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Analysis of characteristics of peripheral blood lymphocytes in endometrial carcinoma: a single-center study based on five-year clinical data

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Abstract

Introduction This study analyzed and discussed the characteristics of peripheral blood lymphocytes (PBLs) in patients with endometrial carcinoma (EC) to explore the PBLs' clinical application value.

Methods This single-center case–control study analyzed the clinical data of patients with EC and uterine fibroids who underwent surgery at the First Affiliated Hospital of Chongqing Medical University between October 2018 and October 2023 retrospectively. The Center for Clinical Molecular Medical Detection of our hospital performed PBLs detection using flow cytometry, and recorded the detection results in the electronic medical records system. Between-group and subgroup comparisons of PBLs in patients with EC were analyzed using t-test or Mann–Whitney U test. The effect of surgery on PBLs in patients with EC was assessed using a paired t-test or the Wilcoxon signed rank test.

Results The immune function of patients with EC was significantly lower than that of healthy people ($P < 0.05$) and those with benign uterine diseases ($P < 0.05$) and was related to body mass index (BMI), hypertension, diabetes, and blood lipids ($P < 0.05$). In patients with EC, the PBLs counts decreased significantly after surgery ($P < 0.05$) and more kinds of lymphocytes were affected in the laparoscopic surgery group than in the open surgery group.

Conclusions With the decrease of PBLs counts, the immune status of patients with EC is impaired. Metabolic syndrome (Mets), including obesity, hypertension, diabetes, and high blood lipids, also affects the immune function of patients with EC. And for EC patients, the effect of laparoscopic surgery is greater than that of open surgery. PBLs has the potential to be one of indicator during the diagnosis and treatment of EC.

Trial registration This study was retrospectively registered by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (approval number K2023-578).

Keywords Endometrial carcinoma, Peripheral blood lymphocytes, Immune function, Surgery, Laparoscopic surgery

Introduction

Endometrial carcinoma (EC) is one of the main malignancies of the female reproductive system and most patients seek medical attention for clinical symptoms such as abnormal vaginal bleeding. Although EC was previously thought to be detectable at an early stage and to have a good prognosis, epidemiological data indicate

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that its incidence is increasing annually, with increasing rates of patients with metabolic diseases and occurrence at younger ages [1]. Although the exact cause of EC is unknown, obesity, hypertension, and diabetes are three high-risk factors, and comprehensive staged surgery is its main treatment.

Immune function is a key research topic and the detection of peripheral blood lymphocytes (PBLs), which were initially used to monitor immunodeficiency diseases such as acquired immune deficiency syndrome (AIDS), has been increasingly used in oncology to monitor patients' immune function and guide disease prediction, diagnosis, and treatment. Tumor cells use self-modification or metabolic changes to significantly reduce the ability of the immune system to monitor and kill tumor cells. Moreover, changes in the composition and function of various components of the tumor microenvironment reduce the ability of the immune system to effectively eliminate tumor cells and may promote cancer progression [2]. Immune escape plays an important role in the occurrence and development of EC. Because the counts and ratios of PBLs may reflect the number of immune cells in the tumor microenvironment and immune function, they may have therapeutic and prognostic value in cancer. To some extent, PBLs can reflect the immune function of the body. A 2022 case–control study involving noncancer patients proposed that natural killer (NK) cell in peripheral blood may predict Federation International of Gynecology and Obstetrics (FIGO) staging in patients with EC [3]. However, the relatively few studies that have examined PBLs in patients with EC are controversial. Based on case data from a single center, this study retrospectively analyzed the PBL counts and ratios of 146 patients with EC, who underwent surgery at the First Affiliated Hospital of Chongqing Medical University and assessed the immune function characteristics of patients with EC and how they were influenced by surgery.

Materials and methods

Clinical data

This study involved 146 patients with EC who were hospitalized at the Department of Gynecology at the First Affiliated Hospital of Chongqing Medical University from October 2018 to October 2023. The control group consisted of 180 healthy individuals and 112 patients with uterine fibroids.

