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Original Article

Tumor-associated macrophages (TAMs) and tumor-infiltrating lymphocytes (TILs) in pretherapeutic breast cancer core biopsies: Anti-tumoral effect of immune cells associated with neoadjuvant chemotherapy

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Abstract: Purpose: Infiltration of immune cells may be associated with response to chemotherapy. To assess the relationship between tumor-associated macrophages (TAMs) or tumor-infiltrating lymphocytes (TILs) and short-term response to neoadjuvant chemotherapy (NAC), we analyzed TAM/TILs in pre-NAC breast cancer core biopsy (PNB) and their therapeutic contribution to the effectiveness of NAC based on the curative surgical specimen after NAC. Methods: In 154 neoadjuvant cohort cases of primary invasive breast cancer, we immunohistochemically measured subtypes (M1 and M2) of TAMs and histologically valued stromal TILs in PNB samples and assessed their correlation with tumor response by pathologic complete response (pCR) and clinicopathological charateristics. Results: Although higher CD163-positive M2 score was significantly associated with aggressive clinicopathological features including older age (≥45) (P=0.041), higher tumor grade (P≤0.001), higher Ki-67 proliferative index (≥20%) (P<0.001), ER negativity (P<0.001), PR negativity (P<0.001) and triple negative type (P=0.023), it was associated with better NAC outcome such as smaller post-NAC tumor size (<1.5 cm) (P=0.007), low post-NAC pathologic T stages (P=0.021), absence of post-NAC nodal metastasis (P=0.048) and more frequent pCR (P=0.018). High TIL score was also related to smaller post-NAC tumor size (<1.5 cm) (P=0.021), low post-NAC pathologic T stages (P=0.003) and high pCR rate (P=0.037). Conclusion: A greater number of TAMs and TILs in the pretherapeutic core biopsy specimen were associated with better outcome after NAC for breast cancer and have antitumoral effect associated with NAC.

Keywords: Breast cancer, macrophages, lymphocytes, tumor-Infiltrating, CD163 protein, HLA-DR Antigens

Introduction

Breast cancer is the most common cancer and the leading cause of death in women worldwide [1-4]. The microenvironment of the breast, composed of adipocytes, fibroblasts, inflammatory cells, endothelial cells, and extracellular matrix, plays a crucial role in breast cancer development. Among those components, tumor-associated macrophages (TAMs) and tumor-infiltrating lymphocytes (TILs) have recently been highlighted as prognostic markers and potential targets for adjuvant therapy [1, 2].

TAMs act like a double-edged sword, in that they can suppress tumor cells and yet also produce cytokines that promote tumor progression [5-7]. Macrophages have plasticity and can change their phenotype depending on the microenvironment. M1 macrophages (HLA-DR-positive by immunohistochemistry [5]) are characterized by high expression of pro-inflammatory factors and exhibit high microbicidal and tumoricidal activity. M2 macrophages (CD163-positive by immunohistochemistry [5]) are immunosuppressive and produce high levels of anti-inflammatory cytokines and low levels of

