Journal of Cancer Research and Clinical Oncology (2024) 150:28
https://doi.org/10.1007/s00432-023-05508-1

RESEARCH

Expression analysis of lymphocyte subsets and lymphocyte-to-monocyte ratio: reveling immunosuppression and chronic inflammation in breast cancer

Hao Zhang[1] · Yan Li[2] · Gang Liu[1] · Xin Chen[1]

Received: 20 October 2023 / Accepted: 6 November 2023 / Published online: 23 January 2024
© The Author(s) 2024

Abstract

Objective To explore the immune status and chronic inflammation of breast cancer patients, this study aims to analyze the diagnostic value of peripheral blood lymphocyte subsets ($CD3^+T$, $CD4^+T$, $CD8^+T$, $CD3^+CD4^-CD8^-T$, $CD19^+B$, and NK cells) and lymphocyte-to-monocyte ratio (LMR) for breast cancer. Furthermore, it seeks to examine the correlation between these subsets and LMR with clinicopathological features.

Methods A total of 100 breast cancer patients were selected as the experimental group, while 55 patients with benign breast diseases were included in the control group. Statistical analysis, including the Wilcoxon test, Kruskal–Wallis test and the receiver operating characteristic curve, was employed to investigate the association between these serum indexes and the clinicopathological characteristics of the patients.

Results The levels of $CD3^+T$ cells, $CD4^+T$ cells, $CD8^+T$ cells, $CD4^+/CD8^+$ ratio, NK cells, $CD3^+CD4^-CD8^-T$ cells, and LMR were found to be related to the occurrence of breast cancer when analyzing data from patients with benign and malignant breast diseases. Among these biomarkers, $CD3^+T$ cells, $CD4^+T$ cells, $CD4^+/CD8^+$ ratio, $CD3^+CD4^-CD8^-T$ cells, and LMR were identified as independent risk factors for breast cancer development, and the AUCs were 0.760, 0.750, 0.598, 0.697, and 0.761 ($P < 0.05$), respectively. Furthermore, we observed varying degrees of differences in the expression of $CD3^+T$ cells, $CD4^+T$ cells, $CD8^+T$ cells, $CD4^+/CD8^+$ ratio, and LMR in lymph node metastasis, clinical staging, molecular typing, Ki-67 level ($P < 0.05$). However, statistical differences in histologic grade and pathology type were not found ($P \geq 0.05$).

Conclusion Lymphocyte subsets and LMR reflect the immune status and chronic inflammation of the body, respectively. They have certain value in the diagnosis of benign and malignant breast diseases, and correlate with lymph node metastasis, clinical staging, molecular typing and other clinicopathological features of breast cancer. Therefore, monitoring the expression of lymphocyte subsets and LMR in the body may help the auxiliary diagnosis and condition analysis of breast cancer in the clinic.

Keywords Lymphocyte subsets · Lymphocyte-to-monocyte ratio · Immunity · Chronic inflammation · Breast cancer · Immunosuppression · Diagnosis

Introduction

Breast cancer has surpassed lung cancer as the malignant tumor with the highest incidence rate among women, with approximately 2.26 million new cases per year globally and 682,000 deaths, accounting for 15.5% of deaths from malignant tumors among women (Sung et al. 2021), which is yet another serious challenge for the global healthcare sector. The understanding of breast cancer has grown, revealing factors such as genetic predisposition, early menarche, late menopause, and infertility that contribute to

✉ Xin Chen
593644382@qq.com

[1] Department of Breast and Thyroid Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

[2] School of Public Health, Chongqing Medical University, Chongqing, China

its development. Furthermore, as the study of tumor immunity progresses, researchers have discovered that immune cells are vital in the progression of breast cancer, from the initial transformation of normal breast epithelial cells to the subsequent development of the disease (Goff and Danforth 2021). Lymphocytes, including $CD3^+$T cells, $CD4^+$T cells, $CD8^+$T cells, $CD3^+CD4^-CD8^-$T cells, $CD19^+$B cells, and NK cells, are the main effector cells of the body's immune response. $CD3^+$T cells, the general term for T lymphocytes in the body, include $CD4^+$T cells and $CD8^+$T cells, which directly respond to the strength of cellular immunity. Studies have demonstrated that elevated levels of $CD3^+$T cells in breast cancer patients are indicative of improved chemotherapy response rates and overall prognosis (Schmidt and Heimes 2021). $CD4^+$T cells, as the hub of the immune system, secrete cytokines with anti-tumor effects and activate other immune effector cells (Kurozumi et al. 2017). $CD4^+$T cell can be divided into Th1, Th2, regulatory T cells (Treg), etc. It is currently believed that Th1 cells mediate anti-tumor immunity, while Th2 and Treg cells have pro-tumorigenic effects due to their immunosuppressive properties (Ruffell et al. 2010; Zamarron and Chen 2011). Gu-Trantien et al. (Gu-Trantien et al. 2013) observed that $CD4^+$T cells in the organism are an important immune-acting component in breast cancer, and that the stronger the immune response they generate, the better the disease-free survival (DFS) may be obtained. In a general way, high level of $CD8^+$T cells is associated with better survival as they play a key role in the anti-tumor effects. $CD8^+$T cells are activated to release granzyme and perforin, leading to the killing of target cells or induce apoptosis through the fas/fasl pathway. Additionally, $CD8^+$T cells can differentiate into cytotoxic T-lymphocytes (CTLs), which directly exert anti-tumor effects (Mahmoud et al. 2011, 2012; Koretzky 2010). In an organism that normally executes an immune response, the majority of $CD8^+$T cells are CTLs, while a few are suppressor T-lymphocytes (Ts) that inhibit the body's immune process (Appay et al. 2008). The $CD4^+/CD8^+$ ratio reflects the balance in T-cell subsets. In patients with malignant tumors, this ratio can decrease or even reverse, indicating a disruption of immune homeostasis (Zacharakis et al. 2018). $CD3^+CD4^-CD8^-$T cells, also known as DNT cells (double negative T cells), are a rare subset of T-cells of as yet unspecified origin. They have been found to play a role in inflammation, autoimmunity, and tumorigenesis (Wu et al. 2022). It has been suggested that DNT cells primarily exert their anti-tumor effects through various mechanisms such as secreting cytokines and the expression of tumor necrosis factor-associated apoptosis-inducing ligand (TRAIL) (Chen et al. 2019). Additionally, the reduced number of DNT cells may be associated with the decreased function of effector $CD4^+$T cells with CTLs in tumor patients (Li et al. 2019). $CD19^+$B cells, in contrast to the aforementioned lymphocytes, can convert $CD4^+$T cells into Treg cells and enhance the immune-suppressive responses of tumor cells (Tadmor et al. 2011). NK cells are lymphocytes that have immunosurveillance and antitumor effects. They can directly kill tumor cells through nonspecific killing without needing specific sensitizers to activate them (Caligiuri 2008; Maxwell et al. 2018). However, tumor cells, in order to escape the immunocidal effect of the body, also produce immunosuppressive factors to inhibit the differentiation and proliferation of immune cells, researchers have detected decreased levels of immune cells and $CD4^+/CD8^+$ ratio in patients with lung, esophageal, colorectal, and gastric cancers (Chraa et al. 2019; Wang et al. 2021).