The inclusion criteria for the EC group were as follows: 1) had pathologically confirmed endometrial carcinoma; 2) did not receive any preoperative radiotherapy or chemotherapy; and 3) had complete clinical and pathological data. The inclusion criteria for the healthy individuals were as follows: 1) underwent a physical examination at the health examination center of our hospital, and the results were normal; and 2) had immune function test results that were interpreted as normal immune function. The inclusion criteria for the uterine fibroid group were as follows: 1) no malignant lesions in the endometrium; and 2) having complete clinical and pathological data. The exclusion criteria for the study were as follows: 1) had an incomplete pathological diagnosis; 2) had other malignant tumors or precancerous lesions; and 3) had chronic inflammatory disease, immune system diseases (e.g., autoimmune diseases, acquired immunodeficiency syndrome), or other severe complications (e.g., severe infection, shock, and organ failure before treatment). Based on the guidelines on EC diagnosis and treatment (2021 edition) [4], all patients with EC underwent surgery to obtain tissue biopsies. Final histopathological diagnoses were based on the pathological reports provided by the Pathology Center of Chongqing Medical University. All patients underwent comprehensive staging surgery, and tissues, including the uterus, fallopian tube, and ovary were pathologically examined and staged based on the FIGO staging system (2009 edition).

The participants' complete medical records, including general information, laboratory reports, pathological reports, and PBL detection reports, were collected. Clinical data, including age, height, weight, menopausal status, pregnancy history, medical history (including hypertension and diabetes), and blood lipid levels, were collected. Because epidemiological studies indicate that in China, EC risk is highest in patients aged 55-<60 years [5], we used 55 years as the cutoff for age grouping. Body mass index (BMI) was calculated using the following formula: BMI = weight (kg)/height^2(m^2), with a BMI of >25kg/m^2 indicating overweight. Hence, 25 kg/ m^2 was set as the BMI cutoff for the statistical analysis. The reference ranges for serum triglycerides and total cholesterol were 0.35-1.70 mmol/L and 2.80-5.20 mmol/L, respectively. The above reference values were provided by the clinical laboratory department of our hospital. For the statistical analysis of the serological indicators, the upper limits of the normal reference ranges were used as cutoff values. This study was approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University and adhered to the principles of the Declaration of Helsinki. This study's reporting followed the STROBE guidelines [6].

Flow cytometric analysis

Two millilitres of fresh early morning fasting peripheral blood were obtained from patients, stored in the blood collection tubes, and sent to the Center for Clinical Molecular Medical Detection at the First Affiliated Hospital of Chongqing Medical University, which conducted the flow cytometry, to complete the analysis. All

Table 1 Clinical characteristics of the 146 patients with endometrial carcinoma

Variable	No. (%)
Age(years)	
<55	76 (52.1)
≥55	70 (47.9)
Menopause	
No	61 (41.8)
Yes	85 (58.2)
BMI(kg/m2)	
<25	83 (56.9)
≥25	63 (43.1)
Hypertension	
No	115 (78.8)
Yes	31 (21.2)
Diabetes	
No	126 (86.3)
Yes	20 (13.7)
Pathological type	
Endometrioid adenocarcinoma	121 (82.9)
Serous adenocarcinoma	8 (5.5)
Clear cell carcinoma	4 (2.7)
Unknown[a]	4 (2.7)
Carcinosarcoma	5 (3.4)
Mixed carcinoma of endometrium[b]	3 (2.1)
Mucinous carcinoma	1 (0.7)
FIGO stage[c]	
I	118 (80.8)
II	5 (3.4)
III	21 (14.4)
IV	2 (1.4)

[a] The unknown included 3 cases of atypical endometrial hyperplasia with cancer, and 1 case of endometrial atypical hyperplasia suspicious cancerous

[b] Mixed carcinoma included 2 cases of clear cell carcinoma - endometrial carcinoma, and 1 cases of clear cell carcinoma - serous carcinoma

[c] All patients were staged according to the FIGO 2009 version

the data were entered into the patient records in the electronic medical records system. A BD Canto II system was used for flow cytometry with a corresponding absolute count microsphere kit. The manipulation was performed according to the manufacturer's instructions.

Detection of absolute lymphocyte counts in peripheral blood [7], named "Body Immune Function Test" : 1) Using the reverse pipetting technique, pipette 50 µl of well-mixed EDTA-K2 anticoagulant whole blood into the bottom of the absolute count microsphere tube. Avoid blood touching the upper part of the tube wall. 2) Add 20 µl of BD Multitest 6-color TBNK reagent (BD Catalog No. 337166) into the bottom of the flow tube. 3) Cap the tube, vortex gently to mix for about 3 seconds. Incubate for 25 minutes in the dark at room temperature (20°C to 25°C). 4) Add 450 µl of hemolytic agent for flow cytometer to the tube. Cap the tube cap, and vortex gently to mix for about 15 seconds. Incubate for 10 minutes in the dark at room temperature (20°C to 25°C). 5) Vortex gently to mix for about 10 seconds. Samples were analyzed on the flow cytometer.