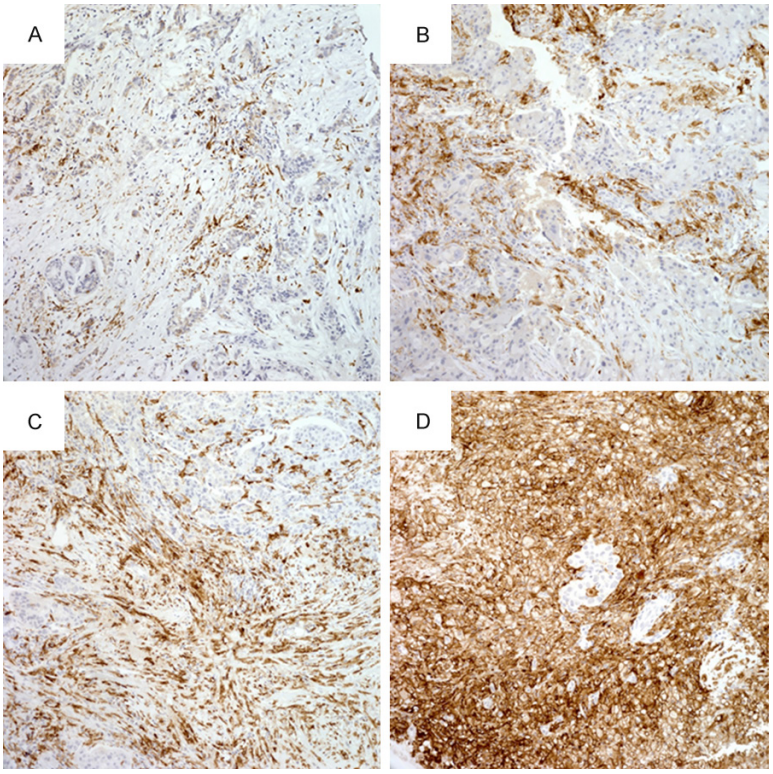

Figure 1. Immunohistochemistry slides of CD163-positive TAMs scored by infiltration density in tumor nests and stroma: A: Grade 1, no/low; B: Grade 2, moderate; C: Grade 3, high; D: Grade 4, massive (×200).

pro-inflammatory cytokines. TAMs are characterized mainly by M2 macrophages [2], which convey mostly pro-tumor functions that promote tumor growth, proliferation and metastasis [1, 2, 5, 8]. In contrast, some reports have described that TAMs with an M1 phenotype display tumoricidal behavior and inhibit tumor progression [1, 2].

TILs are known to be involved in killing tumor cells and are often associated clinically with better outcomes. According to previous studies, stromal TIL have antitumor activity and a favorable prognostic effect in breast cancer [9-14].

Neoadjuvant chemotherapy (NAC) is increasingly used in breast cancer to facilitate conserving surgery, initiate early systemic treatment, and assess response to chemotherapies. However, not all patients respond to NAC, and factors determining clinical response have not yet been defined. TILs appear to be an independent predictor of NAC response [12, 15], but whether TAMs in breast carcinoma tissue predict the response of patients to NAC in breast cancer remains unclear.

In this study, we analyzed the clinicopathologic characteristics and therapeutic effect of different subtypes of TAMs (M1 and M2) and stromal TILs in pre-NAC core biopsy (PNB) specimens.

Methods

Patients

We retrospectively reviewed the records of 154 patients with primary invasive breast cancer who received a sequential schedule of NAC from 2005 to 2011 at Samsung Medical Center, Seoul, Korea. Inclusion criteria were: 1) histologically confirmed invasive carcinoma on PNB and 2) NAC with Anthracycline and/or Taxane-based chemotherapy regimen. After NAC, all patients received standard surgical treatment. Among them, 76 (49.4%) patients were treated with conserving surgery followed by radiation therapy and 78 (50.6%) with modified radical mastectomy (n=74) or total radical mastectomy (n=4).

Clinical data were retrospectively reviewed based on electronic medical records, and pathologic diagnoses were re-reviewed by two pathologists (Yoon N and Cho EY). Tumor stage was determined according to the 7th Edition of the AJCC Cancer Staging Manual.

Clinicopathologic characteristics were assessed, including age at diagnosis, histologic grade, tumor type, tumor size, lymph node stage, Ki-67 proliferative index and protein expression of ER, PR, and HER2. Chemo-response was evaluated with the achievement of pathologic complete response (pCR). pCR was defined as the absence of residual invasive tumor cells in both breast and lymph nodes after NAC and has been associated with favorable clinical outcome. In this study, surgically resected specimens revealed pCR in 19 cases and a failure to

