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Clinicopathologic Features and Immune Cell Subtypes Analysis of Tumor-infiltrating Lymphocytes Rich Invasive Breast Carcinoma of No Special Type

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Abstract: Tumor-infiltrating lymphocytes (TILs) rich invasive breast carcinoma no special type (IBC-NST) is an updated name introduced in the fifth edition WHO classification of breast tumors. Typical medullary breast carcinoma (MBC) represents one end of the spectrum of TILs-rich IBC-NST rather than a distinct morphologic subtype in the new category. A total of 42 cases of MBC and 180 cases of high-grade triple-negative breast cancer (TNBC) without medullary features were included. All samples were stained for CD20, CD4, CD8, and FoxP3 by immunohistochemistry staining. TILs infiltration was more prominent in the MBC tumor nests and in the stroma of high-grade TNBC without medullary features. The average stromal TILs percentage was 78.10% and 61.33%. MBC showed significantly lower numbers of lymphocytes expressing FoxP3 ($P < 0.001$), no significant difference in the number of CD4 ($P = 0.154$), CD8 ($P = 0.199$), and a significantly higher CD8/FoxP3 ratio ($P < 0.001$) than the other high-grade TNBC. MBC cases demonstrated less aggressive features such as lower TNM stage ($P = 0.031$), smaller tumor size ($P = 0.010$), and negative lymph node status ($P = 0.021$) than the other high-grade TNBC. The 5-year disease-free survival and overall survival were significantly higher for MBC 82.50% and 85.00% compared with the other high-grade TNBC(54.49% and 58.68%). MBC is mostly triple-negative with higher nuclear atypia. Despite advanced staging based on cell morphology, it has low malignancy and a good prognosis. Differences in biological features and prognosis between MBC and high-grade TNBC without medullary features may be associated with the composition and function of TILs. Immune cell subtypes are complex in TILs-rich IBC-NST and deserve further investigation.

Key Words: breast cancer, IBC-NST with medullary features, TNBC, clinicopathologic features, TILs

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Breast cancer is the most commonly occurring cancer in women and the most common cancer overall.[1] With the development of tumor immunology, the relationship between tumor microenvironment (TME) and tumor occurrence and development has been widely concerned. Evidence suggested that breast cancer patients with more tumor-infiltrating lymphocytes (TILs) have a better prognosis.[2] However, TILs are not a single type of immunocytes, and a different subpopulation of lymphocytes has different effects on breast cancer. Subtypes of TILs impact both tumor cells and immune cells in different ways, leading to either a protumor or antitumor effect.[3]

Typical medullary breast carcinoma (MBC) is a distinct type in the former WHO classification. The diagnosis criteria for MBC were predominantly circumscribed border, syncytial growth pattern (> 75%), absence of glandular structures, diffuse infiltration of lymphocytes, nuclear pleomorphism, and complete histologic circumscription.[4] However, MBC has suffered from poor interobserver reproducibility and overlapping features with other high-grade triple-negative breast cancer (TNBC). So the fifth edition WHO classification eliminated medullary carcinoma as a distinct histologic type and made it a pattern of invasive breast carcinoma no special type (IBC-NST) that is characterized by a predominance of stromal TILs (sTILs) and high grade.[5] Typical MBC is now represented as one end of the spectrum of TILs-rich IBC-NST rather than a

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distinct morphologic subtype. They are associated with basal phenotype but have a better prognosis than other stage-matched high-grade TNBC.

TNBC is considered to be the most aggressive and heterogenous subtype of breast cancer. Due to its lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (*HER-2*), hormone therapy and drugs that target *HER-2* are not helpful in TNBC. Chemotherapy is the standard systemic treatment option due to the lack of well-accepted targets. TNBC is known to have a poor prognosis and some kinds of TNBC also exhibit rich TILs. In this context, typical MBC and other high-grade TNBC without medullary features are 2 representative lesions in TILs-rich IBC-NST. It is unclear whether there are differences in clinicopathologic features, prognosis, and immune cell subtypes of TILs in typical MBC and other high-grade TNBC.

Increasing clinicopathological data suggests that TILs play an essential role in mediating response to chemotherapy, even predicting that the patient can be treated with immunotherapy.[6] Therefore, the systematic evaluation of TILs and specific populations in IBC-NST may be helpful to guide both prognosis and therapies for breast cancer. In this study, we sought to investigate the clinicopathological features, immune cell subtypes, and prognosis data to improve the understanding of these tumors.

PATIENTS AND METHODS

Patient Cohort and Samples

Forty-two MBC and 180 high-grade TNBC without medullary features at the First Affiliated Hospital of Bengbu Medical College from July 2006 to July 2016 were included in this study. All selected cases were surgically removed specimens without treatment with radiotherapy, chemotherapy, or other treatments before surgery. The patients were all females, without distant metastasis at initial diagnosis, and with typical MBC and high-grade TNBC without medullary features according to the inclusion criteria. MBC must meet the mentioned diagnostic criteria and high-grade TNBC refers to the third grade according to the Nottingham histologic classification.