Detection of the CD4+T cell subsets, named "Ratio of CD4+T subsets": 1) Rewarm the EDTA-K2 anticoagulated peripheral blood sample and the required antibody at room temperature (20°C to 25°C). For each patient sample, label a BD Trucount tube with the sample identification number. 2) Add the appropriate antibodies (CD4-FITC, CD127-PE, CD183-APC, CD3-ECD, CD25-PC7, CD196-BV421) into the numbered tube in turn. 3) Take 70 µl well-mixed blood sample to the bottom of the tube. Avoid blood touching the upper part of the tube wall. Vibrate 2-3 times by the oscillator and incubate for 15 minutes in the dark at room temperature (20°C to 25°C). 4) Add 250 µl OptiLyse C 1x lysing solution to each tube. Shake and mix thoroughly and incubate for 15 minutes in the dark at room temperature (20°C to 25°C). 5) Add 2 ml diluent for blood cell analysis. Shake and mix

Table 2 PBLs comparison between healthy individuals and EC patients

	The healthy	Endometrial carcinoma	t/Z	*P* value
Body immune function test	*N*=180	*N*=146		
Total lymphocyte	2031±399.68	1754.09±581.41	4.893	**<0.001**
CD3+ lymphocyte	1420(1210.75,1637.75)	1229(1029.75,1565)	-4.366	**<0.001**
CD4+T cell	761.5(667.25,895.75)	778.5(593.25,964.25)	-0.159	0.874
CD8+T cell	483(380,644.75)	376(289.75,520.25)	-5.489	**<0.001**
CD19+B lymphocyte	217(168,282.75)	248.5(175.75,353.75)	-2.203	**0.028**
NK cell	289(202.25,406)	194(129,271)	-6.325	**<0.001**
CD4+/CD8+	1.57(1.2,2.01)	1.98(1.55,2.47)	-5.152	**<0.001**

This analysis compared differences between 180 healthy individuals and 146 EC patients for differences between groups (The body immune function test in our hospital' physical examination center only included the above items, so only the same cells were used for data analysis.) The data were compared using a t-test or a Mann-Whitney U test for normally distributed and non-normally distributed data, respectively. Differences between the sample and population means are represented by t or Z, and sample characteristics are expressed as $\bar{x}\pm s$ or M (P25, P75). $P < 0.05$ (in bold) was considered to be statistically significant

Abbreviations: *NK* Natural Killer cell, *PBL* Peripheral Blood Lymphocyte, *EC* endometrial carcinoma

Table 3 Analysis between patients with EC and uterine fibroids

	Age (years)	No. of endometrial carcinoma (%)	No. of uterine fibroids (%)	OR (95% CI)	X^2	P value
Before	<55	76 (52.1)	102 (91.1)	Reference	*1*[a]	<0.001
PSM	≥55	70 (47.9)	10 (8.9)	9.395 (4.545-19.419)		
After	<55	76 (88.4)	76 (88.4)	Reference	*0*[b]	1
PSM	≥55	10 (11.6)	10 (11.6)	1.00 (0.394-2.541)		

[a] The expected count of zero cells(0.0%) was less than 5. The minimum expected count was 34.73

[b] The expected count of zero cells (0.0%) was less than 5. The minimum expected count was 10.00

Abbreviations: *OR* Odds Ratio, *CI* Confidence Intervals

thoroughly, centrifuge at 1100rpm for 5 minutes, and discard the supernatant. Repeat twice. 6) Add 500 μl diluent for blood cell analysis. Shake and mix the suspended cells thoroughly. Samples were analyzed on the flow cytometer immediately.

Patients whose data were not available after surgery were included in the baseline analysis. Due to the limitation of retrospective collection of test results, the "Body Immune Function Test" of the healthy individuals involved only seven main lymphocytes, and the "Ratio of CD4+T subsets" test of some patients was not complete.

Statistical analyses

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 25.0 and GraphPad Prism version 9.5.0. Count data were expressed as Number (%) [N (%)]. The Shapiro-Wilk test was used to determine whether the data were normal distribution or not. Normally distributed data were represented by mean ± standard deviation and differences between groups were analyzed via t-test. Non-normally distributed data were represented by median (inter-quartile ranges) [M (P25, P75)], and differences between groups were analyzed by the Mann-Whitney U test. Propensity score matching (PSM) was used to reduce bias between groups. The data were matched using the 1:1 nearest neighbor matching method and the caliper value was set at 0.05. Before and after surgery data from the same patient with EC were compared using a paired t-test or Wilcoxon signed rank test, respectively. All *P*-values were