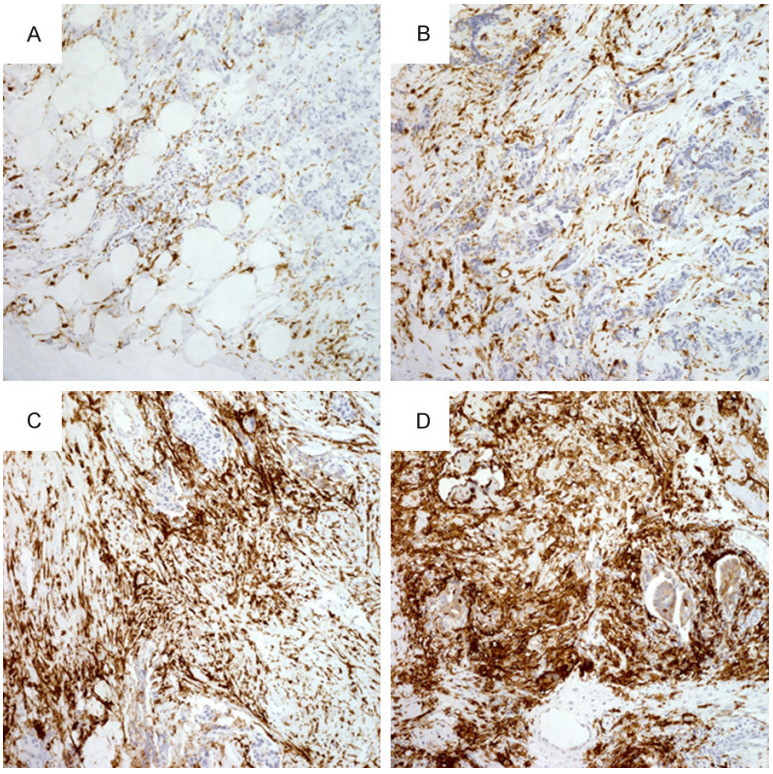

Figure 2. Immunohistochemistry slides of HLA-DR positive TAMs scored by infiltration density in tumor nests and stroma: A: Grade 1, no/low; B: Grade 2, moderate; C: Grade 3, high; D: Grade 4, massive (×200).

respond (non-pCR) to NAC in 135 cases. The mean follow-up time was 48 months. Twenty-eight (18.2%) patients died of disease and 126 (81.8%) patients were alive; among the 126 patients, 8 (5.2%) experienced recurrence, and 43 (27.9%) had metastasis.

Biopsy samples were obtained 7-17 days before the start of NAC, and 3 mm wide serial sections of the biopsy samples were cut, fixed in 10% neutral buffered formalin solution, embedded in paraffin, stained with hematoxylin-eosin, and then evaluated for histology by pathologists.

The study was approved by Institutional Review Board of Samsung Medical Center (IRB No: 2015-11-115-001). All investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Immunohistochemistry and assessment of TAMs and TILs

Formalin-fixed and paraffin-embedded tissue of 154 PNB was used for evaluation of TAMs and TILs.

The distribution and density of TAM subtypes M1 and M2 in PNB samples were evaluated by immunohistochemistry for HLA-DR and CD163. Immunostaining was done on 4 um thick sections of formalin-fixed and paraffin-embedded tissue according to standard procedures. The sections were subsequently deparaffinized using graded alcohol and xylene. Antigen retrieval reactions were performed in a steamer in citrate buffer of pH 10 for 25 minutes. H_2O_2 (3%) solution was applied to block endogenous peroxides at room temperature for 5 minutes. The sections were stained with antibodies against CD163 (dilution 1:200; Novocastra, Newcastle, UK) and HLA-DR (dilution 1:200; DAKO, CA, USA). Sections were counterstained with Mayer's hematoxylin solution for the identification of nuclei. Detection was performed using the DAKO Envision™ system (Envision™, DAKO).

