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Original Article

Clinical significance of regulatory T cells and T lymphocyte subsets in peripheral blood in neoadjuvant chemotherapy for breast cancer

Xiao-Dong Mao[1,2], Su-Zhu Chen[3], Su-Qing Shen[4], Kang-Sheng Liu[4]

[1]Department of Endocrinology, Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing 210028, Jiangsu, China; [2]Key Laboratory of TCM Syndrome & Treatment of Yingbing of State Administration of Traditional Chinese Medicine, Jiangsu Province Academy of Traditional Chinese Medicine, Nanjing 210028, Jiangsu, China; [3]Department of Obstetrics and Gynecology, Suzhou Branch of BenQ Medical Center, The Affiliated BenQ Hospital of Nanjing Medical University, Nanjing 210019, Jiangsu, China; [4]Department of Clinical Laboratory, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing 210029, Jiangsu, China

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Abstract: The impact of the immune response on the therapeutical efficacy of neoadjuvant chemotherapy for breast cancer remains largely unknown. To characterize the role of regulatory T cells ($CD4^+CD25^+CD127^{low}$Treg), T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD4^+/CD8^+$) and NK cells in neoadjuvant chemotherapy, we investigated the correlation patterns of these immune cell subsets with the progression of breast cancer. A total of 120 breast cancer patients receiving neoadjuvant chemotherapy in Nanjing Maternal and Child Health Hospital from May 2019 to November 2021 were retrospectively collected as the breast cancer group, and 46 healthy women were selected as the control group. The number of regulatory T cells, T lymphocyte subsets and NK cells in the peripheral blood were analyzed by flow cytometry. Compared with the control group, $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ ratio and NK cells were significantly decreased in patients with breast cancer ($P < 0.05$), while the levels of Treg and $CD8^+$ cells were significantly increased ($P < 0.05$). In addition, the status of the immune response among breast cancer patients at different clinical stages was obviously different. In higher tumor stages, the level of $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ ratio and NK cell were reduced, while the level of Treg and $CD8^+$ T cells gradually increased. Furthermore, we found a lower percentage of $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ and NK cells in association with lymph node metastasis, accompanied by a higher number of $CD8^+$ T cells. Interestingly, after treatment with neoadjuvant chemotherapy, the levels of Tregs, $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ ratio of patients were all upregulated compared with the levels before treatment, indicating the recovery of cytotoxic lymphocytes and a consolidation of the immunosuppressive microenvironment at the same time ($P < 0.05$). Immune dysfunction is commonly observed in breast cancer patients, which is closely associated with tumor progression and lymph node metastasis. Neoadjuvant chemotherapy was found to highly influence the number of T lymphocytes and improve the immune function of T lymphocyte subsets in breast cancer patients. At the same time, as immunosuppressive cells, the proportion of Tregs ($CD4^+CD25^+CD127^{low}$Treg) also increased after treatment with neoadjuvant chemotherapy. Our results provide guidance for the development of new combination strategies during neoadjuvant chemotherapy to reverse the immunosuppressive microenvironment and achieve better clinical outcomes.

Keywords: Peripheral blood T lymphocytes, Treg cells, NK cells, flow cytometry, breast cancer

Introduction

Breast cancer is one of the most common malignant tumors in women, accounting for about 18% of all female malignant tumors, with the highest incidence and second highest mortality [1]. The 2015 cancer registration reports showed that the risk of breast cancer in the 50-59-year-old age group in China increased by 69.38% from 2005 to 2015 [2, 3]. Recent studies confirmed that one of the determining factors in the progression, response to treatment and prognosis of breast cancer is the immune system, which monitors the occurrence of tumors and eliminates tumor cells through cellular immunity. Hence, when the immune sur-