Table 4 PBLs comparison in patients with endometrial carcinoma and uterine fibroids (After PSM)

	Endometrial carcinoma	Uterine fibroids	t/Z	*P* value
Body immune function test	*N*=86	*N*=86		
Total lymphocyte	1728.72(1368.5,2172.25)	1868(1506.5,2270.25)	-2.17	**0.030**
CD3+ lymphocyte	1270.54±424.41	1479.91±420.09	3.251	**0.001**
CD4+T cell	770.5(593.25,985.25)	852(656,1134)	-2.32	**0.020**
CD8+T cell	384(279.75,502)	442.5(352.5,541.5)	-2.727	**0.006**
Double positive T cell	5(4,10)	7(4,11)	-2.022	**0.043**
Double negative T cell	71.5(46,100.25)	70.5(51.75,117.75)	-1.018	0.309
CD19+B lymphocyte	248.5(174.25,332)	243(176.25,326.5)	-0.155	0.877
NK cell	193.28(122,270.25)	181(104,270)	-0.711	0.477
NKT cell	43(20,74.5)	37(16,61)	-1.576	0.115
CD4+/CD8+	1.95(1.54,2.53)	1.96(1.57,2.33)	-0.17	0.865
Ratio of CD4+T subsets	*N*=39	*N*=82		
Th1/CD4+T	28.41±8.55	24.56±6.32	-2.785	**0.006**
Th2/CD4+T	39.18±10.01	42.56±11.66	1.555	0.123
Th17/CD4+T	13.43(11.02,15.71)	13.42(11,16.47)	-0.250	0.803
Treg/CD4+T	6.88(5.64,8.13)	7.06(5.66,8.22)	-0.017	0.987
Th1/Th2	0.7(0.54,1.02)	0.58(0.4,0.81)	-2.319	**0.020**

Notes: This analysis compared differences between 86 patients with EC (47 did not have data on CD4+ T subset ratios) and 86 patients with uterine fibroids (two did not have data on CD4+ T subsets). The data were compared using a t-test or a Mann-Whitney U test for normally distributed and non-normally distributed data, respectively. Differences between the sample and population means are represented by t or Z, and sample characteristics are expressed as`x±s or M (P25, P75). $P < 0.05$ (in bold) was considered to be statistically significant

Abbreviations: *PSM* Propensity Score Matching, *NK* Natural Killer cells, *NKT* Natural Killer T cells, *PBL* Peripheral Blood Lymphocyte, *EC* endometrial carcinoma

Table 5 Comparison of PBLs in patients of endometrial carcinoma and uterine fibroids (≥55 years old)

	Endometrial Carcinoma	Uterine Fibroids	t/Z	*P* value
Body immune function test	*N*=70	*N*=10		
Total lymphocyte	1754.33±541.69	2143.5±461.11	2.160	**0.034**
CD3+ lymphocyte	1265.96±373.9	1609.7±346.69	2.742	**0.008**
CD4+T cell	789.63±258.27	1092.7±348.93	3.317	**0.001**
CD8+T cell	363(293,522.25)	444(344.25,575.5)	-1.098	0.272
Double positive T cell	5.5(4,10.25)	11(4.75,28.75)	-1.927	0.054
Double negative T cell	67(47.5,94.5)	57.5(31.75,111.75)	-0.306	0.760
CD19+B lymphocyte	217.5(175,354)	324(252.5,373.75)	-1.819	0.069
NK cell	238.27±144.19	185.5±123.38	-1.100	0.275
NKT cell	28(12,57.25)	41(12.75,75.25)	-0.655	0.513
CD4+/CD8+	2.05(1.64,2.48)	2.5(1.64,3.7)	-1.135	0.256
Ratio of CD4+T subsets	N=45	N=10		
Th1/CD4+T	32.3±9.35	26.13±8	-1.930	0.059
Th2/CD4+T	35.25(29.36,44.6)	40.12(33.36,48.65)	-1.178	0.239
Th17/CD4+T	13.4±4.99	14.51±6.52	0.597	0.553
Treg/CD4+T	7.09±1.62	6.55±1.32	-0.986	0.328
Th1/Th2	0.85(0.66,1.11)	0.61(0.49,0.81)	-1.943	0.052

Notes: This analysis compared differences between older patients with EC and with uterine fibroids. The data were compared using a t-test or a Mann-Whitney U test for normally distributed and non-normally distributed data, respectively. Differences between the sample and population means are represented by t or Z, and sample characteristics are expressed as`x±s or M (P25, P75). *P* < 0.05 (in bold) was considered to be statistically significant

Abbreviations: *NK* Natural Killer cells, *NKT* Natural Killer T cells, *PBL* Peripheral Blood Lymphocyte, *EC* endometrial carcinoma

two-sided, and *P*-value < 0.05 indicated a statistically significant difference.