All patients were followed up by phone call using outpatient records. This study was conducted following the Declaration of Helsinki (as revised in 2013). It was approved by the institutional ethics board of The First Affiliated Hospital of Bengbu Medical College (No. BBMEC-2021088), and informed consent was taken from all the patients.

Hematoxylin and Eosin Staining and Evaluation

Hematoxylin and eosin (*H* and *E*) staining slides were double-blind pathologic reviewed by 2 pathologists. Histologic evaluation standards refer to the fifth edition of WHO and TNM staging of breast cancer developed by the American Joint Committee on Cancer (seventh edition).

TILs were assessed by 2 pathologists on *H* and *E*-stained representative whole-tissue sections on glass slides, according to the system recommended by the International TILs Working Group (2014).[7] It is evaluated in 2 different localizations: sTILs and intratumoral TILs (iTILs). While iTILs represent the density of mononuclear cells in direct interaction with tumor cells, sTILs are the percentage of the area occupied by mononuclear cells (lymphocyte and plasma cells) within the tumor stroma. Scoring is done within the borders of tumor invasion. Normal lobules, atypical hyperplasia, and crush artifact with ductal carcinoma in situ surrounding inflammatory response, necrosis, and intense hyalinization are excluded from the evaluation. Compared with iTILs, the evaluation of sTILs is reported to be more accessible, reliable, and reproducible.[8] In our study, the sTILs were assessed by 2 pathologists on the *H* and *E* section containing sufficient tumor area from each case.

Immunohistochemical Staining

Formalin-fixed paraffin-embedded tissue sections were used in each case using a standard protocol. H and E-stained sections (4 μm thickness) were reexamined to evaluate the tumor's histologic features, and immunohistochemistry was performed with the Elivision technique. Mouse anti-human FoxP3 (1:100; Abcam) was used, and other antibodies ER (Monoclonal, cloneSP1), PR (Monoclonal, clone1A6), *HER-2* (Monoclonal, cloneCB11), Ki67 (Monoclonal, clone MIB-1), CD8 (Monoclonal, cloneSP16), CD4(Monoclonal, clone4B12), and CD20 (Monoclonal, cloneL26) were obtained from Maixin Biotech, Inc. and were ready to use. The threshold for positive ER and PR expression was any nuclear labeling 1% or higher.[9] *HER-2* immunoreactivity was evaluated on a standardized scale from 0 to 3 based on the intensity of staining of the cell membrane and the proportion of invasive tumor cells followed by a recommendation of the American Society of Clinical Oncology/College of American Pathologists clinical practice guideline. Strong complete membrane staining in $>10\%$ of tumor cells (score, 3+) was considered positive. Intensity patterns with scores of 0 to 1+ were considered negative, and samples scored as 2+ were further assessed by the fluorescence in situ hybridization test. A fluorescence in situ hybridization ratio of 2.0 or more was considered positive for *HER-2* gene amplification.[10]

TILs of all samples were stained for CD20, CD4, CD8, and FoxP3. Positive cells per high-power field (HPF) by manually inspecting stained sections with 5 areas of high staining intensity. Each slide was initially screened under low magnification (×100), and the most significant number of positively stained cells (hot spot area) was then selected for the subsequent analysis. The mean tumor-infiltrating immune cells in these areas for each case were evaluated carefully.[11] For statistical analyses, the number of positive cells was divided into lower and higher groups based on cutoff points according to the median.

Statistical Analyses

The test for the number of TILs uses the Student *t* test. The association of clinicopathologic factors was

evaluated using the χ^2 test. The primary clinical outcomes for this study were disease-free survival (DFS) and overall survival (OS). Survival time was calculated from the surgery date to these endpoints. DFS was defined as the time from the first diagnosis of primary breast cancer to local recurrence or metastasis. OS was defined as the same starting point to death from any cause. The 5-year survival rate was calculated using the Life Tables method. Survival curves were obtained using the Kaplan-Meier method, and the log-rank test was used to determine the statistical significance in relative survival for various patient and tumor characteristics. All of the statistically significant variables observed in univariate analysis were investigated by means of multivariate analysis using the Cox proportional hazards model. All P values <0.05 were considered statistically significant. All P values are 2-sided. The SPSS 26.0 software package (IBM) was used for statistical analyses.

RESULTS

Clinicopathological Characteristics of Samples

The cases of MBC were at the age of 32 to 61 years (median, 43 y). The high-grade TNBC without medullary features ranged from 23 years to 84 years (median, 52 y). MBC cases demonstrated less aggressive tumor features such as lower TNM stage (P = 0.031), smaller tumor size (P = 0.010), and a lower proportion of nodal involvement (P = 0.021), whereas more aggressive growth patterns were observed in high-grade TNBC without medullary features. Among MBC patients, there were 4 cases of luminal type (9.52%) (including 1 case of luminal *A* type and 3 cases of luminal *B* type), 7 cases of *HER-2* gene overexpression type (16.67%), and 31cases of triple-negative type (73.81%). MBCs are mostly triple-negative phenotypes. The clinicopathologic characteristics of the 2 groups are presented in Table 1.