veillance function of the body is challenged, the risk of tumor occurrence and progression increases [4]. Reports indicated that although the body's immune system may generate a specific immune response against a specific tumor antigen, the complex and orchestrated network of the tumor environment always leads to changes in the function of immune cells in the tumor, preventing effective tumor suppression. This tolerogenic microenvironment for tumor antigens is mainly mediated by the activation of a series of immunosuppressive cells. Among them, $CD4^+CD25^+$ regulatory T cells, as the most typical immunosuppressive cells, can promote the occurrence and development of malignant tumors. $CD4^+CD25^+$ regulatory T cells are mainly responsible for maintaining autoimmune tolerance and regulating adaptive immune responses in normal tissue, mainly by inhibiting $CD4^+CD25^-$ T cells, NK cells, dendritic cells, monocytes, and other immune cells, to prevent the occurrence of autoimmune phenomena [5]. In addition to Treg cells, other T lymphocyte subsets play important roles in antitumor immune surveillance. Relevant studies found that T lymphocyte subsets were associated with the age and tumor stage of cancer patients. Moreover, there are certain changes in T lymphocyte subsets before and after treatment, which are closely related to tumor progression and the treatment effect [6, 7]. At present, there are few studies on the relationship between T lymphocyte subsets in the peripheral blood and the efficacy of neoadjuvant chemotherapy for breast cancer. Therefore, monitoring the immune function of breast cancer patients is of great significance for formulating an effective treatment regimen. In this study, we mainly analyzed the distribution of regulatory T cells and T lymphocyte subsets in the peripheral blood of breast cancer patients, and further explored the relationship of changes in peripheral T lymphocyte subsets with the efficacy of neoadjuvant chemotherapy for breast cancer, in order to provide a reference for the further development of clinical therapy.

Materials and methods

General information

A total of 120 patients with breast cancer admitted to our hospital from May 2019 to November 2021 were collected, all of whom were female and confirmed by pathohistological examination. Neoadjuvant chemotherapy was administered to all enrolled patients. The patients were 25-72 years old, with an average of 50 ± 11 years old. The tumor was on the left side in 58 cases, and on the right side in 62 cases. Ipsilateral axillary lymph node metastasis was found in 80 cases and no lymph node metastasis was found in 40 cases.

According to the TNM staging standards in the New Standard for Diagnosis and Treatment of Common Malignant Tumors, there were 25 patients with intermediate stage I, 45 patients with stage II, 38 patients with stage III, and 12 patients with stage IV disease. None of 120 breast cancer patients received radiotherapy or chemotherapy. A total of 46 healthy female patients were selected as the control group.

Inclusion criteria

Breast cancer group: (a) Female breast cancer patients confirmed by clinicopathological examination admitted to our hospital in 2019.5-2021.11, (b) No other tumors, acute or chronic inflammation, hematological or autoimmune diseases, (c) No chemotherapy or radiotherapy was performed before blood collection.

Control group: Subjects who served as negative controls (NCs) were inquired about their physical condition, medication, smoking, and alcohol consumption. The NCs, showing normal blood routine examinations, liver functions (AST and ALT), renal function (SCr), blood glucose levels, and without tumors, were considered healthy.

Exclusion criteria

Breast cancer group: (a) Pregnancy or lactation, (b) Severe lung infection or systemic infection, (c) Fever, inflammatory disease, and rheumatoid disease, (d) Mass resection biopsy before admission, (e) Allergy to chemotherapy drugs, (f) Immunological diseases or immunosuppressant use. The control group was 20-65 years old with an average of 49 ± 12 years.

Control group: Healthy physical examinees with breast disease, immune system diseases, metabolic diseases, acute infection, or tumors were excluded.

There was no significant difference in age between the two groups ($P > 0.05$). Venous blood was collected before and after neoadjuvant chemotherapy. The control group donated venous blood for the study at the time of physical examination.

Ethics

The present study was approved by the Ethics Committee of Women's Hospital of Nanjing Medical University, and was conducted in accordance with the Declaration of Helsinki. An information sheet was provided to all participants. Written informed consent was obtained from all participants. The relevant guidelines and regulations of the local institute were strictly followed when conducting the study. Participants were informed that they could withdraw from the trial without giving a reason.