All slides were evaluated by two pathologists (Yoon N and Cho EY) who had no access to the clinical data. In case of disagreement, the slides were re-examined until a consensus was reached. For evaluation of TAMs, CD163 and HLA-DR staining was scored from 1 to 4 by the infiltration density in tumor nests and stroma: 1, no/low; 2, moderate; 3, high; and 4, massive (**Figures 1** and **2**).

Analysis of TILs was performed on hematoxylin and eosin-stained sections. TILs were defined as lymphocytes located within the stroma. The density of TILs was scored into 3 categories as: 0, no TILs positive infiltrate; 1, low and 2, high (**Figure 3**).

Immunohistochemistry and Evaluation for ER, PR, Ki-67, and HER2

Additional immunohistochemistry for ER, PR, HER2, and Ki-67 was also conducted using standard procedures. Serial sections of formalin-fixed and paraffin-embedded tumor tissues

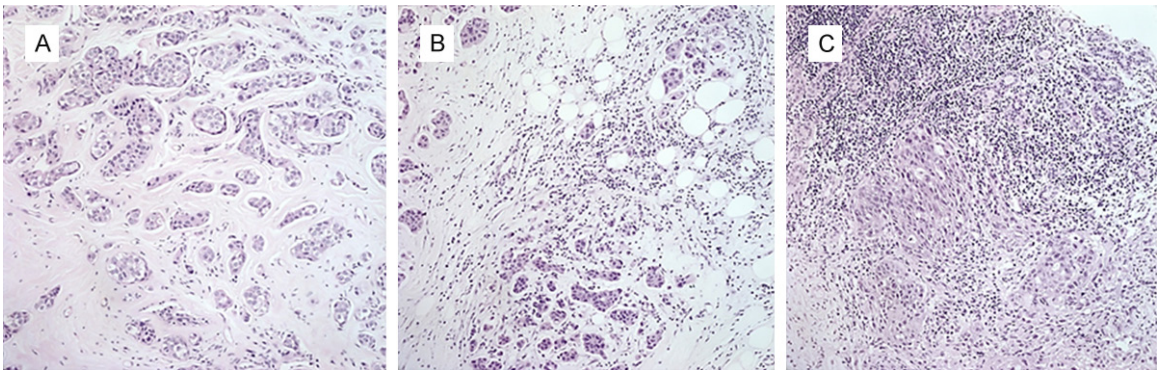

Figure 3. H&E slides of lymphocytes located within the stroma scored by infiltration density: A: Grade 0, no TILs positive infiltrate; B: Grade 1, low; C: Grade 3, high (×200).

were stained using antibodies to ER (clone 6F11, Novocastra), PR (clone 16, Novocastra), Ki-67 (clone MIB-1, Dako, Glostrup, Denmark), and HER2 (clone 4B5, Ventana Medical Systems Inc., Tucson, AZ, USA) according to the manufacturer's instructions. ER and PR expression was analyzed using ASCO/CAP guidelines of a threshold of 1% and also evaluated by Allred score, a semi-quantitative method. HER2 expression was defined according to ASCO/CAP guidelines. IHC grades of 0 and 1 for HER2 were defined as negative, and HER2 3+ tumors were considered HER2 positive. All HER2 2+ cases were confirmed by Fluorescence in-situ hybridization (FISH) using a dual-color probe DNA-specific probe kit from PathVision™ (Abbott/Vysis: LSI® HER2 SpectrumOrange™ and CEP 17 SpectrumGreen™, Des Plaines, IL, USA). Tumors with HER2 FISH amplification of more than 2.0 HER2/CEP17 ratios were considered HER2 positive.