Results

Characteristics of PBLs in the lesional intima vs the normal intima

This retrospective study involved 146 patients with EC. The average age of the EC patients was 54.92±9.91 years. Most patients were at stage FIGO I and II and the main pathological type was endometrioid carcinoma (Table 1). To clarify the role of immune cells in EC, we investigated the PBLs between patients with EC and healthy individuals or patients with uterine fibroids.

The results of normal immune function detection in 180 healthy individuals were compared with those in EC patients. As shown in Table 2, the numbers of total lymphocytes, CD3+, CD8+T, and NK cells were significantly lower ($P < 0.001$), and the numbers of CD19+B lymphocytes ($P = 0.028$), and the ratios of CD4+/CD8+ ($P < 0.001$) were greater in EC patients than in healthy individuals.

Uterine fibroids are among the most readily available normal intima samples and they are benign hormone-dependent uterine tumors. This study included 112 patients who underwent surgery for uterine fibroids during the same period as the normal endometrial control group. After PSM to reduce inter-group bias (Table 3), the baseline analysis included 86 patients (Table 3). Analysis revealed significant differences in the numbers of total lymphocytes, CD3+, CD4+T, CD8+T, and double-positive T cells, as well as in the ratios of Th1/CD4+T and Th1/Th2 between patients with EC and those with uterine fibroids ($P < 0.05$, Table 4). To reduce the bias caused by screening older patients with EC after PSM, we compared the two groups of older patients again and found that the counts of total lymphocytes, CD3+, and CD4+T were significantly lower in the EC patients ($P < 0.05$, Table 5).

Factors influencing PBL in patients with EC

The PBLs of 146 patients with EC were grouped by age at diagnosis, menopausal status, BMI before treatment, hypertension or diabetes status, total cholesterol and triglyceride levels, pathological type, and FIGO stage. The analyses showed that the PBLs of patients with EC correlated with age, BMI, hypertension status, diabetes status, blood lipids, and pathological type (endometrioid adenocarcinoma and non-endometrioid adenocarcinoma) ($P < 0.05$, shown in Figure 1) but not with menopausal status or FIGO stage (data not shown).

The effect of surgery on PBLs in patients with EC

For the 146 patients with EC, post-surgery PBLs data were available for 79 patients, and the average duration for obtaining postoperative samples was 5.08±2.25 days. A comparison of the preoperative and postoperative results indicated that all kinds of T lymphocytes,

CD19+B lymphocytes, NK cells, and NKT cells were significantly lower after surgery ($P < 0.001$, < 0.001, < 0.001, and < 0.05, respectively). However, the ratios of CD4+ T cell subsets ($P > 0.05$), including the Th1/Th2, which increased relatively ($P < 0.10$), did not change significantly. A total of 59 patients underwent laparoscopic surgery, whereas 20 underwent open surgery (the choice of surgery type was random). In the laparoscopic group, all lymphocyte counts, except for those of NKT cells, decreased after surgery ($P < 0.05$). However, the ratios of CD4+ T subsets did not change significantly ($P > 0.05$). In the laparotomy group, after surgery, only the counts of total lymphocytes, CD3+, CD4+ T, CD8+ T, and double positive T cells were significantly different from those before surgery ($P < 0.05$, Table 6).