Treatment methods

Blood cell analysis, liver function and renal function examinations were performed in both groups before treatment. According to the results, the obviously abnormal indexes were corrected, and the patients with malnutrition were provided with nutritional support. Water, electrolyte and acid-base balance were maintained during treatment. Hydration was adopted the day before chemotherapy. After chemotherapy, the blood routine examinations were conducted weekly, and the whole-body condition was evaluated.

The observation group received neoadjuvant chemotherapy based on the TAC regimen [8] as follows: Docetaxel (75 mg/m^2) through intravenous drip, D1; cyclophosphamide (500 mg/m^2) and epirubicin (70 mg/m^2), D2-D21; weekly for 21 days, and all patients received three cycles of chemotherapy. Dexamethasone was taken orally 24 h before docetaxel administration. Antiemetics were routinely given during chemotherapy to alleviate gastrointestinal reactions, and granulocyte colony-stimulating factor was given if leukopenia above grade III occurred.

Clinical efficacy

Before chemotherapy and at the end of the third cycle, the maximum diameter and vertical diameter of tumors in the observation group were measured by clinical physical examination and breast B-ultrasonography or mammography. The clinical efficacy of measurable solid tumors was evaluated by referring to WHO standards, including complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). CR+PR were combined as the effective group, while SD+PD constituted the ineffective group [9].

Laboratory methods

T lymphocyte subsets

A sample comprising 100 μL of anticoagulated peripheral blood was mixed evenly with monoclonal antibodies (anti-CD3-FITC, anti-CD4-PE, anti-CD8-APC) and incubated for 20 min at room temperature in the dark. Then, 1.5 ml of erythrocyte lysate was added and mixed. The solution was incubated at room temperature in the dark for 10 min and centrifuged at 1500 r/min for 5 min. The supernatant was discarded, and 2 mL 0.1% sodium azide phosphonate buffer was added to each tube and mixed evenly, then centrifuged at 1000 r/min for 5 min. The supernatant was discarded, and 1 ml PBS was added to resuspend the cells. The solution was analyzed by flow cytometry (BD Company, USA) [9]. Anti-CD3-FITC, anti-CD4-PE, anti-CD8-PE were purchased from BD Company in the United States. Erythrocyte lysate was purchased from BD Company (United States of America). The reference values for the percentage of $CD3^+$ T cells, $CD4^+$ T cells and $CD8^+$ T cells is ranged from 61.1 to 77%, 25.8 to 41.6% and 18.1 to 29.66%, respectively, and the ratio of $CD4^+/CD8^+$ T cells was 0.98-1.94.

Regulatory T cells in the peripheral blood

The anticoagulated peripheral blood was fully mixed and 50 μl of whole blood was added to the bottom of the flow tube using the reversing sampling method (taking care not to touch the tube wall). Then, $CD4^+$ antibody (8 μl), CD25 antibody (8 μl) and CD127 antibody (2 μl) were added to the whole blood mixed for 5 s. After incubation for 15-20 min at room temperature in the dark, 450 μl of pre-diluted 1× FACS lysis solution was added and mixed for 5 s, followed by incubation at room temperature in the dark for 10-15 min. The cells were washed with PBS, the supernatant was discarded, and 300 μl PBS was added to resuspend the cells. The cells were analyzed by flow cytometry (BD Company, USA).

Statistical analysis

SPSS19.0 statistical software (IBM Corp., USA) was used for data analysis. The measurement data conformed to a normal distribution and were expressed as means ± standard deviations ($\bar{x}\pm s$). The independent samples *t*-test was used for comparisons between two groups, the paired samples *t*-test was used for intragroup comparisons, and single factor analysis of variance (one-way ANOVA) was used for comparisons between multiple groups. Differences with $P < 0.05$ were considered statistically significant.