TABLE 1. Clinicopathological Characteristics of MBC and High-grade TNBC Without Medullary Features

Characteristics	MBC (%)	High-grade TNBC (%)	χ^2	*P*
Age (y)				
≤40	13 (30.9)	21 (11.7)	5.829	0.030
>40	29 (69.1)	159 (88.3)	—	—
Tumor size (cm)				
≤2	19 (45.2)	42 (23.4)	9.165	0.010
2-5	22 (52.4)	105 (58.3)	—	—
>5	1 (2.4)	33 (18.3)	—	—
Nodal status				
Positive	10 (23.8)	87 (48.3)	6.292	0.021
Negative	32 (76.2)	93 (51.7)	—	—
TNM stage				
I	16 (38.1)	30 (16.7)	6.933	0.031
II	24 (57.1)	126 (70.0)	—	—
III	2 (4.8)	24 (13.3)	—	—
KI67				
<30%	6 (14.3)	84 (46.7)	11.657	0.001
≥30%	36 (85.7)	96 (53.3)	—	—
P53				
Positive	25 (59.5)	117 (65.0)	0.317	0.723
Negative	17 (40.5)	63 (35.0)	—	—

MBC indicates medullary breast carcinoma; TNBC, triple-negative breast cancer.

Analysis of Immune Cell Subtypes

TILs infiltration was more prominent in the MBC tumor nests and in the stroma of high-grade TNBC without medullary features. The average sTILs percentage was 78.10% in MBC whereas the average sTILs percentage was 61.33% in other high-grade TNBC. Further analysis showed that *T* lymphocytes were the main subpopulation in almost all breast cancer patients with MBC or without medullary features. The density of *T* lymphocytes in MBC tumors was higher than that in high-grade TNBC cases. There were few *B* lymphocytes within MBC and high-grade TNBC tumors. But tertiary lymphoid structures that include *B* cells can be identified in MBC whole sections, particularly those taken from the interface between carcinoma and adjacent normal tissues. However, this phenomenon is rarely seen in high-grade TNBC without medullary features (Fig. 1).

In MBC, the average number of CD8 positive lymphocytes was 74.76 per HPF and the average number of FOXP3 was 41.43/HPF. Similarly, in high-grade TNBC without medullary features, the average number of CD8 positive lymphocytes was 65.10/HPF and the average number of FOXP3 was 67.47/HPF. The average number of CD20-positive lymphocytes was 20.38/HPF in MBC and 16.08/HPF in high-grade TNBC. The average number of CD4-positive lymphocytes was 32.49/HPF in MBC and 28.20/HPF in high-grade TNBC (Fig. 2).

More characteristically, in comparison with high-grade TNBC without medullary features, there were significantly lower numbers of FoxP3-positive lymphocytes detected in MBC and a higher CD8/FoxP3 ratio was observed in this tumor. The mean CD8/FoxP3 ratios for MBC and controls were 1.77 and 0.96 respectively (Table 2).

Outcome, Recurrence, and Prognosis

All patients were followed up until July 2021. The median follow-up time was 78 months (range, 34 to 89 mo). Contact with 2 patients in the MBC group and 13 patients in the high-grade TNBC group was lost during the follow-up period.

It was found that 7 patients with MBC developed disease progression. Of them, 4 patients had local recurrence. Three cases had distant metastasis (1 of lung metastasis; 1 of liver metastasis; and 1 patient with multiple metastases with lung, liver, and brain). To the endpoint, 6 patients died due to cancer recurrence. In high-grade TNBC without a medullary pattern, there were 76 patients with disease progression. Of them, 35 patients had local recurrence.41 patients had distant metastases (21 of lung metastasis; 12 of liver metastasis; and 8 with multiple metastases to lung, liver, brain, and bone). To the endpoint, 69 cases died from tumor recurrence. Finally, the 5-year DFS of patients with MBC and high-grade TNBC without a medullary pattern was 82.50% and 54.49%, respectively.