Molecular subtypes were defined as follows by the definition of St Gallen International Expert Consensus 2011 [16]. Luminal A subtype: ER and/or PR positive and HER2 negative with low Ki-67 labeling index (<14%); Luminal B subtype: ER and/or PR positive and HER2 positive with any Ki-67 labeling index or ER and/or PR positive and HER2 negative with high Ki-67 labeling index (≥14%); HER2 positive subtype: HER2 over-expressed or amplified and ER, PR negative; Triple negative subtype: ER and PR absent and HER2 negative.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 statistical software (SPSS, Chicago, IL, USA). Correlation analyses between M1 TAMs, M2 TAMs, or TILs in PNB samples and clinicopathologic parameters were done using Pearson's chi-square test. A p-value of <0.05 was regarded as significant. The probability of pCR as a function of inflammatory parameters was determined by logistic regression analysis.

Results

Patient characteristics

The average age at diagnosis was 46.8 years (range from 26 to 73 years) with surgical staging from ypT0 to ypT4. Nodal stage was variable from ypN0 to ypN3. ER was expressed in 79 (51.3%) of 154 patients and not in 75 (48.7%). PR was expressed in 65 (42.2%) of 154 patients and not in 89 (57.8%). HER2 was expressed in 63 (40.9%) of 154 patients and not in 91 (59.1%). As for molecular subtype, 27 (17.5%) cases were luminal A, 54 (35.1%) cases were luminal B, 31 (20.1%) cases were HER2-positive, and 42 (27.3%) cases were triple negative.

Expression of the M2 macrophage marker CD163 and M1 macrophage marker HLA-DR in breast cancer

Macrophages were stained in cytoplasm strongly and diffusely by CD163 and HLA-DR. For CD163-positive M2 TAM staining, 58 (37.7%) patients were graded 1+, 53 (34.4%) patients were graded 2+, 30 (19.5%) patients were graded 3+ and 13 (8.4%) patients were graded 4+. For HLA-DR positive M1 TAM staining, 13 (8.4%) patients were graded 1+, 81 (52.6%)

Table 1. Relationships between CD163-positive TAM in PNB and clinicopathological characteristics

Characteristics	CD163 grade No. (%)				*P*-value
	1	2	3	4	
Age					
<45	32 (55.2)	23 (43.4)	11 (36.7)	4 (30.8)	0.041
≥45	26 (44.8)	30 (56.6)	19 (63.3)	9 (69.2)	
Post-NAC tumor size					
<1.5 cm	19 (32.8)	18 (34.0)	14 (46.7)	10 (76.9)	0.007
≥1.5 cm	39 (67.2)	35 (66.0)	16 (53.3)	3 (23.1)	
Nuclear grade					
1	15 (31.9)	5 (9.4)	1 (3.3)	1 (7.7)	<0.001
2	37 (63.8)	28 (52.8)	14 (46.7)	5 (38.5)	
3	6 (10.3)	20 (37.7)	15 (50.0)	7 (53.8)	
Post-NAC LN metastasis					
Absent	24 (41.4)	21 (39.6)	12 (40.0)	8 (61.5)	0.048
Present	34 (58.6)	32 (60.4)	18 (60.0)	5 (38.5)	
Ki-67					
<20%	23 (39.7)	14 (26.4)	7 (23.3)	1 (7.7)	<0.001
≥20%	35 (60.3)	39 (73.6)	23 (76.7)	12 (92.3)	
ypT stage					
ypT0	4 (6.9)	7 (13.2)	7 (23.3)	5 (38.5)	0.021
ypTis	5 (8.6)	2 (3.8)	0 (0.0)	0 (0.0)	
ypT1	18 (31.0)	15 (28.3)	9 (30.0)	6 (46.2)	
ypT2	19 (32.8)	15 (28.3)	7 (23.3)	2 (15.4)	
ypT3 or 4	12 (20.7)	14 (26.4)	7 (23.3)	0 (0.0)	
ER expression					
Negative	15 (25.9)	31 (58.5)	19 (63.3)	10 (76.9)	<0.001
Positive	43 (58.6)	22 (41.5)	11 (36.7)	3 (23.1)	
PR expression					
Negative	22 (37.9)	35 (66.0)	21 (40.0)	10 (76.9)	<0.001
Positive	36 (62.1)	18 (34.0)	9 (60.0)	3 (23.1)	
HER2 expression					
Negative	33 (56.9)	34 (64.2)	16 (53.3)	8 (61.5)	0.975
Positive	25 (43.1)	19 (35.8)	14 (46.7)	5 (38.5)	
Type					
Non-triple negative	50 (86.2)	35 (66.0)	19 (63.3)	8 (61.5)	0.023
Triple negative	8 (13.8)	18 (34.0)	11 (36.7)	5 (38.5)	