Results

Comparison of immune cell profiles between breast cancer patients and healthy controls

The levels of $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ and NK cells were significantly decreased in breast cancer patients ($P < 0.05$), while $CD8^+$ cells were significantly increased ($P < 0.05$). The proportion of Treg cells in the breast cancer group was statistically different from the control group ($P < 0.05$; **Figure 1** and **Table 1**).

Comparison of the immune cell profiles of breast cancer patients at different disease stages

There were statistically significant differences in cellular immune function between patients at different disease stages ($P < 0.05$). With the progression of clinical stages, the levels of $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ T cells and NK cells gradually decreased ($P < 0.05$). At the same time, the level of $CD8^+$ T cell increased gradually ($P < 0.05$). The proportion of Treg cells in the peripheral blood of patients with breast cancer increased with the progression of clinical stages, and the proportion of Treg cells in patients with stage I-IV breast cancer of all stages was statistically different from the healthy control group ($P < 0.05$). The proportion of Tregs in stage III and IV patients was significantly increased compared with stage I patients, and in stage IV patients compared with stage II patients ($P < 0.05$), as shown in **Table 2** and **Figure 2**.

Comparison of immune cell profiles in breast cancer patients with and without lymph node metastasis

The levels of $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ and NK cells in breast cancer patients with lymph node metastasis were significantly lower than in those without lymph node metastasis ($P < 0.05$), while the levels of $CD8^+$ cells were significantly higher than in patients without lymph node metastasis ($P < 0.05$). The proportion of Tregs in the peripheral blood of breast cancer patients with lymph node metastasis was significantly higher than in patients without lymph node metastasis ($P < 0.05$), as shown in **Table 3** and **Figure 3**.

Comparison of lymphocyte subsets in the peripheral blood of breast cancer patients before and after neoadjuvant chemotherapy

After chemotherapy, $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ ratio in the peripheral blood of breast cancer patients was significantly higher than before treatment, while $CD8^+$ T cells were significantly less abundant than before treatment ($P < 0.05$), as shown in **Table 4**.

Comparison of lymphocyte subsets in the peripheral blood of breast cancer patients with different treatment effects

After neoadjuvant chemotherapy, there were 82 cases of DC and 38 cases of PD. Among that, there were no cases of CR, 27 cases of PR and 55 cases of SD. The ratio of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ was significantly higher in the DC group than in the PD group ($P < 0.05$), while the abundance of $CD8^+$ T cells was slightly lower than in the PD group, with no statistical significance ($P > 0.05$), as shown in **Table 5**.

Discussion

Human antitumor immunity includes cellular and humoral components, among which cellular immunity is dominant. The cellular immune system mainly consists of T cells, B cells, and NK cells, which mount an immune response through complex intercellular interactions [10]. According to their phenotypes and functions, T lymphocytes can be divided into $CD4^+$ T cells (T helper cells) and $CD8^+$ T cells (cytotoxic T cells) [11, 13]. Studies revealed that T cells are one of the main populations infiltrating early tumors. The dominant subset comprises $CD4^+$ T cells, which can secrete cytokines that directly affect tumor cells or indirectly exert antitumor effects through activation of $CD8^+$ T cells [12]. Activated $CD8^+$ T cells bind to the major histocompatibility complex I (MHC I) - antigen complex on target cells through their receptors,