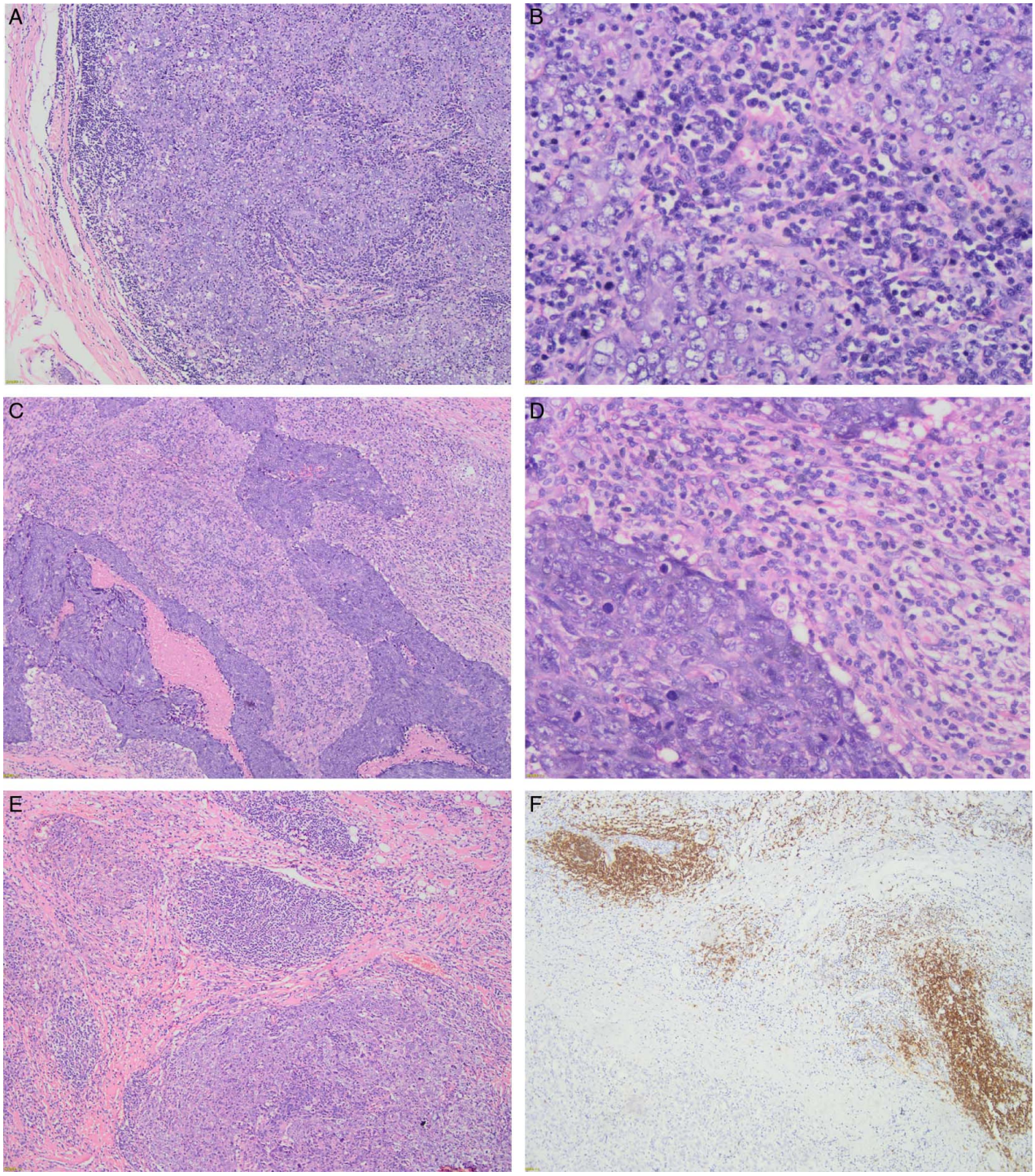

FIGURE 1. TILs infiltration in MBC and TNBC. Hematoxylin and eosin staining slides showing TILs infiltration were more prominent in the MBC tumor nests [(A) ×100 and (B) ×400] and in the stroma of high-grade TNBC without medullary features [(C) ×100 and (D) ×400]. Tertiary lymphoid structures can be seen between the MBC tumor and normal tissue and CD20 marks the *B* cells in them [(E) ×100 and (F) ×100]. MBC indicates medullary breast carcinoma; TIL, Tumor-infiltrating lymphocyte; TNBC, triple-negative breast cancer.

There was a statistical difference between the two groups ($P = 0.032$). Similarly, the OS of patients with MBC was 85.00%, which was significantly better than the control group (58.68%) ($P = 0.031$) (sFig. 3).