In addition, the role of chronic inflammation in tumor biology is gradually being recognized. Inflammatory response can release various inflammatory mediators that boost tumor microangiogenesis and hinder tumor cells senescence and death (Rius et al. 2008). Monocytes, as a type of inflammatory cells, which enter tumor tissues and differentiate into tumor associated macrophages (TAMs), form part of the inflammatory infiltration of tumors. It is currently believed that TAMs are predominantly classified as M2-type macrophages (Sica et al. 2006). TAMs secrete vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), etc., which are involved in peritumor angiogenesis and promote tumor infiltration and metastasis (Pollard 2004). Additionally, TAMs release soluble factors that attenuate the cytotoxic function of T cell subsets, thereby suppressing the body's anti-tumor immune response. High level of TAMs in breast cancer tissues is associated with high vascular density, a higher incidence of lymphatic metastasis, and poorer overall survival (OS) (Rius et al. 2008). In addition, monocytes themselves secrete pro-inflammatory cytokines, such as interleukins IL-1, IL-6, IL-10, and tumor necrosis factor (TNF-α) (Watari et al. 2014). Therefore, monocytes, as precursor components of macrophages, can be considered as a marker of the level of inflammatory response. Recently, many researchers have proposed combining the application of body immune function indexes and chronic inflammatory response indexes to obtain new predictive indexes like the lymphocyte-to-monocyte ratio (LMR). LMR not only reflects the level of anti-tumor immune response but also indicates the level of chronic inflammation in tumors, providing a more comprehensive assessment of the intra-organismal status of tumor patients. Studies have shown that high preoperative LMR is associated with a significant increase in time to recurrence (TTR) and OS in colon cancer patients, while patients with low LMR do not benefit much from adjuvant chemotherapy (Stotz et al. 2014). Additionally, Hu et al. (Hu et al. 2018) discovered a significant decrease in LMR among breast cancer patients. This decline was strongly linked to worse DFS and OS, particularly among Asian populations and TNBC patients.

The current clinical diagnosis of breast malignant tumors mainly relies on imaging and pathology examinations. However, imaging diagnosis is economically costly and has limitations such as lag. Pathologic diagnosis mainly consists of needle aspiration cytology and crude needle aspiration biopsy, which are invasive operations. Although pathology is considered the gold standard for diagnosis, some patients may experience psychological burden. Therefore, there is a need for another diagnostic method for early detection of breast malignant tumors. Currently, serological tests such as tumor markers have been successfully tried and widely used in clinics. Our research aims to explore another serological test index with diagnostic value. We will assess the predictive value of lymphocyte subsets and LMR on the occurrence, metastasis, and invasiveness of breast malignant tumors by integrating them with clinical features. This research will provide a new effective serological index for the early diagnosis of breast malignant tumors, offering a new diagnostic method to supplement imaging and pathology examinations.

Patients and methods

Patients and data collection

The test group for this study consisted of 120 patients with pathologically confirmed breast malignant tumors who attended the First Affiliated Hospital of Chongqing Medical University between August 2022 and July 2023. In the same period, 55 patients with benign breast lesions were selected as the control group. The studies involving human participants were reviewed and approved by the institutional ethics committee of the First Affiliated Hospital of Chongqing Medical University, and all experiments were performed in accordance with relevant guidelines and regulations. Pathological biopsy operations of all patients with breast malignant tumors were performed by experienced surgeons, and pathological results were determined by two experienced pathologists in our center. The inclusion criteria for the test group were as follows: (1) women aged 18–80 years, (2) histologically confirmed breast malignancy, and (3) no history of radiotherapy, chemotherapy, or targeted therapy prior to enrollment. The exclusion criteria were as follows: (1) prior anti-inflammatory drug therapy, (2) no history of acute or chronic inflammation, other neoplastic and hematological disorders, autoimmune diseases such as hyperthyroidism, rheumatoid arthritis, diabetes mellitus, and steroid medications, and (3) the presence of other major co-morbidities. Ultimately, 100 patients with breast malignant tumors were included in the study. The average age of the experimental group was 52.47 ± 10.40 years. Among the included patients, 52 cases (52.0%) were menopausal. Tumor sizes were as follows: 29 cases (29.0%) had a size of ≤ 2 cm, 57 cases (57.0%) had a size of > 2 cm and ≤ 5 cm, and 14 cases (14.0%) had a size of > 5 cm. Additionally, 68 cases (68.0%) had lymph node metastasis, and 5 cases (5.0%) had distant metastasis. According to the 8th edition of AJCC TNM staging, 57 cases (57.0%) were classified as $\leq$ TNM stage II, and 43 cases (43.0%) were classified as $\geq$ TNM stage III. Furthermore, 41 cases (41.0%) were hormone receptor-positive, 43 cases (43.0%) were HER-2 receptor-positive (regardless of hormone receptor expression), and 16 cases (16.0%) were triple-negative breast cancer (TNBC). According to the 2012 edition of the WHO classification of breast tumors, there were 8 cases (8.0%) of carcinoma in situ and 87 cases of invasive ductal carcinoma, including 4 cases (4.0%) of histologic grade I, 59 cases (59.0%) of grade II, 24 cases (24.0%) of grade III, and 5 cases (5.0%) of special type of invasive carcinoma. Based on a Ki-67 expression cut-off of $\leq 20\%$, Ki-67 low expression (Ki-67 $\leq 20\%$) in 36 cases (36.0%), and high expression (Ki-67 $> 20\%$) in 64 cases (64.0%). In the control group of 55 women with benign breast diseases, the average age was 49.18 ± 11.14 years. Among them, 26 cases (47.3%) were menopausal. The distribution of tumor sizes in the control group was as follows: 21 cases (38.2%) had a size of ≤ 2 cm, 24 cases (43.6%) had a size of > 2 cm and ≤ 5 cm, and 10 cases (18.2%) had a size of > 5 cm. Furthermore, the differences in age, menstrual status, and tumor size between the two groups were not statistically significant ($P > 0.05$) and were comparable (Table 1).

Detection of lymphocyte subsets

Before treatment, all study subjects were fasted and 3 ml of peripheral venous blood was drawn into a disposable blood collection tube. The blood was then immediately sent for examination and stored at room temperature after anticoagulation with EDTA-K2, while avoiding shaking. The flow cytometry steps involved adding 20 μL of murine anti-human CD3, CD4, CD8, CD16, CD19, CD56 fluorescently labeled monoclonal antibody to the bottom of the flow tube, thoroughly mixing it, and allowing it to label for 30 min at a temperature range of 20–25 °C, all while avoiding exposure to light. Following that, 3 mL of PBS was added for washing purposes. After centrifugation at 1500 r/min for 5 min, an additional 1 mL of PBS was added for further mixing. Subsequently, $CD3^+$T cells, $CD3^+CD4^+$T cells, $CD3^+CD8^+$T cells, $CD3^+CD4^-CD8^-$T cells, $CD3^-CD16^+CD56^+$ NK cells, and $CD19^+$B cells were detected using the flow cytometer. The mouse monoclonal antibodies and flow cytometers were purchased from BD, USA, and were utilized as per the provided instructions.