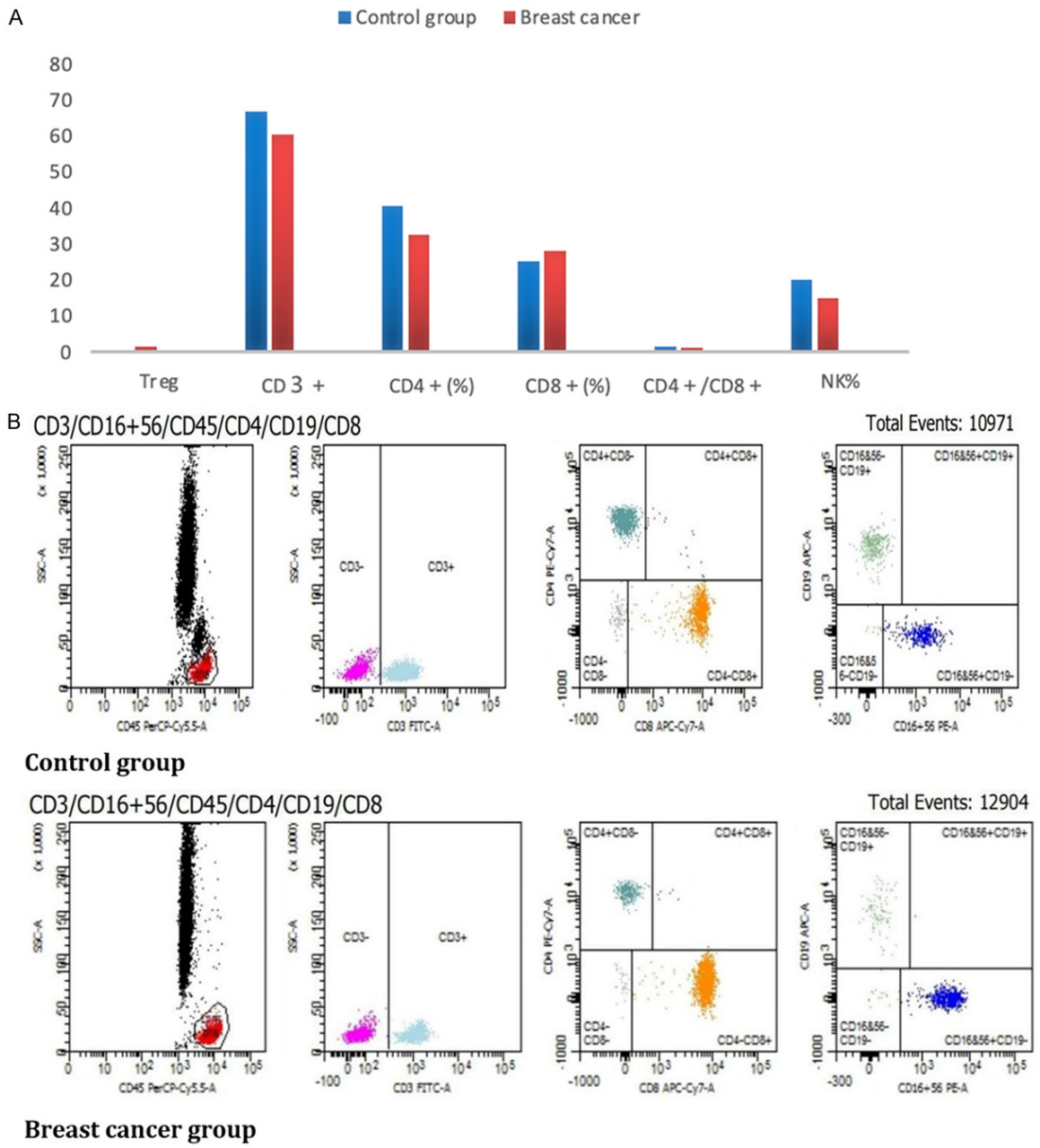

Figure 1. Comparison of immune indices between the two groups. A: Comparison of cellular immune function between breast cancer patients and healthy controls. B: The number of regulatory T cells, T lymphocyte subsets and NK cells in then peripheral blood of breast cancer patients and healthy controls were analyzed by flow cytometry.

inducing $CD8^+$ T cells to release granzymes and perforin, thereby killing tumor cells [14, 15].