Univariate and multivariate Cox regression analysis of DFS and OS were performed using clinicopathological prognostic factors and expressions of immune cell subtypes in MBC and high-grade TNBC

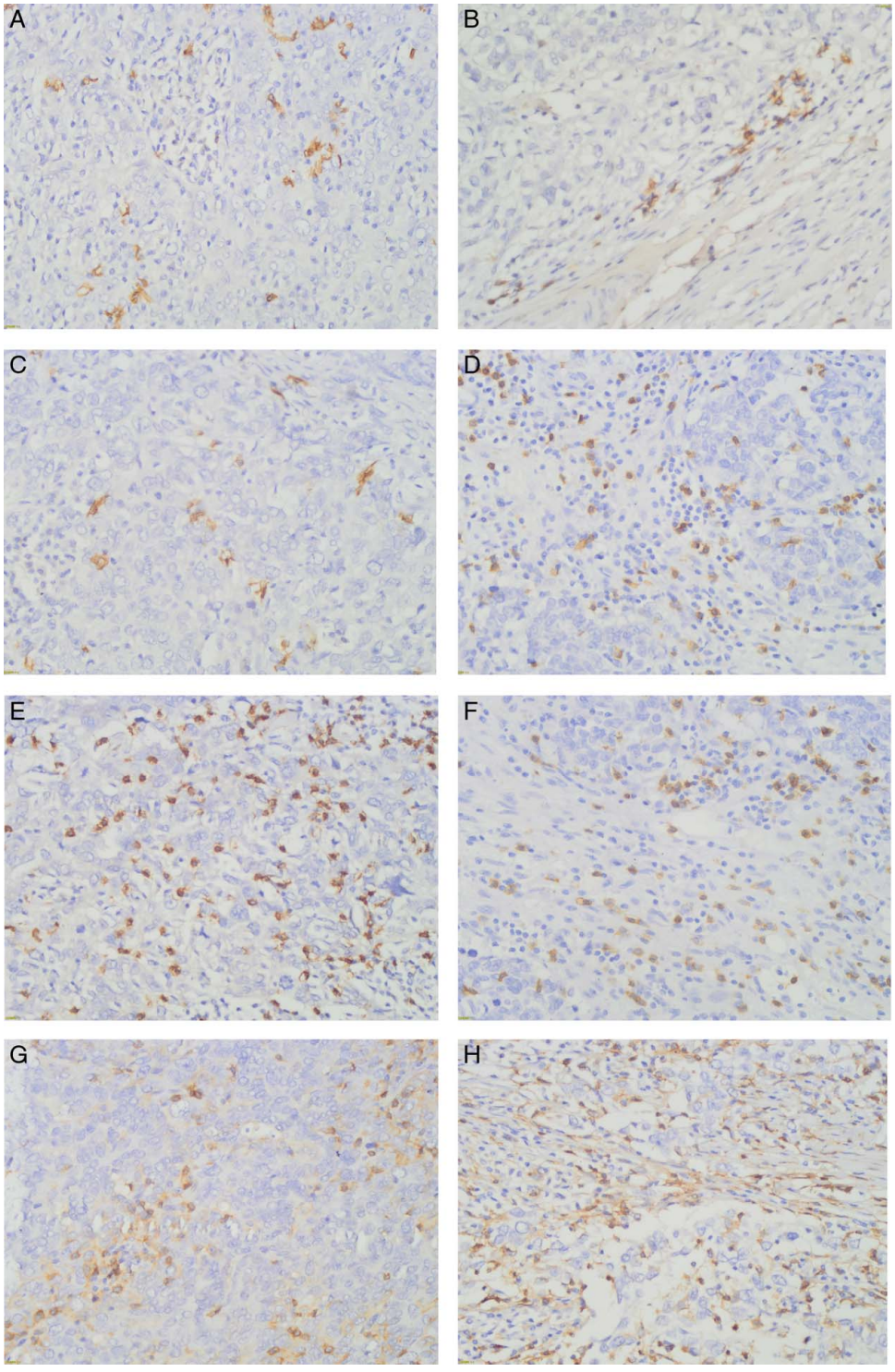

FIGURE 2. The immunohistochemistry expression of different immune cells in MBC and TNBC. CD20 positive lymphocytes are shown for MBC [(A) ×400] and TNBC [(B) ×400]. CD4-positive lymphocytes are shown for MBC [(C) ×400] and TNBC [(D) ×400]. CD8-positive lymphocytes are shown for MBC [(E) ×400] and TNBC [(F) ×400]. FOXP3-positive lymphocytes are shown for MBC [(G) ×400] and TNBC [(H) ×400]. MBC indicates medullary breast carcinoma; TNBC, triple-negative breast cancer. full color online

without medullary pattern group. The data revealed that tumor size, lymph node status, TNM stage, CD8, FoxP3, CD8/FoxP3 were statistically significant factors for survival in the MBC group, and age, tumor size, lymph node status, TNM stage, CD8, FoxP3, and CD8/FoxP3 were statistically significant factors for survival in

TABLE 2. Immunophenotyping of TILs in MBC and High-grade TNBC Without Medullary Pattern

	MBC	TNBC (high grade)	*t*	*P*
sTILs	78.10 ± 2.209	61.33 ± 2.922	3.100	0.0035
CD20	20.38 ± 1.861	16.08 ± 1.547	1.785	0.081
CD4	32.49 ± 3.482	28.20 ± 4.491	1.675	0.147
CD8	74.76 ± 4.671	65.10 ± 5.661	1.303	0.199
FoxP3	41.43 ± 3.229	67.47 ± 5.285	4.205	0.0001
CD8/FoxP3	1.77 ± 0.131	0.96 ± 0.072	5.127	< 0.0001

MBC indicates medullary breast carcinoma; sTIL, stromal TIL; TIL, tumor-infiltrating lymphocyte; TNBC, triple-negative breast cancer.

high-grade TNBC without medullary pattern group. (Tables 3 and 4).