patients were graded 2+, 48 (31.2%) patients were graded 3+ and 12 (7.8%) patients were graded 4+.

Table 1 shows the correlations between CD-163 positive TAMs in PNB and clinicopathologic variables. A high score of CD-163 positive TAMs in PNB correlated with old age (≥45) (*P*=0.041), high nuclear grade (*P*<0.001), and high Ki-67 proliferative index (≥20%) (*P*<0.001) and was associated with ER negativity (*P*<0.001), PR negativity (*P*<0.001) and triple negative type (*P*=0.023). In addition, a high score of CD-163 positive TAMs in PNB was correlated with smaller post-NAC tumor size (<1.5 cm) (*P*=0.007), low post-NAC pathologic T stages (*P*=0.021) and absence of post-NAC nodal metastasis (*P*=0.048). HER2 expression was not significantly correlated with CD-163 positive TAMs in PNB.

Table 2 demonstrates the correlations between HLA-DR positive TAMs in PNB and clinicopathologic variables. A high score of HLA-DR positive TAMs in PNB was correlated with ER negativity (*P*=0.004), PR negativity (*P*=0.044), smaller post-NAC tumor size (<1.5 cm) (*P*=0.001), and low post-NAC pathologic T stages (*P*=0.012). Age, nuclear grade, Ki-67 proliferative index, post-NAC nodal status, HER2 expression and triple negative type were not significantly correlated with HLA-DR positive TAMs in PNB.

Evaluation of TILs and association with clinicopathologic characteristics

In the scoring of TILs, 40 (26.0%) patients were graded 0, 81 (52.6%) were graded 1+, and 33 (21.4%) were graded 2+. The correlations between TILs of PNB and clinicopathologic variables are summarized in **Table 3**. A high TILs score of PNB was correlated with ER negativity (*P*=0.002), PR negativity (*P*=0.005), smaller post-NAC tumor size (<1.5 cm) (*P*=0.021), and low post-NAC pathologic T stages (*P*=0.003). Age, nuclear grade, Ki-67 proliferative index, post-NAC nodal status, HER2 expression and

Table 2. Relationships between HLA-DR-positive TAM in PNB and clinicopathological characteristics

