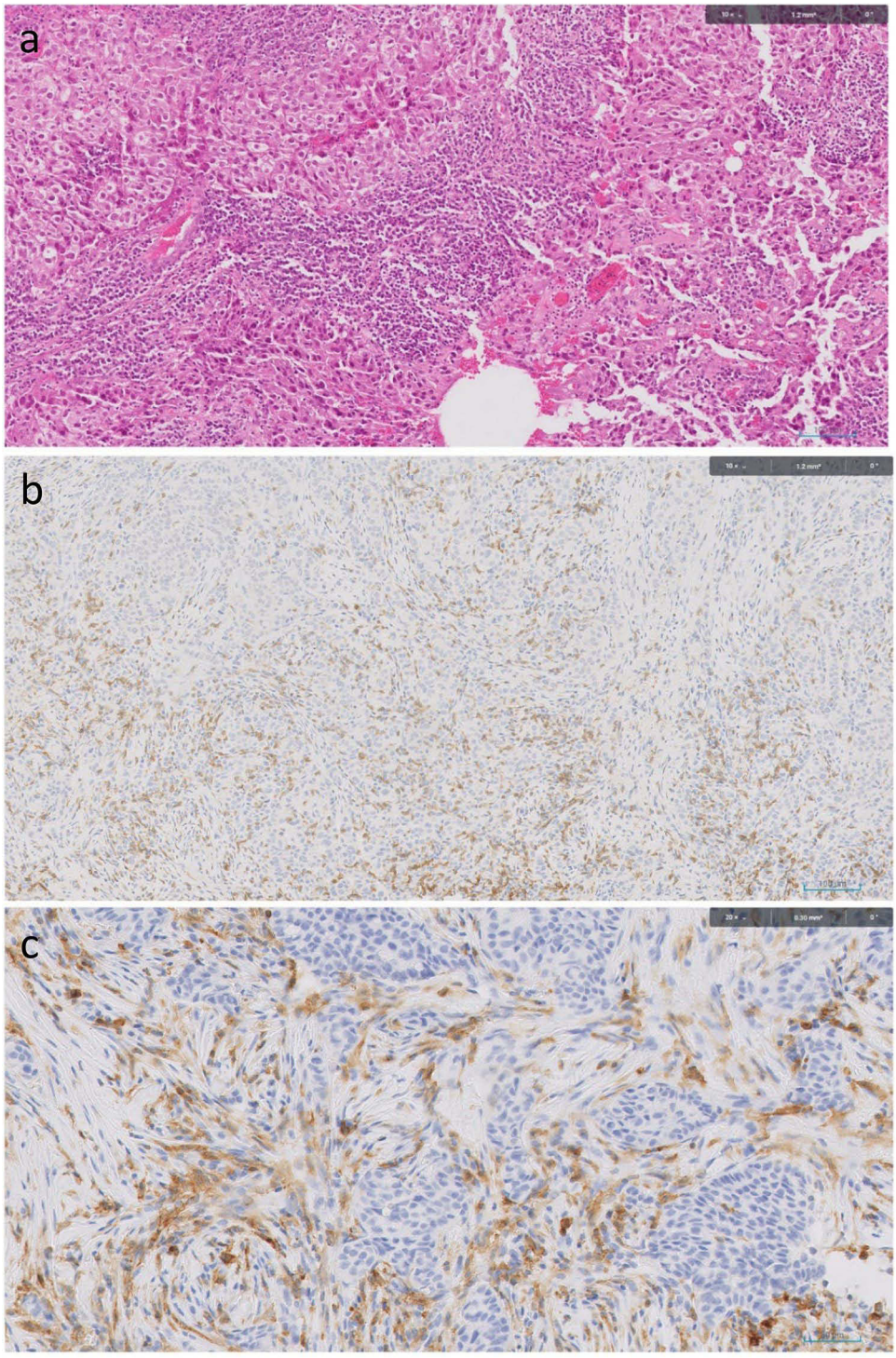

Figure 1 Immunohistochemistry (IHC) parameters.(**A**) Histological section of a breast carcinoma with abundant tumor infiltrating lymphocytes (H&E stain, 100×; scale bar = 1.2 mm). The tumor microenvironment is markedly inflamed, consistent with a lymphocyte predominant phenotype.(**B**) IHC for programmed cell death protein 1 (PD 1) showing membranous staining in approximately 30% of infiltrating lymphocytes (PD 1 IHC, 100×; scale bar = 1.2 mm), indicative of an activated/exhausted T cell population.(**C**) Immunohistochemical staining for T cell immunoglobulin and mucin domain containing protein 3 (TIM 3 IHC, 200×; scale bar = 0.30 mm) demonstrating strong expression in tumor infiltrating immune cells and cancer associated fibroblasts within the tumor microenvironment, with no detectable staining in epithelial tumor cells.

Associations Between BMI and Other Parameters

Associations between BMI category and clinicopathological and immune variables are summarized in Table 1. Patients with BMI ≥25 kg/m^2 demonstrated significantly higher stromal TIL density compared with patients with BMI <25 kg/m^2 (median 15.0% [IQR, 10.0–30.0] vs 10.0% [IQR, 5.0–20.0]; $p = 0.011$).