DISCUSSION

As one end of the spectrum of TILs-rich IBC-NST, MBC has unique clinicopathologic characteristics and has continued to garner interest. In this study, we found that a higher fraction of MBCs occurred among young patients and showed less aggressive features such as lower tumor stage, smaller tumor size, and a lower proportion of nodal involvement than the control group. We also found that 37 cases of MBCs in this study were triple-negative (73.81%), which is in line with the fact that MBCs frequently display a profile of TNBC. As we observed in our experiments, MBCs were generally associated with "aggressive" histopathological features—high mitotic index, enriched cytoplasm, easy syncytia formation, and a high level of *TP53* mutations. What is more, MBCs were usually made of poorly differentiated cells characterized by the presence of large nuclei and prominent nucleoli. Surprisingly, patients with MBC presented significantly higher 5-year DFS and OS than high-grade TNBC without medullary features. The study by Sabatier et al[12] suggested that MBCs enhanced tumor cell apoptosis, elevated levels of metastasis-inhibiting factors, and low levels of metastasis-promoting factors. Using whole-genome oligonucleotide microarrays, Bertucci et al[13] compared gene expression profiles of MBCs and grade 3 DBCs. They found that the important process associated with MBCs was apoptosis. Over-expressed genes encode members of the tumor necrosis factor (TNF) receptor, TNF ligand superfamilies, and TNFα-induced proteins TNFAIP2 and TNFAIP3, all involved in the extrinsic apoptosis pathway. At the same time, they found that the immune response was a remarkably represented biological process and contained many T-cell–associated genes. TILs are mainly composed of cytotoxic CD8+ T cells and indicate the outcome of patients with this tumor type would be the result of the joint action of tumor cells and effective T cells in TME.

Nowadays, the contribution of TMEs, especially TILs, to cancer evolution and treatment response has attracted more attention in the field of translational research and clinical practice, especially for prognostic evaluation. TILs are primarily composed of *T* lymphocytes, *B* lymphocytes, natural killer cells, and macrophages. Some of these cells (CD8+ *T* lymphocytes, Th1 CD4+ lymphocytes, and M1 macrophages) exert antitumor properties to control cancer development, whereas some other immune cells such as CD4+ Th2 *T* lymphocytes and M2 macrophages may promote cancer growth or evasion of immune surveillance.[14] This suggests that a complex and dynamic interaction occurs between tumor cells and the immune system during cancer progression. Hence, detection of TILs in the tumor immune microenvironment, which controls tissue homeostasis and activates innate and