Table 1 Baseline information table and univariate analysis

	Total (n = 155)	Benign (n = 55)	Malignant (n = 100)	P-value
Patients	155	55	100	–
Age	51.30 ± 10.75	49.18 ± 11.14	52.47 ± 10.40	0.068
Menopausal status				0.573
Menopausal	78 (50.3%)	26 (47.3%)	52 (52.0%)	
Non-menopausal	77 (49.7%)	29 (52.7%)	48 (48.0%)	
Tumor size				0.576
≤ 2 cm	50 (32.3%)	21 (38.2%)	29 (29.0%)	
> 2 cm and ≤ 5 cm	81 (52.2%)	24 (43.6%)	57 (57.0%)	
> 5 cm	24 (15.5%)	10 (18.2%)	14 (14.0%)	
$CD3^+$	1202.00 (963.00,1462.00)	1404.00 (1159.00,1701.00)	1089.00 (838.50,1342.75)	**< 0.001***
$CD3^+CD4^+$	716.00 (561.00,890.00)	816.00 (669.00,1052.00)	636.50 (470.75,763.00)	**< 0.001***
$CD3^+CD8^+$	39.40 (321.00,506.00)	429.00 (360.00,559.00)	384.00 (298.50,459.75)	**0.009***
$CD4^+/CD8^+$	1.72 (1.37,2.23)	1.80 (1.42,2.61)	1.60 (1.30,2.09)	**0.043***
$CD3^+CD4^-CD8^-$	74.00 (50.00,116.00)	96.00 (69.00,178.00)	66.00 (43.25,96.00)	**< 0.001***
$CD19^+B$	213.00 (166.00,293.00)	224.00 (180.00,323.00)	210.00 (161.25,287.00)	0.068
NK	214.00 (132.00,298.00)	251.00 (174.00,347.00)	205.00 (122.25,264.25)	**0.019***
LMR	4.57 (3.46,5.75)	5.46 (4.54,6.52)	3.96 (3.07,5.08)	**< 0.001***

*Values in bold represent statistically significant results (P < 0.05)

Blood tests

5 mL of venous blood was drawn on an empty stomach and placed in an anticoagulation tube. The blood was then subjected to centrifugation at a centrifugal rate of 1500 r/min and a centrifugal radius of 15 cm for 10 min. Using a Mindray BC5180 automatic hematology analyzer and accompanying reagents, the levels of white blood cells, lymphocyte, and monocyte were detected. The test was repeated 3 times, and the average of the 3 results was taken as the final examination result. Subsequently, the lymphocyte and monocyte counts were collected, and the lymphocyte-to-monocyte ratio (LMR) was calculated, defined as the ratio of lymphocyte count to monocyte count.

Statistical analysis

The clinical characteristics of patients in the two groups were expressed as frequencies or descriptive analysis. If the quantitative information satisfied normal distribution, it was expressed as mean ± standard deviation, and comparisons between two groups were conducted using the independent samples t-test. On the other hand, if the quantitative information was skewed, it was expressed as M (P25, P75), and comparisons between two groups were made using the Wilcoxon (Mann–Whitney U) test. For comparisons between multiple groups, the Kruskal–Wallis (KW test) was utilized. Qualitative information was expressed as n (%), and comparisons between groups were performed using the chi-square test or the KW test. Multifactorial analysis was conducted using binary logistic regression. Additionally, the diagnostic value of the above indexes for breast malignant tumors was analyzed using the receiver operating characteristic curve (ROC curve). Statistical analysis was performed using SPSS 25.0.

Result

Univariate analysis reveals possible factors in the development of breast malignancy

The results showed that age, menstrual status, and tumor size did not demonstrate statistical significance in both the benign and malignant lesion groups (P ≥ 0.05). On the other hand, statistically significant differences were observed in $CD3^+T$, $CD4^+T$, $CD8^+T$, $CD4^+/CD8^+$, DNT, NK, and LMR between the two groups of patients (P < 0.05) (Table 1).

Multifactorial analysis reveals independent risk factors for the development of breast malignancy

Indicators with statistically significant results in the univariate analysis were included as independent variables in a multifactorial logistic regression model, which showed that $CD3^+T$, $CD4^+T$, $CD4^+/CD8^+$, DNT, and LMR were independent risk factors for the development of malignant tumors in the breast (P = 0.001, < 0.001, = 0.043, = 0.025, < 0.001) (Table 2).

Table 2 Comparison of independent risk factors for breast malignancy

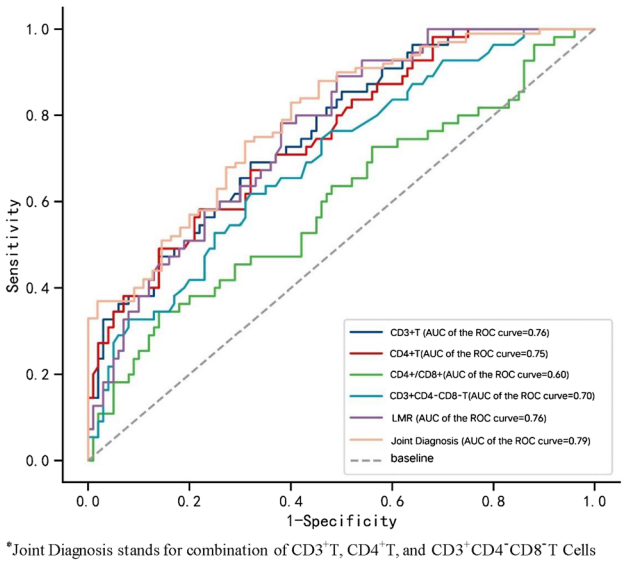
Indicators	B	SR	Wald	P-value	OR	OR 95% CI	
						Lower limit	Upper limit
$CD3^+$	− 0.002	0.001	10.257	0.001	0.998	0.996	0.999
$CD3^+CD4^+$	− 0.004	0.001	19.251	< 0.001	0.996	0.994	0.998
$CD4^+/CD8^+$	− 0.631	0.311	4.109	0.043	0.532	0.289	0.979
$CD3^+CD4^-CD8^-$	− 0.007	0.003	4.982	0.025	0.993	0.987	0.999
LMR	− 0.543	0.153	12.540	< 0.001	0.581	0.430	0.785

Table 3 ROC analysis of lymphocyte subsets and LMR in differentiating breast cancer from benign control group

Indicators	AUC	Sensitivity	Specificity	Yoden Index	Threshold
$CD3^+$	0.760	0.691	0.680	0.371	1248.000
$CD3^+CD4^+$	0.750	0.582	0.780	0.362	782.000
$CD4^+/CD8^+$	0.598	0.345	0.860	0.205	2.300
$CD3^+CD4^-CD8^-$	0.697	0.618	0.680	0.298	83.000
LMR	0.761	0.782	0.620	0.402	4.511
Joint diagnosis	0.790	0.740	0.691	0.431	0.640

ROC curve reveals the diagnostic value of each index for breast malignant tumors

The results of ROC curve analysis demonstrated that the AUCs for $CD3^+T$, $CD4^+T$, $CD4^+/CD8^+$, DNT, and LMR were 0.760 (95% CI 0.684–0.836), 0.750 (95% CI 0.672–0.828), 0.598 (95% CI 0.503–0.694), 0.697 (95% CI 0.612–0.781), and 0.761 (95% CI 0.686–0.835), respectively. Sensitivities for these indicators were 69.1%, 58.2%, 34.5%, 61.8%, and 78.2%, while specificities were 68.0%, 78.0%, 86.0%, 68.0%, and 62.0%. Moreover, when T-lymphocyte subsets $CD3^+T$, $CD4^+T$, and DNT were combined for diagnostic purposes, the resulting AUC was 0.790 (95% CI 0.717–0.862), with a sensitivity of 74.0% and specificity of 69.1% (Table 3; Fig. 1). Hence, serum levels of $CD3^+T$, $CD4^+T$, $CD4^+/CD8^+$, DNT, and LMR have significant associations with the development of breast malignant tumors, demonstrating their diagnostic value. Furthermore, the combined application of these indicators can further enhance their diagnostic value.