In the healthy body, $CD4^+$ and $CD8^+$ T cells are in a balanced state of mutual induction and restriction. However, when the number or proportion of the two subsets is out of balance, the proportion of $CD4^+$ T cells decreases while that of $CD8^+$ T cells increases, leading to a decrease of their immune function. As a result, the ability of the inducing organ to resist tumors is weakened, which is also a major cause of carcinogenesis [16]. NK cells participate in the antitumor immune response by producing pro-inflammatory cytokines, which exert antitumor effects by recruiting and promoting the proliferation of other immune cells. NK cells are immune cells with innate and adaptive immune characteris-

Table 1. Comparison of immune cell profiles between breast cancer patients and healthy controls ($\bar{x}\pm s$)

Group	n	$CD4^+CD25^+CD127^{low}$Treg	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$	NK%
Control group	46	0.63±0.12	66.81±5.68	40.72±4.36	25.19±3.87	1.59±0.21	20.13±3.55
Breast cancer	120	1.53±0.31	60.54±4.20	32.65±4.55	28.16±3.51	1.26±0.16	15.06±2.50
t		8.215	8.021	9.207	9.182	10.01	10.219
P		0.003	0.006	0.001	0.005	0.013	0.005

Table 2. Comparison of the levels of different immune cells in patients at different stages of breast cancer ($\bar{x}\pm s$)

TNM	n	$CD4^+CD25^+CD127^{low}$Treg	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$	NK%
I	25	0.92±0.15	65.36±6.21	35.21±5.81	25.41±3.62	1.55±0.32	18.33±2.68
II	45	1.18+0.21*	61.76±5.02*	32.68±5.62*	27.80±2.91*	1.32±0.29*	16.72±2.90*
III	38	1.92±0.36*,#	58.52±4.92*,#	29.96±3.26*,#	32.50±3.26*,#	1.16±0.20*,#	13.50±2.95*,#
IV	12	2.10±0.51*,#,Δ	55.19±3.86*,#,Δ	28.50±3.52*,#,Δ	35.29±3.02*,#,Δ	0.82±0.18*,#,Δ	10.25±1.88*,#,Δ
F		5.10	11.268	6.158	7.316	10.321	9.217
P		0.013	0.003	0.005	0.018	0.006	0.012

Note: Compared with stage I, $*P < 0.05$; Compared with stage II, $\#P < 0.05$; Compared with stage III, $\Delta P < 0.05$.

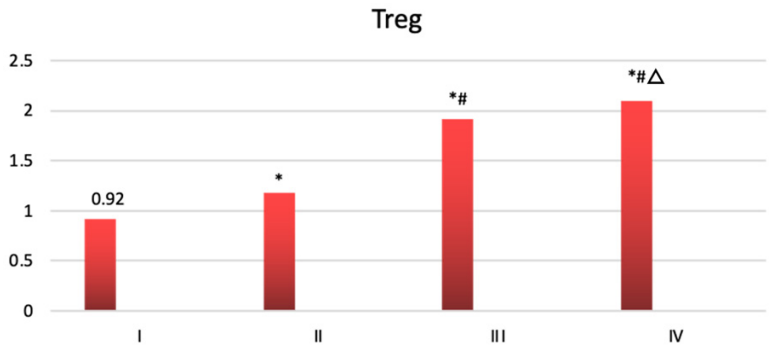

Figure 2. Comparison of cellular immune function in patients with different stages of breast cancer ($\bar{x}\pm s$). Compared with stage I, $*P < 0.05$; Compared with stage II, $\#P < 0.05$; Compared with stage III, $\Delta P < 0.05$.