PD-1 positivity was more frequent among patients with BMI ≥25 kg/m^2 than among those with BMI <25 kg/m^2 (69.0% vs 48.8%), showing a trend toward statistical significance ($p = 0.058$). High Ob-R expression was significantly more common in patients with BMI ≥25 kg/m^2 compared with those with BMI <25 kg/m^2 (76.6% vs 57.1%; $p = 0.043$).

No significant differences were observed in hormone receptor status or Ki-67 index according to BMI category. BMI category was not significantly associated with pCR (BMI ≥25 kg/m^2 vs <25 kg/m^2: 60.9% vs 51.9%; $p = 0.373$).

Logistic Regression Analyses

In univariate logistic regression analyses, none of the anthropometric or clinical variables—including age, menopausal status, BMI as a continuous variable, or BMI ≥25 kg/m^2—were significantly associated with pCR (Table 2). Hormone receptor positivity was associated with a significantly lower likelihood of achieving pCR (odds ratio [OR] = 0.24; 95% confidence interval [CI], 0.09–0.61; $p = 0.004$).

Table 2 Univariate and Multivariate Logistic Regression Analysis: Association of Variable(s) with Pathological Complete Response.[a]

Variable	N	Event, n	Univariate			Adjusted Multivariate		
			OR	95% CI	*p* value	OR	95% CI	*p* value
Age	99	56	1.01	0.98, 1.04	0.621			
Weight	98	55	1.01	0.97, 1.04	0.763			
Height	98	55	0.98	0.93, 1.04	0.565			
Menopausal status	99							
No		30	—	—				
Yes		26	1.2	0.54, 2.70	0.65			
BMI	98	55	1.02	0.94, 1.11	0.63	1.14	0.97, 1.37	0.12
BMI categories	98							
<25 kg/m^2		27	—	—				
≥25 kg/m^2		28	1.44	0.65, 3.25	0.374			
BMI categories	98							
<30 kg/m^2		47	—	—				
≥30 kg/m^2		8	1.05	0.34, 3.44	0.934			
ER	99	56	0.98	0.97, 0.99	0.003			
PR	99	56	0.98	0.97, 1.00	0.02			
Hormonal receptors	99							
No		25	—	—				
Yes		31	0.24	0.09, 0.61	0.004	0.15	0.04, 0.51	0.004
Ki67	97	56	1.01	0.99, 1.03	0.376			
TILs	98	55	1.02	1.00, 1.05	0.136	1.37	1.03, 1.93	0.048

(*Continued*)

Table 2 (Continued).

Variable	N	Event, n	Univariate			Adjusted Multivariate		
			OR	95% CI	*p* value	OR	95% CI	*p* value
TILs categories	98							
<30%		43	—	—				
≥30%		12	1.44	0.52, 4.21	0.493			
PD-1	85	51	1.07	1.01, 1.15	0.049	1.07	0.099, 1.17	0.112
PD-1 categories	85							
≤1%		19	—	—				
>1%		32	1.5	0.62, 3.64	0.369			
TIM-3	56							
Low		15						
High		19	1.27	0.43, 3.75	0.667			
Ob-R score	96							
0–1		18						
2–3		38	1.14	0.48, 2.68	0.77			
BMI × TILS						0.99	0.97, 1.00	0.044
BMI × PD-1								
BMI × Ob-R BMI × Score 2–3								
TILS × PD-1								
TILs × Ob-R TILs × Score 2–3								
PD-1 × Ob-R PD-1 × Score 2–3								

Note: [a]Only statistically significant interactions are shown.

Abbreviations: BMI, body mass index; CI, confidence interval; ER, estrogen receptor, Ki67, antigen kiel 67; Ob-R, leptin receptor; OR, odds ratio; PD-1, programmed cell death protein 1; PR, progesterone receptor; TILs, tumor-infiltrating lymphocytes; TIM-3, T-cell immunoglobulin and mucin-domain containing-3 protein.

Among immune-related variables, higher PD-1 expression analyzed as a continuous variable was associated with increased odds of pCR (OR = 1.07; 95% CI, 1.01–1.15; $p = 0.049$). No significant associations with pCR were observed for TIM-3 expression, Ob-R expression, Ki-67 index, or stromal TILs when analyzed individually in univariate models.

In the multivariable logistic regression model, hormone receptor positivity remained independently associated with lower odds of achieving pCR (OR = 0.15; 95% CI, 0.04–0.51; $p = 0.004$). Higher stromal TIL density, analyzed as a continuous variable, was independently associated with increased likelihood of pCR (OR = 1.37; 95% CI, 1.03–1.93; $p = 0.048$).

A statistically significant interaction between BMI category and stromal TIL density was observed (interaction OR = 0.99; 95% CI, 0.97–1.00; $p = 0.044$), indicating that the association between immune infiltration and pCR differed according to BMI.