Discussion

PBLs characteristics in patients with EC

Gynecological tumor studies, including those by our group, have reported lymphocytes changes in patients with ovarian and cervical cancers. Here, we discussed the PBLs from EC patients. These changes indicated that patients with EC were in a state of immunosuppression. Moreover, the CD8+T lymphocytes (inhibitory T lymphocytes) count was lower in the EC group than in the healthy or uterine fibroid group. Whereas the Th1/Th2 ratio was greater, indicating that cellular immunity was more dominant in patients with EC and that anti-infection and anti-tumor capacities were stronger [8]. Additionally, some studies have investigated lymphocytes characteristics in patients with EC by comparing them with those of healthy individuals. Chang et al. reported that the number of CD8+T lymphocytes was decreased in the peripheral blood of patients with EC, while the number of CD4+T lymphocytes was significantly elevated, and that the CD8+T lymphocytes count was significantly greater in tumor tissues than in peripheral blood, whereas the CD4+/CD8+ ratio was significantly lower [9]. Pascual-Garcia et al. reported that in the peripheral blood, CD3+ lymphocytes count was lower than those in healthy donors, without a significant change in the proportion of CD8+T lymphocytes [10]. Furthermore, many studies have closely associated Tregs with EC occurrence and development [11, 12]. We found that in patients with endometrioid carcinoma, the Treg/CD4+ T ratio was markedly greater than that in people with non-endometrioid carcinoma, which led to the hypothesis that patients with estrogen-dependent endometrioid adenocarcinoma have worse immune suppression. Therefore, EC development may be associated with immunity. During EC treatment, dynamic immune function assessment can not only indicate therapeutic effects and disease prognosis but also guide optimal immunotherapy timing and treatment plan formulation. Additionally, before surgery, for patients with suspected benign uterine tumors and a preference for preserving fertility function, immune function may predict the presence of benign or malignant tumors to determine the best surgical strategy. However, current studies have not reached a unified conclusion about lymphocytes characteristics in patients with EC, mainly because most patients with EC are usually diagnosed at an early stage and PBLs are also affected by the patient's basic condition.

EC diagnosis requires an endometrial biopsy. However, intrauterine procedures may cause complications such as intrauterine adhesions and inflammation, which may affect fertility. Therefore, this invasive procedure is only suitable for patients with persistent clinical symptoms or endometrial thickening as indicated by ultrasound. However, ultrasound findings suggest that the specificity of endometrial thickening is limited [13]. Therefore, there are significant challenges in early EC screening and current guidelines do not recommend EC screening indicators, whereas CA125 and HE4, which are commonly used as gynecological tumor markers, have limitations when applied to EC [14]. Recent research indicates that CD8+ T lymphocytes have potential value in the early diagnosis of various cancers, including melanoma and lung cancer [15]. Moreover, Tregs and phosphatase and tensin homolog deleted on chromosome ten (PTEN) expression have been proposed as EC predictors [12], which indicates the potential value of PBLs as non-invasive markers for EC screening, especially when combined with other markers, such as tumor markers.

(See figure on next page.)

Fig. 1 PBLs comparison in different groups of patients with EC. Notes: The data were compared using a t-test or a Mann-Whitney U test for normally distributed and non-normally distributed data, respectively. The results with $P < 0.05$ were shown: Comparison of PBLs in EC patients between different age subgroups(**A**); Comparison of PBLs in EC patients between different BMI subgroups(**B**); Comparison of PBLs in EC patients between different HBP subgroups(**C**); Comparison of PBLs in EC patients between different DM subgroups(**D**); Comparison of PBLs in EC patients between different TC subgroups(**E**); Comparison of PBLs in EC patients between different TG subgroups(**F**); Comparison of PBLs in EC patients between different classification of pathology subgroups(**G**). Abbreviations: NK, Natural Killer cell; NKT, Natural Killer T cell; PBLs, Peripheral Blood Lymphocytes; HBP, High Blood Pressure; DM, Diabetes; TC, Total Cholesterol; TG, Triglycerides