Characteristics	HLA-DR grade No. (%)				*P*-value
	1	2	3	4	
Age					
<45	7 (53.8)	41 (50.6)	15 (31.3)	7 (58.3)	0.299
≥45	6 (46.2)	40 (49.4)	33 (68.8)	5 (41.7)	
Post-NAC tumor size					
<1.5 cm	4 (30.8)	18 (22.2)	23 (47.9)	8 (66.7)	0.001
≥1.5 cm	9 (69.21)	63 (77.8)	25 (52.1)	4 (33.3)	
Nuclear grade					
1	1 (7.7)	14 (17.3)	5 (10.4)	2 (16.7)	0.618
2	10 (76.9)	40 (49.4)	28 (58.3)	6 (50.0)	
3	2 (15.4)	27 (33.3)	15 (31.3)	4 (33.3)	
Post-NAC LN metastasis					
Absent	10 (76.9)	51 (63.0)	40 (83.3)	10 (83.3)	0.074
Present	3 (23.1)	30 (37.0)	8 (16.7)	2 (16.7)	
Ki-67					
<20%	2 (15.4)	29 (35.8)	11 (22.9)	3 (25.0)	0.597
≥20%	11 (84.6)	52 (64.2)	37 (77.1)	9 (75.0)	
ypT stage					
ypT0	1 (7.7)	10 (12.3)	9 (18.8)	3 (25.0)	0.012
ypTis	1 (7.7)	4 (4.9)	1 (2.1)	1 (8.3)	
ypT1	4 (30.8)	21 (25.9)	18 (37.5)	5 (41.7)	
ypT2	2 (15.4)	26 (32.1)	13 (27.1)	2 (16.7)	
ypT3 or 4	5 (38.5)	20 (24.7)	7 (14.6)	1 (8.3)	
ER expression					
Negative	3 (23.1)	37 (45.7)	25 (52.1)	10 (83.3)	0.004
Positive	10 (76.9)	44 (54.3)	23 (47.9)	2 (16.7)	
PR expression					
Negative	6 (46.2)	42 (51.9)	31 (64.6)	9 (75.0)	0.044
Positive	7 (53.8)	39 (48.1)	17 (35.4)	3 (25.0)	
HER2 expression					
Negative	8 (61.5)	46 (56.8)	31 (64.6)	6 (50.0)	0.976
Positive	5 (38.5)	35 (43.2)	17 (35.4)	6 (50.0)	
Type					
Non-triple negative	11 (84.6)	63 (77.8)	30 (62.5)	8 (66.7)	0.057
Triple negative	2 (15.4)	18 (22.2)	18 (37.5)	4 (33.3)	

triple negative type were not significantly correlated with TIL score of PNB.

Correlation between TAMs/TILs and chemoresponse

Table 4 shows the correlation between TAMs and TILs of PNB and pCR rate to chemotherapy. A high score of CD-163 positive TAMs and TILs in PNB correlated with a high pCR rate (P=0.018 and P=0.037, respectively). HLA-DR positive TAMs of PNB were not correlated with pCR rate (P=0.062). In the multivariate analysis combined with subtype, a high TAM or TIL score of PNB was not an independent predictive factor of pCR.

Discussion

Improvement of breast cancer prognosis requires the development of new prognostic and predictive markers for therapy capable of selecting patients most likely to benefit from chemotherapy. PNB offer an excellent sample for the analysis of predictive markers [12].

Although the role of tumor microenvironment, especially TILs and TAMs, is becoming more apparent in breast cancer, the prognostic effect of TILs and TAMs is still poorly defined [17]. Accordingly, we examined the presence of TAMs and TILs in PNB samples from 154 breast cancer patients.

The presence of a large number of TAMs has been reported to be associated with hormone receptor negativity, higher mitotic count, higher tumor grade, larger tumor size, higher stage, and more positive lymph nodes [18-22]. We found that a high TAM score of PNB was correlated with ER and PR negativity. We also revealed that higher M2 macrophage (CD163-positive TAMs) scores of PNB were related aggressive clinicopathological features including older age (≥45), higher tumor grade, higher Ki-67 proliferative index (≥20%) and triple negative type.

It has been known that a greater density of TAMs is associated with higher tumor stage

Table 3. Relationships between tumor-infiltrating lymphocytes in PNB and clinicopathological characteristics