tics, which play a role in cancer suppression during initial tumor immune surveillance [17, 18]. It has been reported that the progression of breast cancer is accompanied by the dysregulation of NK cell [19]. Therefore, the detection of NK cells in tumor patients is also of great significance for judging the occurrence, development, treatment and prognosis of the disease [20]. In this study, the changes of relevant lymphocyte subsets in the peripheral blood of breast cancer patients were investigated. The results showed that the levels of $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ and NK cells in the peripheral blood of breast adenocarcinoma patients were significantly lower than in healthy controls, while the levels of $CD8^+$ cells were significantly higher. These results indicated that the immune function of patients with breast adenocarcinoma was significantly weakened (**Table 1**; **Figure 1**). Previous studies pointed out that cytokines released in a strongly immunosuppressive tumor microenvironment could suppress the immune killing response to further promote tumor progression and metastasis [21]. In addition, our study also revealed that the immune function of patients further deteriorated with tumor progression, which was consistent with a report by Kochi et al. from 2018 [22]. These results indicated that with the increase of tumor burden and advancing disease stage, tumor cells produce more immunosuppressive factors to inhibit the differentiation and proliferation of lymphocytes, resulting in stronger immune escape and lower antitumor efficacy. Accordingly, the cellular immune function of breast cancer patients with lymph node metastasis was significantly lower than that of patients without lymph node metastasis, indicating that once the tumor metastasizes, the immune function of the patients will be further suppressed, resulting in a deteriorating prognosis.

To further understand the role of Tregs in modulating the suppressive tumor microenvironment and cancer development, the levels of Tregs in

Table 3. Comparison of immune cell profiles in the peripheral blood of breast cancer patients with and without lymph node metastasis ($\bar{x}\pm s$)

TNM	n	$CD4^+CD25^+CD127^{low}$Treg	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$	NK%
Without lymph node metastasis	40	1.10±0.23	62.16±5.22	32.61±4.25	28.81±3.61	1.25±0.22	15.72±2.65
Lymph node metastasis	80	1.96±0.38	58.25±4.23	26.91±4.32	30.63±3.02	1.06±0.21	12.55±2.30
t		5.13	2.625	6.218	2.733	3.155	7.235
P		0.013	0.030	0.018	0.042	0.021	0.046

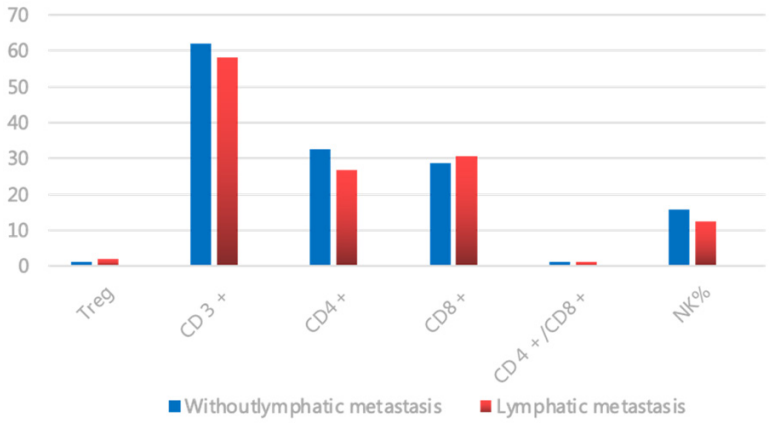

Figure 3. Comparison of immune cell profiles in the peripheral blood of breast cancer patients with and without lymph node metastasis ($\bar{x}\pm s$). The percentage of $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ and NK cells was significantly lower in patients with lymph node metastasis than in patients without lymph node metastasis, while the percentages of $CD8^+$ cells showed the opposite trend ($P < 0.05$).

the peripheral blood of 120 breast cancer patients were compared with healthy controls and patients with benign breast disease. Firstly, we found that the proportion of Tregs was significantly increased in breast cancer patients, indicating that Tregs were upregulated (**Table 1**; **Figure 1**). Moreover, the levels of Tregs in the peripheral blood of breast cancer patients increased with the progression of clinical stages (**Table 2**; **Figure 2**). Accordingly, the proportion of Tregs in stage III and IV patients was significantly higher than in stage I and II patients, which was consistent with an early report by Xu et al. from 2011 [23]. At the same time, the ratio of Tregs in the peripheral blood of breast cancer patients with lymph node metastasis was significantly higher than in patients without lymph node metastasis. This suggests that Tregs may be involved in the occurrence and development of breast cancer at an early stage, thus further mediating immunosuppression and then contributing to the progression and metastasis of breast cancer (**Table 3**; **Figure 3**). These findings indicate that the proportion of Treg cells is of great significance for evaluating the prognosis of breast cancer patients. However, a causal relationship between Treg levels and cancer progression still requires further mechanistic research.