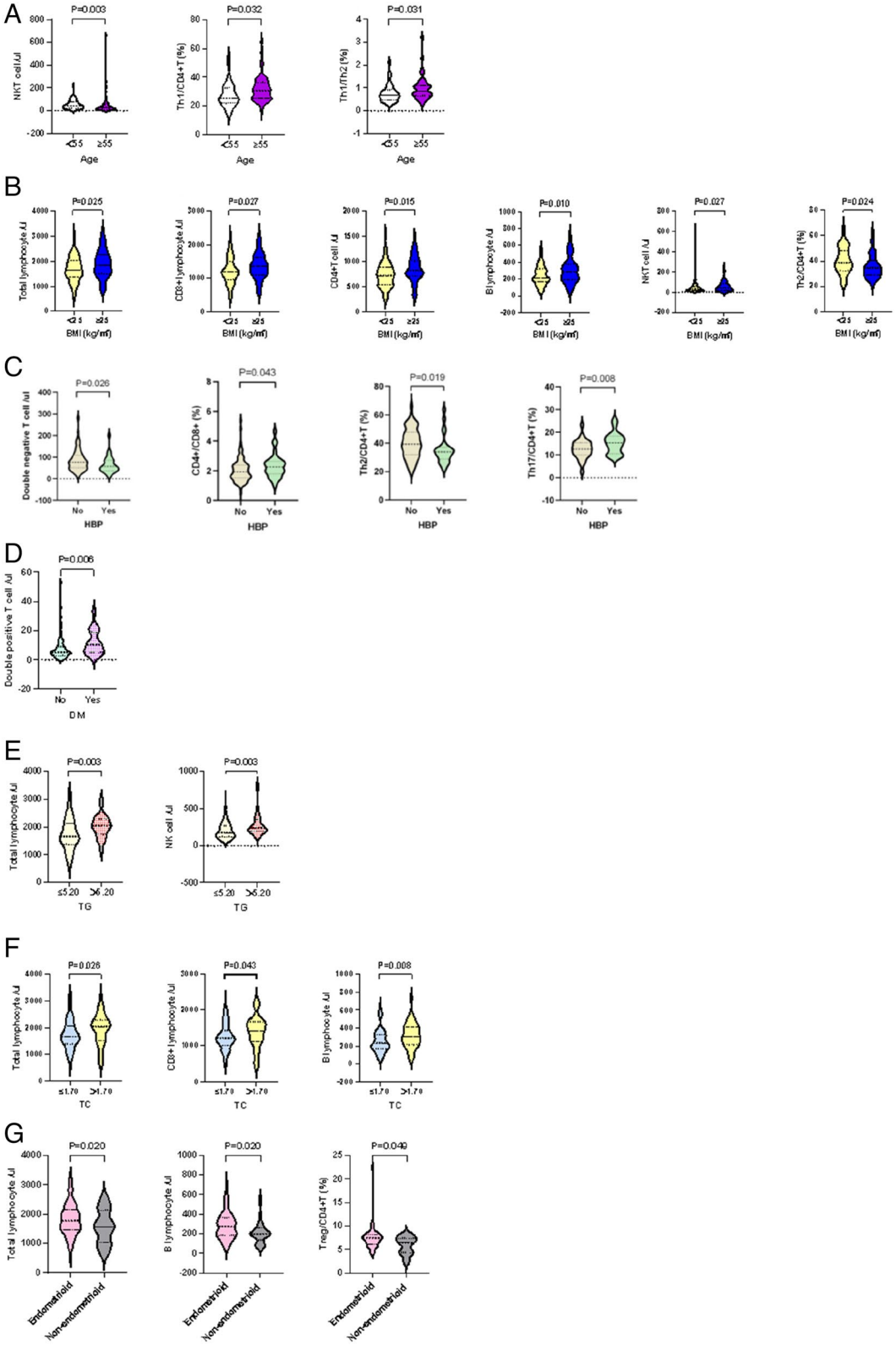

Fig. 1 (See legend on previous page.)

Table 6 PBLs comparison in patients with EC between before and after surgery

	Surgery			Laparoscopic surgery			Open surgery		
	Before operation	**After operation**	***P* value**	**Before operation**	**After operation**	***P* value**	**Before operation**	**After operation**	***P* value**
Body immune function test	*N*=79	*N*=79		*N*=59	*N*=59		*N*=20	*N*=20	
Total lymphocyte	1844.27±575.58	1411.9±487.12	**<0.001**	1871.44±571.98	1404.46±504.27	**<0.001**	1764.12±593.52	1433.85±444.08	**0.002**
CD3+ lymphocyte	1315.85±399.79	993.99±354.48	**<0.001**	1234(1101, 1584)	994(682, 1235)	**<0.001**	1223.12±373.93	978.1±289.11	**0.004**
CD4+T cell	820.67±269.99	614.41±236.44	**<0.001**	835.12±268	613.71±251.4	**<0.001**	778.05±278.27	616.45±191.24	**0.008**
CD8+T cell	430.41±176.27	330.49±145.51	**<0.001**	441.71±186.71	335.27±155.16	**<0.001**	397.05±139.91	316.4±114.69	**0.001**
Double positive T cell	6(4, 9)	4(2, 7)	**<0.001**	5(4, 9)	4(2, 7)	**0.003**	6(4, 7.75)	4.5(2.25, 6.75)	**0.006**
Double negative T cell	67(46, 98)	48(33, 71)	**<0.001**	77(51, 107)	50(34, 91)	**<0.001**	59.1±34.54	50.75±40.44	0.099
CD19+B lymphocyte	282.1±148.48	235.62±125.12	**<0.001**	257(182, 374)	202(126, 324)	**<0.001**	279.8±175.96	236.95±97.88	0.096
NK cell	240.56±146.26	175.34±120.35	**<0.001**	212(129, 302)	148(109, 207)	**<0.001**	183.28(135, 350)	164.5(115, 244)	0.070
NKT cell	35(16, 74)	29(15, 55)	**0.030**	34(15, 70)	25(14, 53)	0.053	38(19.75, 80.75)	31.5(18.25, 61.5)	0.262
CD4+/CD8+	2.1±0.81	2.04±0.82	0.165	2.13±0.87	2.03±0.9	0.081	2.02±0.64	2.05±0.57	0.631
Ratio of CD4+T subsets	*N*=38	*N*=38		*N*=28	*N*=28		*N*=10	*N*=10	
Th1/CD4+T	27.75(24.19, 33.02)	30.01(24, 34.36)	0.101	27.75(24.45, 32.82)	30.1(23.84, 34.07)	0.175	29.81±8.85	29.81±6.58	1.000
Th2/CD4+T	38.38±10.88	37.37±10.72	0.146	38.82±11.59	37.71±11.22	0.179	37.13±9	36.41±9.66	0.602
Th17/CD4+T	13.57±4.61	13.83±4.97	0.436	13.32±5.06	13.67±5.25	0.365	14.26±3.13	14.27±4.32	0.990
Treg/CD4+T	7.02±1.4	6.94±1.51	0.743	7.26±1.34	7.06±1.31	0.444	6.34±1.41	6.6±2	0.659
Th1/Th2	0.72(0.59, 1.03)	0.76(0.62, 1.09)	0.093	0.72(0.59, 1.03)	0.73(0.61, 1.08)	0.147	0.86±0.36	0.87±0.26	0.858