*Joint Diagnosis stands for combination of $CD3^+T$, $CD4^+T$, and $CD3^+CD4^-CD8^-T$ Cells

Fig. 1 The ROC curve graph of lymphocyte subsets and LMR in breast cancer and benign breast disease patients

Relationship between lymphocyte subsets and LMR with clinical features of breast malignancy

We further analyzed 100 patients from the breast malignancy group to find associations between lymphocyte subsets and LMR with clinical features of breast malignancy. Compared with the group without lymphatic metastasis, the group with lymphatic metastasis exhibited a significant decrease in the levels of $CD3^+T$, $CD4^+T$, DNT, and LMR ($P < 0.05$). However, no statistically significant relationship was found between other indexes and lymphatic metastasis ($P \geq 0.05$) (Table 4). We also distinguished whether there was any difference in lymphocyte subsets and LMR levels between different TNM stages. In patients with early stage (≤ stage II) compared to patients with advanced breast malignancy (≥ stage III), statistically significant findings were observed. Specifically, there was a significant increase in $CD8^+T$, and a significant decrease in $CD4^+/CD8^+$ and LMR ($P < 0.05$). However, other indicators did not show statistically significant differences in relation to clinical stage ($P \geq 0.05$) (Table 5).

Table 4 Lymphocyte subsets and LMR associated with lymph node metastasis

Indicators	Presence of lymph node metastasis		P-value
	No lymph node metastasis group (n = 32)	Group with lymph node metastasis (n = 68)	
$CD3^+$	1177.50 (1038.75,1392.75)	1022.00 (793.00,1293.00)	**0.030**
$CD3^+CD4^+$	715.00 (607.00,762.50)	591.00 (444.00,760.00)	**0.014**
$CD3^+CD8^+$	395.50 (317.00,462.75)	380.00 (286.00,445.00)	0.346
$CD4^+/CD8^+$	1.86 (1.32,2.22)	1.59 (1.28,2.00)	0.113
$CD3^+CD4^-CD8^-$	76.50 (61.50,96.00)	61.00 (36.50,99.25)	**0.043**
$CD19^+B$	193.50 (166.25,327.75)	213.00 (150.50,261.50)	0.329
NK	221.50 (148.00,284.50)	196.00 (100.25,260.25)	0.085
LMR	4.71 (3.25,5.72)	3.7 (2.93,4.78)	**0.037**

Values in bold represent statistically significant results ($P < 0.05$)

Table 5 Lymphocyte subsets and LMR associated with TNM staging

Indicators	TNM Staging		P-value
	Stage 0–II (n = 57)	Stage III–IV (n = 43)	
$CD3^+$	1067.00 (859.00,1243.50)	1105.50 (831.00,1494.00)	0.145
$CD3^+CD4^+$	643.00 (493.00,751.50)	630.00 (463.25,861.00)	0.484
$CD3^+CD8^+$	360.00 (275.50,414.00)	399.50 (339.00,540.00)	**0.002**
$CD4^+/CD8^+$	1.84 (1.37,2.19)	1.45 (1.20,1.86)	**0.015**
$CD3^+CD4^-CD8^-$	71.00 (43.50,95.00)	63.00 (40.00,102.00)	0.805
$CD19^+B$	193.00 (156.00,291.00)	214.00 (166.00,288.00)	0.638
NK	214.00 (132.00,279.00)	203.00 (94.00,257.00)	0.356
LMR	4.44 (3.26,5.52)	3.49 (2.77,4.42)	**0.005**

Values in bold represent statistically significant results ($P < 0.05$)

Relationship between lymphocyte subsets and LMR with molecular typing

In this study, 100 patients with breast malignant tumors were divided into three groups: the HR (+) group with 41 cases, the HER-2 (+) group with 43 cases, and the TNBC group with 16 cases. Statistical analysis revealed that there were no statistically significant differences in the levels of $CD4^+/CD8^+$, DNT, NK, and LMR among the three groups ($P \geq 0.05$). However, significant differences were found in $CD3^+$T, $CD4^+$T, and $CD8^+$T between the groups ($P < 0.05$). Specifically, $CD4^+$T was significantly lower in the HER-2 (+) group compared to the HR (+) group and the TNBC group. $CD3^+$ and $CD8^+$T were significantly lower in the HER-2 (+) group compared to the TNBC group. It is worth noting that there were no significant differences in the above indexes between the HR (+) and the TNBC groups ($P \geq 0.05$) (Table 6).

Relationship between lymphocyte subsets and LMR with Ki-67 level

Based on the Ki-67 ≤ 20% threshold, 100 patients with breast malignant tumors were categorized into Ki-67 low-expression and high-expression groups. Specifically, there were 36 cases in the low-expression group and 64 cases in the high-expression group. The results of the statistical analysis indicated that the low-expression group exhibited significantly higher expression levels of $CD3^+$T, $CD4^+$T, and $CD8^+$T compared to the high-expression group ($P < 0.05$). Conversely, no statistically significant differences were observed between the two groups in terms of $CD4^+/CD8^+$, DNT, $CD19^+$B, NK, and LMR ($P \geq 0.05$) (Table 7).

Relationship between lymphocyte subsets and LMR with histologic grading and pathology type

We divided 100 patients with breast malignant tumors into three groups based on the pathological results: ductal carcinoma in situ group, special type invasive carcinoma group, and invasive ductal carcinoma group. The invasive ductal carcinoma group was further divided into histological grade I group, histological grade II group, and histological grade III group, according to the WHO breast tumor classification (2012 version) standard. However, statistical analysis did not show significant differences in the expression levels of the indicators between these five groups ($P \geq 0.05$) (Table 8).

Table 6 Lymphocyte subsets and LMR associated with molecular typing

Indicators	Hormone receptor-positive type (n = 41)			HER-2 receptor-positive type (n = 43)	
$CD3^+$	1137.00 (984.00,1365.00)			931.00 (755.00,1176.00)	
$CD3^+CD4^+$	681.00 (575.50,785.50)			548.00 (444.00,716.00)	
$CD3^+CD8^+$	393.00 (289.50,454.50)			333.00 (286.00,413.00)	
$CD4^+/CD8^+$	1.63 (1.28,2.27)			1.59 (1.38,2.00)	
$CD3^+CD4^-CD8^-$	74.00 (48.50,112.50)			57.00 (40.00,87.00)	
$CD19^+B$	228.00 (172.00,312.00)			185.00 (132.00,245.00)	
NK	213.00 (137.50,263.50)			207.00 (97.00,271.00)	
LMR	3.97 (3.01,5.08)			3.69 (2.73,4.80)	
Indicators	Triple negative type (n = 16)	P-value	P1*	P2*	P3*
$CD3^+$	1333.00 (897.00,1497.00)	**0.004**	0.076	**0.006**	0.460
$CD3^+CD4^+$	754.00 (611.00,921.00)	**0.003**	**0.025**	**0.007**	0.879
$CD3^+CD8^+$	445.00 (368.00,526.00)	**0.017**	0.534	**0.014**	0.212
$CD4^+/CD8^+$	1.61 (1.31,1.88)	0.690	–	–	–
$CD3^+CD4^-CD8^-$	74.50 (57.25,99.25)	0.237	–	–	–
$CD19^+B$	202.50 (161.25,277.25)	0.050	–	–	–
NK	203.00 (122.00,340.00)	0.856	–	–	–
LMR	4.68 (3.67,6.36)	0.113	–	–	–

Values in bold represent statistically significant results ($P < 0.05$)