Finally, we investigated the relationship between the levels of immune cells and therapeutical efficiency. A positive correlation between the enhanced immunity mediated by $CD3^+/CD4^+$ T cells and the efficacy of chemotherapy was observed, while a decreasing trend of $CD8^+$ T cells was observed after treatment. These results suggested that when the tumor is effectively controlled, the immune function can be corrected to a relatively normal level (**Table 4**). In addition, the $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ ratios were significantly higher in DCR than in PD patients, suggesting that the effect of chemotherapy may depend on the immune microenvironment, which further contributed to the inhibition of tumor proliferation, progression and metastasis (**Table 5**).

Conclusions

Studies revealed widespread immune dysfunction in breast cancer patients, which is closely related to tumor progression and lymph node metastasis. To some extent, $CD4^+/CD8^+$ T cells can reflect the changes of cellular immune function. In addition, Tregs are associated with tumor immunosuppression, and the proportion of Tregs may increase with disease progression, which is also of great significance for evaluating the prognosis of breast cancer. After treatment with neoadjuvant chemotherapy, the cellular immune function of patients significantly improved, especially in patients with a

Table 4. Comparison of lymphocyte subsets in the peripheral blood of breast cancer patients before and after neoadjuvant chemotherapy ($\bar{x}\pm s$)

Group	$CD4^+CD25^+CD127^{low}$Treg	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$	NK%
Before chemotherapy	1.53±0.31	60.54±4.20	32.65±4.55	28.16±3.51	1.30±0.16	15.06±2.50
After chemotherapy	1.32±0.23	65.12±4.82	36.23±5.03	24.35±3.16	1.50±0.35	18.68±3.61
t	5.82	5.81	6.83	7.55	8.19	5.31
P	0.011	0.002	0.003	0.003	0.012	0.005

Table 5. Comparison of lymphocyte subsets in the peripheral blood of breast cancer patients with different treatment effects ($\bar{x}\pm s$)

Group	n	$CD4^+CD25CD127^{low}$Treg	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$	NK%
DC	82	1.28±0.19	66.58±4.51	38.31±5.06	23.83±3.13	1.53±0.33	18.60±3.58
PD	38	1.45±0.25	64.21±4.45	32.70±4.56	24.36±3.35	1.18±0.25	15.01±2.63
t		4.533	2.357	5.612	0.055	3.812	5.450
P		0.013	0.038	0.001	0.063	0.001	0.023

good response to chemotherapy, which provides a theoretical basis for rational treatment of breast cancer patients by seizing the optimal treatment opportunity. Monitoring the above immune indicators in patients has a certain reference value for judging the disease progression and prognosis of breast cancer patients.

Here, we characterized the comprehensive pattern of immune cell populations in the peripheral blood of breast cancer patients stratified by disease progression and chemotherapy efficacy. However, this study still has some limitation limitations. Firstly, this was a retrospective study with a relatively small sample size, so that some important prognostic factors could not be included in the analysis, which may lead to bias. Secondly, all data included in the study were from a single center. Multicenter studies with larger sample sizes are needed to generalize our findings. Finally, future studies must analyze and discuss the potential causal relationship between changes in immune cell profiles and the treatment effect of neoadjuvant chemotherapy in breast cancer.

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Disclosure of conflict of interest

None.

Address correspondence to: Kang-Sheng Liu, Department of Clinical Laboratory, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing 210029, Jiangsu, China. E-mail: Kanshengliu@163.com

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