Characteristics	TIL grade No. (%)			*P*-value
	0	1	2	
Age				
<45	16 (40.0)	38 (46.9)	16 (48.5)	0.455
≥45	24 (60.0)	43 (53.1)	17 (51.5)	
Post-NAC tumor size				
<1.5 cm	10 (25.0)	26 (32.1)	17 (51.5)	0.021
≥1.5 cm	30 (75.0)	55 (67.9)	16 (48.5)	
Nuclear grade				
1	5 (12.5)	14 (17.3)	3 (9.1)	0.1
2	28 (70.0)	39 (48.1)	17 (51.5)	
3	7 (17.5)	28 (34.6)	13 (39.4)	
Post-NACLN metastasis				
Absent	24 (60.0)	63 (77.8)	24 (40.0)	0.189
Present	16 (40.0)	18 (22.2)	9 (60.0)	
Ki-67				
<20%	12 (30.0)	26 (32.1)	7 (21.2)	0.448
≥20%	28 (70.0)	55 (67.9)	26 (78.8)	
ypT stage				
ypT0	5 (12.5)	10 (12.3)	8 (24.2)	0.003
ypTis	1 (2.5)	4 (4.9)	2 (6.1)	
ypT1	9 (22.5)	25 (30.9)	14 (42.4)	
ypT2	13 (32.5)	23 (28.4)	7 (21.2)	
ypT3 or 4	12 (30.0)	19 (23.5)	2 (6.1)	
ER expression				
Negative	14 (35.0)	37 (45.7)	24 (72.7)	0.002
Positive	26 (65.0)	44 (54.3)	9 (27.3)	
PR expression				
Negative	18 (45.0)	44 (54.3)	26 (78.8)	0.005
Positive	22 (55.0)	37 (45.7)	7 (21.2)	
HER2 expression				
Negative	23 (57.5)	51 (63.0)	17 (51.5)	0.658
Positive	17 (42.5)	30 (37.0)	16 (40.9)	
Type				
Non-triple negative	31 (77.5)	60 (74.1)	21 (63.6)	0.23
Triple negative	9 (22.5)	18 (34.0)	12 (36.4)	

and nodal metastasis [8, 22, 23]. However, CD163-positive M2 TAMs from PNB were correlated with smaller post-NAC tumor size, low post-NAC pathologic T stage and negative post-NAC nodal status and showed more frequent pCR in our study. HLA-DR-positive M1 TAMs scores of PNB were also correlated with smaller post-NAC tumor size and low post-NAC pathologic T stage. This result suggests that when chemotherapy is applied TAMs have anti-tumoral properties and may be a sensitive predictor of chemotherapy effectiveness.

In addition, high TIL score of PNB was correlated with smaller post-NAC tumor size, lower post-NAC pathologic T stage and higher pCR rate. These results support a previous hypothesis of Denkert *et al.* that TILs enhance chemo-agents to eliminate cancer cells and are a predictor of response to NAC [12].

We observed a significant association of TAMs and TILs in PNB with ER and PR negativity. Calabro *et al.* discovered that immune response genes are over-expressed more frequently in ER-negative compared to ER-positive breast cancer [4, 25], suggesting that ER might have an inhibitory effect on immune response [25].

In this study, infiltration density of M1 and M2 TAMs tend to go along with that of TILs ($P<0.001$) suggesting that humoral immune reaction against tumor occurred simultaneously through complex and diverse process. TAMs can switch phenotype and interact with TILs according to the stage of tumor development [26-28].

The present study was limited by the small number of cases of each molecular subtype. There is no pCR case in lumnal A subgroup, thus, we statistically failed to figure out that TAMs and TILs are independent predictive factors for pCR.

In summary, TAMs and TILs in PNB have an anti-tumoral effect in breast cancer associated with NAC. Our result suggests possible new therapeutic approaches using combined immunologic therapeutic agents and classical chemotherapy to improve NAC response rates.

Disclosure of conflict of interest

None.

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Table 4. Relationships between TAMs/TILs in PNB and pCR

	pCR No. (%)		*P*-value
	Complete	Incomplete	
CD163 grade			
1	4 (6.9)	54 (93.1)	0.018
2	6 (11.3)	47 (88.7)	
3	5 (16.7)	25 (83.3)	
4	4 (30.8)	9 (69.2)	
HLA-DR grade			
1	1 (7.7)	12 (92.3)	0.062
2	7 (8.6)	74 (91.4)	
3	8 (16.7)	40 (83.3)	
4	3 (25.0)	9 (75.0)	
TILs grade			
0	3 (7.5)	37 (92.5)	0.037
1	8 (9.9)	73 (90.1)	
2	8 (24.2)	25 (75.8)	

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