*P1, Difference in lymphocyte subsets and LMR between hormone receptor-positive and HER-2 receptor-positive types. *P2, Difference in lymphocyte subsets and LMR between HER-2 receptor-positive and triple-negative types. *P3, Difference in lymphocyte subsets and LMR between hormone receptor-positive and triple-negative types

Table 7 Lymphocyte subsets and LMR associated with Ki-67 expression

Indicators	Ki-67		P-value
	Low expression (n = 36)	High expression (n = 64)	
$CD3^+$	1220.00 (979.75,1472.25)	1038.00 (779.00,1231.00)	**0.004**
$CD3^+CD4^+$	727.00 (568.25,901.25)	611.00 (428.00,728.00)	**0.005**
$CD3^+CD8^+$	397.50 (331.25,529.25)	368.00 (280.00,430.00)	**0.042**
$CD4^+/CD8^+$	1.61 (1.32,2.22)	1.60 (1.28,2.01)	0.638
$CD3^+CD4^-CD8^-$	74.50 (44.50,100.75)	63.00 (39.50,90.00)	0.432
$CD19^+B$	214.50 (161.25,309.50)	204.00 (159.75,277.00)	0.573
NK	196.00 (135.75,247.75)	216.00 (118.75,271.00)	0.566
LMR	4.35 (3.09,5.47)	3.90 (3.03,4.80)	0.291

Values in bold represent statistically significant results ($P < 0.05$)

Discussion

More attention has been paid to the tumor microenvironment of breast cancer due to the progress of molecular immunology. Tumor infiltrating lymphocytes (TILs) have emerged as a significant area of interest in this regard. However, the current detection methods for TILs are complex and expensive, limiting their widespread implementation. Ruffell et al. (2012) discovered that TILs in solid tumors primarily consist of $CD3^+$T-lymphocytes, with a small population of B-lymphocytes and NK cells. Conversely, lymphocytes in peripheral blood, which serve as the main source of TILs, can indirectly reflect the TILs level in patients. This alternative approach offers several advantages, including simplicity, cost-effectiveness, and the ability to dynamically monitor the patient's immune response at different disease stages, presenting promising clinical potential. Furthermore, our research also explores the chronic inflammatory response caused by tumors, in addition to focusing on the patient's immune status. Interestingly, we found that the relationship between lymphocytes levels and chronic inflammation in breast cancer patients may have intriguing implications.

Table 8 Lymphocyte subsets and LMR associated with histologic grading and pathology type

Indicators	Histopathological grade (n = 86)			DCIS (n = 9)	Infiltrative non-specific cancer (n = 5)	P-value
	I (n = 3)	II (n = 59)	III (n = 24)			
$CD3^+$	1535.00 (1035.00,1552.50)	1061.00 (823.00,1305.00)	1107.00 (853.00,1431.50)	1137.00 (1014.00,1322.00)	972.0 (669.00,1395.50)	0.435
$CD3^+CD4^+$	743.00 (415.00,963.50)	627.00 (465.00,749.00)	674.50 (476.50,851.25)	728.00 (602.00,846.00)	628.00 (373.50,788.00)	0.527
$CD3^+CD8^+$	367.00 (286.00,556.50)	393.00 (298.00,466.00)	374.00 (301.00,490.00)	395.00 (270.00,431.50)	341.00 (256.50,573.50)	0.991
$CD4^+/CD8^+$	1.45 (1.00,2.34)	1.53 (1.27,2.15)	1.85 (1.45,2.00)	1.78 (1.41,2.41)	1.37 (1.26,1.92)	0.612
$CD3^+CD4^-CD8^-$	88.00 (63.00,210.50)	63.00 (40.00,102.00)	68.50 (54.00,116.5)	75.00 (30.00,102.00)	55.00 (22.00,66.50)	0.286
$CD19^+B$	246.00 (148.00,294.00)	193.00 (158.00,268.00)	235.00 (181.25,300.00)	170.00 (143.00,245.50)	255.00 (158.00,295.00)	0.554
NK	165.00 (92.00,204.50)	213.00 (122.00,271.00)	205.00 (125.75,265.00)	176.00 (127.00,257.00)	123.00 (90.50,318.50)	0.925
LMR	5.08 (4.26,5.25)	3.78 (2.91,5.08)	4.27 (3.06,4.79)	3.80 (3.18,4.78)	4.73 (3.90,5.55)	0.753

Although decreased levels of $CD3^+$T cells, $CD4^+$T cells, $CD8^+$T cells, NK cells and $CD4^+/CD8^+$ ratio have been detected in the peripheral blood of patients with malignant tumors such as lung, esophageal, colorectal, and gastric cancers, the degree of immune suppression may also be inconsistent across tumors. In this study, we demonstrated that significantly reduced levels of $CD3^+$T cells, $CD4^+$T cells, $CD8^+$T cells, DNT cells, NK cells, $CD4^+/CD8^+$ ratio, and LMR were detected in the peripheral blood of patients with breast cancer relative to patients with benign breast lesions. Reduced $CD3^+$T cells, $CD4^+$T cells, DNT cells, CD4/CD8 ratio, and LMR were considered to be independent risk factors for the development of breast cancer. $CD3^+$T cells, which represent the overall level of cellular immunity in the body, were found to be significantly decreased in breast cancer patients. This indicates the existence of immunosuppression in patients with breast cancer, potentially related to tumorigenesis. On the other hand, the levels of $CD4^+$T cells, $CD8^+$T cells, $CD4^+/CD8^+$ ratio, DNT cells, and NK cells in breast cancer patients have been reduced to different degrees within the lymphocyte subsets. This reduction can be attributed to the tumor releasing immunosuppressive factors, such as TGF-β, IL-10, during its growth. These factors inhibit the differentiation and proliferation of lymphocytes, leading to an imbalance of Th1/Th2 within the $CD4^+$T cells, favoring Th2 cells, and promoting the generation of Ts cells. Consequently, the number and activity of lymphocytes with anti-tumor effects are suppressed to varying degrees (Janneh et al. 2009; Yuan et al. 2013). In addition, the increase in Ts and Th2 cells inhibits the formation and maturation of $CD4^+$T cells and other immune cells, resulting in a decrease in $CD4^+$T cells and an increase in $CD8^+$T cell reactively. The predominance of Ts cells in the $CD8^+$T cells ultimately leads to a decrease or even inversion of the $CD4^+/CD8^+$ ratio, indicating dysfunction in the body's immune function and compromised anti-tumor efficacy (Mamessier et al. 2011; Trédan et al. 2013). Studies have shown that a lower $CD4^+/CD8^+$ ratio is associated with a higher recurrence and metastasis rate of tumors, which has implications for poor prognosis (Xu et al. 2017). Recent studies have found that DNT cells exert anti-tumor effects in xenograft models of leukemia, lung cancer, and pancreatic cancer. (Lee, et al. 2018; Yao et al. 2019; Lu et al. 2019). Our research has also revealed a significant decrease in the number of DNT cells in breast cancer patients. This suggests that the reduction of DNT cells may play a promoting role in the development of breast cancer. Additionally, researchers have discovered that the addition of allogeneic DNT cells in vitro models does not lead to significant rejection (Lee et al. 2018), indicating the potential of DNT cells in anti-tumor immunotherapy. The decrease in LMR observed in breast cancer patients is partly due to tumor-induced reduction of lymphocytes caused by tumor immunosuppression as mentioned above and partly due to an increase in monocyte production resulting from inflammation. During the process of tumor transformation to malignancy, the body's repair mechanism is disrupted, leading to chronic inflammation. At the same time, tumor cells consume a significant amount of oxygen during their growth, resulting in a localized hypoxic environment that induces hypoxic stress in the body. This state of stress triggers the release of mononuclear chemokine ligand 2 (CCL2). As a consequence, numerous monocytes are recruited from the vasculature to the tumor microenvironment, where they differentiate into TAMs (Hagemann et al. 2006). Moreover, the stress induces Treg production that can both enhance tumor angiogenesis and inhibit anti-tumor responses (Facciabene et al. 2011). TAMs produce a variety of pro-angiogenic growth factors and proteases, such as VEGF, MMP-9, and uPA, to promote angiogenesis and oxygenation of tumors. Furthermore, TAMs restrain the