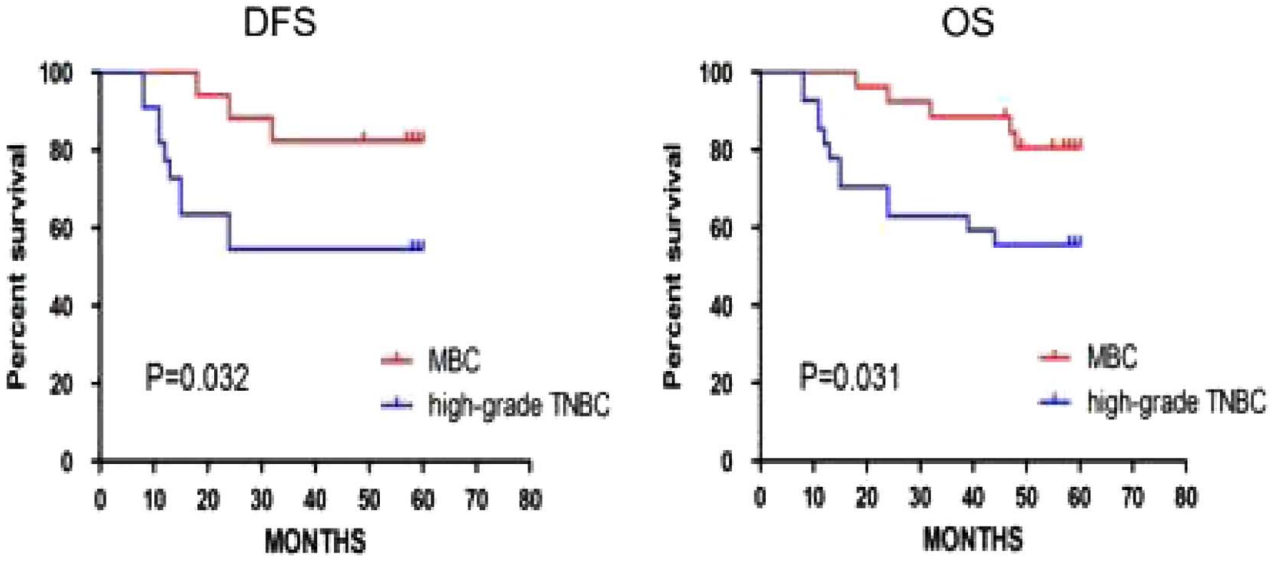

FIGURE 3. Comparison of survival rate between MBC and TNBC. DFS (A) and OS (B) of patients with MBC as compared with high-grade TNBC without medullary features. DFS indicates disease-free survival; MBC, medullary breast carcinoma; OS, overall survival; TNBC, triple-negative breast cancer. full color online

TABLE 3. Univariate and Multivariate Cox Regression Analyses for DFS and OS of MBC

Clinicopathological features	Univariate analysis DFS HR (95% CI)	*P*	Multivariate analysis DFS HR (95% CI)	*P*	Univariate analysis OS HR (95% CI)	*P*	Multivariate Analysis OS HR (95% CI)	*P*
Age (<40 vs ≥40)	2.962 (0.191–4.873)	0.116	—	—	2.692 (2.256–2.056)	0.326	—	—
Tumor size (≤2 cm vs >2 cm)	2.687 (1.399–3.825)	0.006	3.625 (2.753–6.569)	0.005	3.332 (1.325–4.628)	0.012	3.677 (1.849–3.602)	0.010
Lymph node status (absent vs present)	3.923 (1.921–3.245)	0.021	3.874 (1.951–3.532)	0.023	3.093 (1.561–3.243)	0.035	3.608 (1.876–3.894)	0.023
TNM stage (I vs II, III)	2.209 (1.281–5.238)	0.008	3.351 (1.046–5.624)	0.012	2.033 (1.088–6.875)	0.032	3.023 (1.408–4.929)	0.004
CD4 (low vs. high)	2.945 (0.261–3.292)	0.874	—	—	3.201 (1.931–4.345)	0.748	—	—
CD8 (low vs high)	3.355 (1.826–3.345)	0.012	3.252 (1.092–3.662)	0.001	2.784 (1.976–3.930)	0.008	2.406 (2.943–3.965)	0.001
FoxP3 (low vs high)	3.298 (1.874–3.234)	<0.001	2.945 (1.824–3.243)	0.001	3.452 (1.051–3.076)	0.001	3.732 (1.941–3.025)	0.012
CD8/FoxP3 (low vs high)	3.626 (1.245–3.234)	0.063	3.925 (1.252–6.023)	0.023	3.836 (1.832–3.895)	0.013	3.670 (1.943–3.686)	0.020

DFS indicates disease-free survival; HR, hazard ratio; MBC, medullary breast carcinoma; OS, overall survival.

adaptive immune cells may provide a vital indicator for monitoring the immune interaction between host and tumor, and effective predictive biomarkers of cancer immunogenicity, response to immunotherapy and clinical outcome.[15] Immunohistochemistry is a widely used detection method to identify specific immune populations of TILs in formalin-fixed paraffin-embedded tissues because it is a relatively easy, inexpensive, and widely available method. In this study, more CD8+ T cells were observed in MBC, indicating antitumor activities may be prominent in this kind of breast cancer.

The balance between antitumor-effector *T* cells and regulatory *T* cells (T_{regs}), is critical for antitumor effects and the prognosis of cancer.[16] Higher effector T-cell infiltration in the tumors is associated with a better prognosis, whereas the opposite effects may be the function of Tregs. Cytotoxic *T* lymphocytes (CTLs) are the most critical players in TILs and can directly target tumor cells by the release of effector cytokines such as perforin and granzyme *B*.[17] However, regulatory *T* lymphocytes (T_{regs}) may in part attenuate the tumor-specific immunity by suppressing the activity of CTLs. FOXP3 is a crucial regulator for the development of T_{regs} that play an immunosuppressive role by decreasing the response to self-antigens.[18–20] In this study, we found that the density of FoxP3-positive lymphocytes was lower in MBC than that in the high-grade TNBC without medullary features, and a higher CD8/FoxP3 ratio was observed in this tumor, indicating de novo antitumor immunity may function in the tumor that would benefit the clinical outcome of patients with MBC.