Discussion

In this retrospective cohort of patients with early-stage HER2-positive breast cancer treated with neoadjuvant HER2-targeted therapy, associations were evaluated between BMI, stromal TIL density, selected immune checkpoint markers, Ob-R expression, and pCR. Our findings confirm the prognostic relevance of immune-related features such as stromal

TILs and PD-1 expression, and suggest that BMI may modify the relationship between immune infiltration and treatment response, although BMI itself was not directly associated with pCR.

We observed that patients with BMI ≥25 kg/m^2 exhibited higher stromal TIL density compared with patients with BMI <25 kg/m^2. This finding contrasts with a prior report showing no association or even lower immune infiltration in tumors from overweight or obese patients,[31] but aligns with emerging evidence that host body composition may influence immune cell recruitment in a context-dependent manner.[32] Importantly, BMI was used in this study as a pragmatic clinical surrogate of body composition and does not directly reflect adiposity, fat distribution, or metabolic health. Therefore, these results should be interpreted as associations rather than evidence of adiposity-driven immune mechanisms.

Consistent with previous studies in HER2-positive breast cancer and TNBC,[33–35] higher stromal TIL density was independently associated with increased likelihood of achieving pCR. Notably, this association became evident only in the multivariable model, underscoring the importance of accounting for tumor biological features such as hormone receptor status. The observed interaction between BMI and TIL density suggests that the predictive value of immune infiltration may vary according to host-related factors. While this interaction does not establish causality, it supports the hypothesis that host characteristics may influence the functional relevance of tumor immune infiltration.

PD-1 expression, when analyzed as a continuous variable, was positively associated with pCR. This observation is in line with prior reports linking PD-1 or PD-L1 expression to increased immunogenicity and improved response to systemic therapy in breast cancer.[36,37] The lack of significance when PD-1 was analyzed as a dichotomous variable highlights the limitations of threshold-based classification and suggests that quantitative assessment may better capture biologically relevant variation. Although PD-1 positivity was more frequent in patients with elevated BMI, BMI itself was not associated with pCR, indicating that these relationships are likely multifactorial.

TIM-3 expression did not show a significant association with TIL density or pCR in this cohort. In addition, TIM-3 assessment was feasible in only a subset of cases due to tissue availability. As a result, no definitive conclusions can be drawn regarding the role of TIM-3 in early-stage HER2-positive breast cancer based on the present data. Future studies incorporating broader immune profiling and functional assays will be necessary to clarify the relevance of TIM-3 and other exhaustion markers in this setting.

Ob-R expression was more frequently observed in tumors from patients with higher BMI, but it was not independently associated with immune infiltration or pCR. Although leptin signaling has been implicated in immune modulation in preclinical models,[32] leptin levels were not assessed in this study. Therefore, our findings regarding Ob-R expression should be considered descriptive, and no mechanistic inferences regarding leptin-mediated immune effects can be made. Additional studies integrating metabolic biomarkers and immune phenotyping are required to elucidate these relationships.

This study has several limitations. Its retrospective design and modest sample size may limit statistical power, particularly for subgroup and interaction analyses. BMI is an indirect and imperfect measure of body composition and does not account for metabolic heterogeneity. Immune characterization was restricted to selected markers, without evaluation of CD4+ or CD8+ T-cell subsets or alternative exhaustion pathways. Furthermore, clinical outcomes beyond pCR, such as recurrence or survival, were not available. These limitations restrict causal interpretation and underscore the hypothesis-generating nature of the analysis.

Despite these limitations, our findings reinforce the clinical relevance of stromal TILs and PD-1 expression in early-stage HER2-positive breast cancer and suggest that host-related factors such as BMI may influence immune-tumor interactions. Prospective studies integrating detailed metabolic, immunological, and clinical data are warranted to further clarify these associations and their potential implications for personalized treatment strategies.

Conclusions

In this retrospective analysis of early-stage HER2-positive breast cancer treated with neoadjuvant HER2-targeted therapy, stromal TIL density and PD-1 expression were associated with pCR, reinforcing the relevance of immune-related biomarkers in this disease setting. While BMI was not directly associated with treatment response, it was related to differences in immune infiltration and modified the association between TIL density and pCR.

These findings suggest that host-related factors, as captured by BMI, may influence the tumor immune microenvironment and the predictive value of immune biomarkers. Given the use of BMI as an indirect measure of body

composition and the retrospective design of the study, these observations should be considered hypothesis-generating. Prospective studies incorporating comprehensive metabolic and immune profiling, as well as long-term clinical outcomes, are warranted to further elucidate the interplay between host characteristics, tumor immunity, and treatment response in HER2-positive breast cancer.

Data Sharing Statement

Data analyzed in this study are available upon reasonable request from the corresponding author, LGE.

Ethics Approval and Informed Consent

The study was approved by the Institutional Review Board of Hospital Ramón y Cajal (approval number 3-2022-0131) and conducted in accordance with the Declaration of Helsinki (2013 amendment). Due to the retrospective nature of the study, informed consent was waived.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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