proliferation of T-lymphocytes by releasing mediators such as prostaglandins and IL-10. The increased survival rate is associated with low level of TAMs in the tumor microenvironment (Sica et al. 2008). Wilcox et al. (Wilcox et al. 2009) also found that monocytes and their progeny in the tumor microenvironment are able to stimulate Treg proliferation by expressing PD-L1. Consequently, chronic inflammation can lead to immunosuppression, resulting in tumor escape. Statistically, chronic inflammation has been found to be related to around 15%-20% of tumor pathogenesis in various cancer types, such as hepatitis B virus and liver cancer, helicobacter pylori and gastric cancer, and inflammatory bowel disease and colon cancer (Allavena et al. 2008; Mantovani et al. 2008), highlighting its role in promoting tumorigenesis. Moreover, NSAIDs can reduce the production of inflammatory mediators such as prostaglandins and thromboxane A2, thus reducing the inflammatory response to exert a cancer-suppressive effect (Balkwill and Coussens 2004). Some studies have shown that taking NSAIDs can reduce the risk of colon cancer by 40–50% compared with the control group (Rodríguez and Huerta-Alvarev 2001). This have further illustrated the role of chronic inflammation in promoting tumorigenesis (Greten and Grivennikov 2019). As a result, Hal Dvorak says, "Tumors are wounds that don't heal." (Dvorak 2015). Therefore, controlling chronic inflammation through some interventions like NSAIDs has shown promise in reducing the risk of certain cancers.

Currently, clinical factors such as tumor stage, lymph node metastasis, and molecular typing impact the severity and prognosis of breast cancer. To establish a better understanding of the relationship between breast cancer, immune function, and chronic inflammation, we conducted additional analyses.

Lymph node metastasis, being the most common metastatic component of breast cancer, has been shown to affect the prognosis of patients. Studies indicate that compared with breast cancer patients without lymph node metastasis, patients with lymph node metastasis have a higher degree of malignancy and a significantly lower survival rate (To et al. 2020). Consequently, immune escape is more severe in these patients. In this study, we observed that patients with lymphatic metastasis exhibited significantly lower levels of $CD3^+T$ cells, $CD4^+T$ cells, and $CD3^+CD4^-CD8^-T$ cells. Particularly a notable decrease in the main effector cells, $CD4^+T$ cells. This decrease in $CD4^+T$ cells is likely to lead to a significant reduction in the tumor-eliminating effect, consequently facilitating the tumor cells evasion from the primary site. Moreover, we noted a significant decrease in the LMR among patients with lymph node metastasis. We hypothesize that this reduced LMR is associated with an intensified chronic inflammatory response in these patients. NF-kB is activated by chronic inflammation, leading to an increase in the metastatic potential of cancer cells. This occurs through the promotion of angiogenesis to provide oxygen to cancer cells, as well as by stimulating proliferation and inhibiting apoptosis (Cao and Prescott 2002). In a study comparing mice with NF-kB activation to those with NF-kB inactivation, comparable levels of cell proliferation and atypical hyperplasia were observed at the tumor initiation stage. However, as mice mature and tumors progress, tumor cells in the mice with activated NF-kB exhibited significantly proliferative activity (Pikarsky et al. 2004). Additionally, COX-2 also secretes substances such as VEGF, PGE2 and TXA2, which promote tumor cells proliferation and metastasis in a manner similar to NF-kB activation (Ono 2008). This mechanism may primarily impact on the tumor progression rather than its initiation or early stages. We further analyzed the relationship between clinical stage and the levels of lymphocyte subsets and LMR. Patients with stage III-IV may observe an increase in $CD8^+T$ cells and experience a significant decrease $CD4^+/CD8^+$ ratio and LMR when compared to patients with stage I–II. This could be due to the continuous release of immunosuppressive molecules during tumor development, which leads to the dominance of lymphocytes with an inhibitory effect and exacerbates the inflammatory response. The immune damage becomes more severe as the tumor stage progresses (Chen et al. 2014). Ts cells phenotyped as $CD8^+$ are expressed in large numbers, while Th cells activities are reduced. The dominance of Ts and Treg cells with pro-tumorigenic effects aids in immune escape by tumor cells. Consequently, patients with advanced breast cancer may witness an increase in $CD8^+T$ cells and experience a significant decrease or even reversal of the $CD4^+/CD8^+$ ratio. However, there is no statistically significant difference in $CD4^+T$ cells level between the two groups. It is hypothesized that although Th1 cells are reduced in advanced patients, Treg cells are increased within the $CD4^+$ subset due to the hypoxic environment and chronic inflammation (Hagemann et al. 2006), resulting in no significant change in overall $CD4^+T$ cells levels. This is supported by a study by Burugu et al. (2017) who found that patients with advanced breast cancer had predominantly mesenchymal infiltrating $CD4^+T$ cells with Treg and Th2. Treg in $CD4^+T$ cells could reach 20–40% of total $CD4^+T$ subset. However, in this study, the level or ratio of Treg and Ts cells in peripheral blood was not tested separately. Future studies cananalyze the composition of $CD4^+T$ and $CD8^+T$ cells internal lymphocytes in patients with breast cancer.

In this study, it was found that HER-2 (+) patients had significantly lower levels of $CD3^+T$ and $CD8^+T$ cells compared to TNBC patients. Additionally, the level of $CD4^+T$ cells was lower in HER-2 (+) patients compared to both TNBC and HR (+) patients. Consequently, the researchers conclude that HER-2 (+) patients experience the most apparent immunosuppression. The overexpression of HER-2, which is a proto-oncogene with tyrosine kinase activity,

promotes tumor cell proliferation, inhibits apoptosis, and facilitates tumor angiogenesis, thus increasing tumor invasiveness. Datta et al. (2016) reported an increased proportion of Treg cells in HER-2 (+) patients with advanced breast cancer, and this proportion decreased after trastuzumab treatment. Similarly, Liu et al. (2011) observed that Treg cells were significantly elevated in HER-2 (+) patients, and the higher CTL/Treg ratio in the paraneoplastic tissues was associated with better progression-free survival (PFS) and OS. Hence, while blocking the HER-2 pathway, using Treg cells expression as a target to counteract the cancer-associated immunosuppression is a potential therapeutic approach that may improve the prognosis of HER-2 (+) patients. Furthermore, assessing the levels of Treg and $CD4^+$T cells in HER-2 (+) patients can be valuable for monitoring the condition of breast cancer patients and predicting the efficacy of anti-HER-2 treatment.