Increasing evidence suggests that *B* lymphocytes may contribute to antitumor immunity when organized lymphoid aggregates known as tertiary lymphoid structures are found in the tumor mass.[21,22] In this study, the tertiary lymphoid structures can be identified in the interface between carcinoma and adjacent normal tissues of MBC despite few *B* lymphocytes within the tumor. In contrast, almost the same density of both CD8+ and FoxP3+ T cells was found in high-grade TNBC without medullary features with a decrease of CD8/FoxP3 ratios. Moreover, CD20+ *B* lymphocytes within the tumor and tertiary lymphoid structures were invisible. These findings strongly suggest that robust immune response against the tumor was more boosted in MBC than that in high-grade TNBC without medullary features. The different immune reactions between the two groups of TNBC may add significant evidence to explain the differences in clinicopathologic features and prognosis in clinical practice. In contrast, infiltrating lymphocytes both in the stroma and epithelial components of MBC may lead to direct contact of CTLs with tumor cells that facilitate attacking tumor cells by activating *T* cells. In contrast, the decrease of T_{reg} lymphocytes in MBC may certainly augment T-cell activation. These findings were also in line with the data from several recent studies that the quantity and subpopulations of infiltrating lymphocytes are the key determinants for clinical outcomes.[15,18,19,23,24] Importantly, it has been shown that a subset of $CD8^+$ *T* cells with resident memory T-cell phenotype infiltrated within peripheral tumor tissue without recirculation is positively associated with increased survival

TABLE 4. Univariate and Multivariate Cox Regression Analyses for DFS and OS of High-grade TNBC Without Medullary Pattern

	DFS				OS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
Clinicopathological features	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (<40 vs ≥40)	1.769 (0.851–3.567)	0.026	2.353 (0.516–2.749)	0.014	1.802 (1.756–3.261)	0.002	2.283 (1.851–5.015)	0.012
Tumor size (≤2 cm vs >2 cm)	3.387 (1.809–3.536)	0.025	3.653 (2.729–4.749)	0.012	3.623 (1.321–4.656)	0.022	3.651 (1.511–3.661)	0.008
Lymph node status (absent vs present)	3.603 (1.821–3.985)	0.031	3.427 (1.031–3.605)	0.002	2.934 (1.934–3.262)	0.020	3.053 (2.351–4.545)	0.001
TNM stage (I vs II, III)	2.863 (1.488–7.225)	0.005	2.753 (1.093–5.765)	0.004	3.019 (1.150–6.854)	0.002	2.726 (1.601–4.935)	0.002
CD4 (low vs high)	2.469 (0.851–3.567)	0.198			2.233 (1.854–3.651)	0.103		
CD8 (low vs high)	2.387 (1.809–3.536)	0.005	2.952 (1.042–3.952)	0.004	2.515 (1.951–4.515)	0.010	2.606 (2.723–3.032)	0.002
FoxP3 (low vs high)	3.603 (1.821–3.985)	0.025	3.495 (1.752–4.963)	0.022	3.512 (3.011–3.025)	0.005	3.984 (1.961–3.051)	0.012
CD8/FoxP3 (low vs high)	3.603 (1.821–3.985)	0.002	3.954 (1.731–3.017)	0.010	3.345 (1.025–3.571)	0.001	3.150 (2.613–5.618)	0.002

DFS indicates disease-free survival; HR, hazard ratio; OS, overall survival; TNBC, triple-negative breast cancer.

of patients with TNBC, and similar results also found in TNBC with granzyme B^+ $CD8^+$ TIL infiltration.[19,25,26] Indeed, we have observed higher DSF and OS in patients with MBC than that in other subtypes of TNBC (Fig. 3). But a more detailed function of infiltrating immune cells in MBC or other types of breast cancer needs to be further investigated. It has been demonstrated that dysfunction or exhaustion of infiltrating CD8+ *T* cells has been reported in most patients with breast cancer and displays a negative correlation of exhausted CD8+ *T* cells with clinical outcomes in ER+ breast cancer, highlighting the role of exhausted CD8+ *T* cells in regulating treatment response and prognosis, particularly immunotherapy.[24,27] Exhausted *T* cells are a group of dysfunctional *T* cells, which are present in chronic infections or tumors. The most significant characteristics of exhausted *T* cells are attenuated effector cytotoxicity, reduced cytokine production, and upregulation of multiple inhibitory molecular receptors (eg, PD-1, TIM-3, and LAG-3).[28] It is clear that tumor-infiltrating lymphocytes are the most important group of immune cells in the TME and are a direct force to suppress or eliminate tumor cells. However, even these special cells, which are supposed to be an important immune monitoring and clearance force, can be gradually weakened or exhausted to varying degrees in the TME. Hence, the complex of the breast cancer microenvironment implies that exhausted CD8+ *T* cells may function as a negative regulator for treatment response in high-grade TNBC without medullary features. Furthermore, it is worth speculating that further investigation of the phenotype and spatial function of T-cell subsets in breast cancer may provide more precision insights into the personalized management of this disease, and that is also worthy of further investigation.

In summary, this study further confirmed that the function and immune cell subtypes of TILs are different in MBC and high-grade TNBC without medullary features, and the differences are associated with the clinical outcome. These findings further suggest that there is the importance of patient stratification to tailor therapeutic regimens for breast cancer patients based on the activated biomarkers of T-cell subsets, especially exhausted $CD8^+$ T-cell immune signature.[29]

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