Ki-67 is a nuclear protein discovered by Gerdes et al. (1983) as a cell proliferation marker, which responds to the proliferative activity of tumor cells. In this study, we found that the levels of $CD3^+$T, $CD4^+$T and $CD8^+$T cells were significantly reduced in the peripheral blood of breast cancer patients with high Ki-67 expression. This finding confirms that immunosuppressive factors are constantly released during tumor proliferation, which is consistent with earlier descriptions. The degree of decrease in the level of immune effector cells is more pronounced with stronger proliferative activity of the tumor cells. It is worth noting that the overall survival rate of breast cancer has been reported to be correlated with histologic grade. High histologic grade is identified as a risk factor for recurrent metastasis of breast cancer (Bianchini and Gianni 2014). Nevertheless, our study did not find a statistical relationship between lymphocyte subsets and LMR with histologic grade and pathology type. This lack of correlation may be attributed to the insufficient sample size or the selection bias resulting from the inclusion of a high percentage of patients with histologic grades II and III. Notably, this high percentage may be due to the heavy tumor load of most of the patients enrolled in our study.

Recent studies have found that $CD19^+$B cells promote tumor development in vitro models. Inoue et al. (2006) showed that B-cells depletion may enhance the anti-tumor immune response in a B-cells-deficient mouse model. $CD19^+$B cells are thought to promote breast cancer development and metastasis through the secretion of IL-10 and induction of the conversion of $CD4^+$T cells to Treg in the mouse models (Olkhanud et al. 2011; Gray et al. 2007). However, our study has not found a significant elevation in the expression level of $CD19^+$B cells in breast cancer patients. Additionally, there is no significant correlation between $CD19^+$B cells and clinical features of breast cancer patients. Yang et al. (Yang et al. 2019) found that $CD3^+$T and $CD4^+$T cells are independent predictors of PFS and OS in metastatic breast cancer patients with different molecular phenotypes, but no predictive value is found for $CD19^+$B cells in relation to prognosis, nor any association between $CD19^+$B cells and clinical factors in breast cancer. However, considering the potential role of $CD19^+$B cells in promoting Treg, future research can further measure Treg level to elucidate the inherent connection between $CD19^+$B cells and Treg. These efforts may uncover the potential reasons for discrepancies in conclusions about $CD19^+$B cells obtained from experimental models and clinical research.

This study indicates the formation of a closed loop connecting breast cancer, chronic inflammation, and the immune status of the body. Although malignant tumor primarily originates from genetic mutations, but it prompts changes in the body's microenvironment. These changes then trigger chronic inflammation, which can further promote tumor progression. Both the tumor cells and chronic inflammation simultaneously cause damage to the body's immune function through various pathways. And suppression of immune function correspondingly accelerates the progression of tumors and diminishes the elimination of inflammation. Blocking any of the pathways on this closed loop may be a strategy to improve the therapeutic efficacy and prognosis of breast cancer patients. In particular, the development of bioimmunology highlights cellular immunotherapy as an important direction for breast cancer patients who respond poorly to conventional therapy. Nevertheless, there are limitations to our study. Firstly, this is a single-center study with a small sample size. To confirm our current conclusions, a large-sample multicenter clinical study is needed. Secondly, this study only collected venous blood once during the hospitalization of the patients for examination. However, it is important to note that the immune damage caused by chronic inflammation and tumors is a long-lasting process. Therefore, a dynamic follow-up is required to clearly observe this chronic damage process. Furthermore, long-term follow-up can also collect the survival status of the patients, thus aiding in determining the prognostic value of lymphocyte subsets and LMR in breast cancer patients. This work can serve as a foundation for further diagnostic studies.

Conclusion

The results of this paper revealed the role of immunosuppression and chronic inflammatory state in the transformation of normal epithelial cells of the breast into breast cancer, which is complementary to the mechanism of present-day breast cancer development. It showed that $CD3^+$T cells, $CD4^+$T cells, $CD8^+$T cells, $CD4^+/CD8^+$ ratio, DNT cells, NK cells, and LMR were significantly lower in the breast cancer group than in the group with benign breast lesions. In patients with lymph node metastasis and high

Ki-67 expression, $CD3^+$T cells, $CD4^+$T cells, $CD8^+$T cells or LMR appeared to be reduced to different degrees, suggesting that immunosuppression and chronic inflammation also have a promotional role in the proliferation of tumor cells and the escape of tumors from the primary site. Moreover, in clinically advanced patients, $CD4^+$/$CD8^+$ ratio and LMR decreased, while $CD8^+$T cells levels increased, indicating that different immune cells within the $CD8^+$T cells may play an inconsistent role at different stages of tumor progression. Suppressive $CD8^+$T cells will dominate when the tumor progresses to advanced stages, which will exacerbate the already existing immunosuppression. HER-2 (+) patients were found to exhibit prominent immune suppression, characterized by lower levels of $CD3^+$T, $CD4^+$T, and $CD8^+$T cells. This may be one of the reasons why patients with HER-2 overexpression have a more aggressive tumor status. Combining immunotherapy with blockade of HER-2 pathway may enhance treatment efficacy in these patients. However, the study found no significant correlation between the expression levels of lymphocyte subsets and LMR and histologic grade and pathology type. Additionally, $CD19^+$B cells were not found to have a significant correlation with breast cancer in this study. Further exploration on the role of $CD19^+$B cells in breast cancer can be conducted by starting from their mechanism in immune function and expanding the sample size. In conclusion, serum lymphocyte subsets and LMR are valuable for the early diagnosis of breast cancer and can provide insight into certain clinicopathological features of breast cancer. Therefore, the relationship between these factors and tumor characteristics in breast cancer patients deserves further study. By doing so, we can better understand the connection between breast cancer, the immune function of the body, and chronic inflammatory state. This knowledge may lead to the identification of brand-new therapeutic targets and offer alternative treatment options beyond the conventional approaches for breast cancer.

Acknowledgements The authors thank all the patients who participated in this study.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by HZ, YL, and GL. The first draft of the manuscript was written by HZ, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This study was supported by the Research Fund of the First Affiliated Hospital of Chongqing Medical University.

Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval The studies involving human participants were reviewed and approved by the institutional ethics committee of the First Affiliated Hospital of Chongqing Medical University, and all experiments were performed in accordance with relevant guidelines and regulations. As this was a retrospective non-interventional study, written informed consent was not required from participants in accordance with national legislation and institutional requirements, and the application for exempted informed consent for the study has been approved by the Ethics Committee. Oral informed consent was obtained from participants.

Consent to participate Informed consent was obtained from all individual participants included in the study.

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

References

Allavena P, Garlanda C, Borrello MG, Sica A, Mantovani A (2008) Pathways connecting inflammation and cancer. Curr Opin Genet Dev 18:3–10

Appay V, Douek DC, Price DA (2008) $CD8^+$ T cell efficacy in vaccination and disease. Nat Med 14:623–628

Balkwill F, Coussens L (2004) M. Cancer: an inflammatory link. Nature 431:405–406

Bianchini G, Gianni L (2014) The immune system and response to HER2-targeted treatment in breast cancer. Lancet Oncol 15:e58-68

Burugu S, Asleh-Aburaya K, Nielsen TO (2017) Immune infiltrates in the breast cancer microenvironment: detection, characterization and clinical implication. Breast Cancer Tokyo Jpn 24:3–15

Caligiuri MA (2008) Human natural killer cells. Blood 112:461–469

Cao Y, Prescott SM (2002) Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. J Cell Physiol 190:279–286

Chen EP et al (2014) Myeloid Cell COX-2 deletion reduces mammary tumor growth through enhanced cytotoxic T-lymphocyte function. Carcinogenesis 35:1788–1797

Chen J, Hu P, Wu G, Zhou H (2019) Antipancreatic cancer effect of DNT cells and the underlying mechanism. Pancreatol off J Int Assoc Pancreatol IAP Al 19:105–113

Chraa D, Naim A, Olive D, Badou A (2019) T lymphocyte subsets in cancer immunity: Friends or foes. J Leukoc Biol 105:243–255

Datta J et al (2016) Association of depressed anti-HER2 T-helper type 1 response with recurrence in patients with completely treated HER2-positive breast cancer: role for immune monitoring. JAMA Oncol 2:242–246

Dvorak HF (2015) Tumors: wounds that do not heal-redux. Cancer Immunol Res 3:1–11
Facciabene A et al (2011) Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T (reg) cells. Nature 475:226–230
Gerdes J, Schwab U, Lemke H, Stein H (1983) Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 31:13–20
Goff SL, Danforth DN (2021) The role of immune cells in breast tissue and immunotherapy for the treatment of breast cancer. Clin Breast Cancer 21:e63–e73
Gray M, Miles K, Salter D, Gray D, Savill J (2007) Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells. Proc Natl Acad Sci 104(35):14080–14085
Greten FR, Grivennikov SI (2019) Inflammation and cancer: triggers, mechanisms, and consequences. Immunity 51:27–41
Gu-Trantien C et al (2013) $CD4^+$ follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest 123:2873–2892
Hagemann T et al (2006) Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype. J Immunol Baltim Md 1950(176):5023–5032
Hu R-J, Liu Q, Ma J-Y, Zhou J, Liu G (2018) Preoperative lymphocyte-to-monocyte ratio predicts breast cancer outcome: a meta-analysis. Clin Chim Acta Int J Clin Chem 484:1–6
Inoue S, Leitner WW, Golding B, Scott D (2006) Inhibitory effects of B cells on antitumor immunity. Cancer Res 66:7741–7747
Janneh O et al (2009) Intracellular accumulation of efavirenz and nevirapine is independent of P-glycoprotein activity in cultured CD4 T cells and primary human lymphocytes. J Antimicrob Chemother 64:1002–1007
Koretzky GA (2010) Multiple roles of CD4 and CD8 in T cell activation. J Immunol Baltim Md 1950(185):2643–2644
Kurozumi S et al (2017) Significance of evaluating tumor-infiltrating lymphocytes (TILs) and programmed cell death-ligand 1 (PD-L1) expression in breast cancer. Med Mol Morphol 50:185–194
Lee J et al (2018) Allogeneic human double negative T cells as a novel immunotherapy for acute myeloid leukemia and its underlying mechanisms. Clin Cancer Res off J Am Assoc Cancer Res 24:370–382
Li SX et al (2019) Alteration of liver-infiltrated and peripheral blood double-negative T-cells in primary biliary cholangitis. Liver Int off J Int Assoc Study Liv 39:1755–1767
Liu F et al (2011) $CD8^+$ cytotoxic T cell and $FOXP3^+$ regulatory T cell infiltration in relation to breast cancer survival and molecular subtypes. Breast Cancer Res Treat 130:645–655
Lu Y et al (2019) Double-negative T cells inhibit proliferation and invasion of human pancreatic cancer cells in co-culture. Anticancer Res 39:5911–5918
Mahmoud SMA et al (2011) Tumor-infiltrating $CD8^+$ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol off J Am Soc Clin Oncol 29:1949–1955
Mahmoud S, Lee A, Ellis I, Green A (2012) $CD8^+$ T lymphocytes infiltrating breast cancer: a promising new prognostic marker? Oncoimmunology 1(3):364–365
Mamessier E et al (2011) Human breast cancer cells enhance self tolerance by promoting evasion from NK cell antitumor immunity. J Clin Invest 121:3609–3622
Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-related inflammation. Nature 454:436–444
Maxwell AJ et al (2018) Risk factors for the development of invasive cancer in unresected ductal carcinoma in situ. Eur J Surg Oncol J Eur Soc Surg Oncol Br Assoc Surg Oncol 44:429–435
Olkhanud PB et al (2011) Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting $CD4^+$ T cells to T-regulatory cells. Cancer Res 71:3505–3515
Ono M (2008) Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. Cancer Sci 99:1501–1506
Pikarsky E et al (2004) NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 431:461–466
Pollard JW (2004) Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 4:71–78
Rius J et al (2008) NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. Nature 453:807–811
Rodríguez LAG, Huerta-Alvarez C (2001) Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal antiinflammatory drugs. Epidemiology 12:88–93
Ruffell B, DeNardo DG, Affara NI, Coussens LM (2010) Lymphocytes in cancer development: polarization towards pro-tumor immunity. Cytokine Growth Fact Rev 21:3–10
Ruffell B et al (2012) Leukocyte composition of human breast cancer. Proc Natl Acad Sci USA 109:2796–2801
Schmidt M, Heimes A-S (2021) Immunomodulating therapies in breast cancer—from prognosis to clinical practice. Cancers 13:4883
Sica A, Schioppa T, Mantovani A, Allavena P (2006) Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. Eur J Cancer Oxf Engl 1990(42):717–727
Sica A, Allavena P, Mantovani A (2008) Cancer related inflammation: the macrophage connection. Cancer Lett 267:204–215
Stotz M et al (2014) The preoperative lymphocyte to monocyte ratio predicts clinical outcome in patients with stage III colon cancer. Br J Cancer 110:435–440
Sung H et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71:209–249
Tadmor T, Zhang Y, Cho H-M, Podack ER, Rosenblatt JD (2011) The absence of B lymphocytes reduces the number and function of T-regulatory cells and enhances the anti-tumor response in a murine tumor model. Cancer Immunol Immunother CII 60:609–619
To B, Isaac D, Andrechek ER (2020) Studying lymphatic metastasis in breast cancer: current models, strategies, and clinical perspectives. J Mamm Gland Biol Neoplasia 25:191–203
Trédan O et al (2013) Patients with metastatic breast cancer leading to $CD4^+$ T cell lymphopaenia have poor outcome. Eur J Cancer Oxf Engl 1990(49):1673–1682
Wang Q et al (2021) Changes in T lymphocyte subsets in different tumors before and after radiotherapy: a meta-analysis. Front Immunol 12:648652
Watari K et al (2014) Tumor-derived interleukin-1 promotes lymphangiogenesis and lymph node metastasis through M2-type macrophages. PLoS ONE 9:e99568
Wilcox RA et al (2009) Monocytes promote tumor cell survival in T-cell lymphoproliferative disorders and are impaired in their ability to differentiate into mature dendritic cells. Blood 114:2936–2944
Wu Z et al (2022) $CD3^+CD4^-CD8^-$ (double-negative) T cells in inflammation. Immune Disord Cancer Front Immunol 13:816005
Xu T et al (2017) The predictive and prognostic role of stromal tumor-infiltrating lymphocytes in HER2-positive breast cancer with trastuzumab-based treatment: a meta-analysis and systematic review. J Cancer 8:3838–3848
Yang J, Xu J, Ying E, Sun T (2019) Predictive and prognostic value of circulating blood lymphocyte subsets in metastatic breast cancer. Cancer Med 8:492–500
Yao J et al (2019) Human double negative T cells target lung cancer via ligand-dependent mechanisms that can be enhanced by IL-15. J Immunother Cancer 7:17

Yuan H et al (2013) Destructive impact of T-lymphocytes, NK and Mast cells on basal cell layers: implications for tumor invasion. BMC Cancer 13:1–5

Zacharakis N et al (2018) Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. Nat Med 24:724–730

Zamarron BF, Chen W (2011) Dual roles of immune cells and their factors in cancer development and progression. Int J Biol Sci 7:651–658

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