This analysis was on the basis of 79 EC patients (59 with laparoscopic surgery, 20 with open surgery) for differences between groups. The data were compared using a two-paired t-test or a two-paired Mann-Whitney U test for normally distributed and non-normally distributed data, respectively. Sample characteristics are expressed as $\bar{x}\pm s$ or M (P25, P75). $P < 0.05$ (in bold) was considered to be statistically significant

Abbreviations: NK Natural Killer cell, *NKT* Natural Killer T cell, *PBL* Peripheral Blood Lymphocyte, *EC* endometrial carcinoma

The relationship between immune function and metabolic syndrome in patients with EC

Obesity, diabetes, hypertension, and lipid metabolism disorders are the main clinical manifestations of metabolic syndrome (Mets). In recent years, many studies have associated Mets with the occurrence of various cancers, including EC [16], with studies showing that people with obesity [17–19], diabetes [20, 21], hypertension [22], or lipid metabolism disorders [23] have an increased relative risk of EC. We found that Mets also had an impact on immune function in patients with EC. A high BMI of EC patients was reported to be inversely correlated with CD8+T lymphocyte infiltration [24]. This study focused mainly on PBLs, which have not come such features. But we found the counts of CD3+, CD4+T, CD19+B lymphocytes, which were thought to be associated with infection, were greater in patients with obesity and EC. Chronic inflammation triggers endometrial carcinogenesis [25]. Patients with obesity have high levels of inflammatory factors, such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α(TNF-α), and the endometrium lacks protective immune cells [26]. Hence, people with obesity are more likely to have inflammation [27] and endometrial lesions, which can be reflected in their peripheral blood. Moreover, although lymphocytes were closely associated with the development of diabetes [28], hypertension [29], and hyperlipidemia [30], there is a lack of research on the relationship between lymphocytes and EC. Lymphocyte imbalance is known to lead to abnormal cytokine secretion and inflammation, which leads to disease progression [10, 31–33]. Therefore, from an immune function perspective, Mets may lead to an imbalance of immune function and an increased risk of EC. Moreover, these findings indicate that in addition to blood pressure, glucose, and lipids, PBLs can also be used to predict EC risk in patients on medication to control underlying disease. It is reported that improving the basic situation or immunotherapy may reverse the anti-tumor effects of CD8+T lymphocytes [24], which might have therapeutic target potential. Therefore, comprehensive treatment may prevent EC occurrence.

Effect of surgical treatment on the immune function of patients with EC

Surgery, which aims for complete surgical and pathological lesion staging, is the main treatment for EC. In this study, we found that patients who received surgical treatment had a significant reduction in the PBLs, indicating that in the short term, surgery affects immune function. Previous studies have shown that surgery can cause immunosuppression [34]. The reason is that peritoneal exposure to the gas (air or CO2) can stimulate the immune response of the body, promote the production of inflammatory factors such as TNF-α, and subsequently alter the number of lymphocytes such as Treg cells [35]. Hence, different surgical approaches affect PBLs differently. Because of its benefits, such as less tissue damage and fewer complications, surgeons and patients favor laparoscopy, which is minimally invasive. Early studies suggested that laparoscopic surgery causes less trauma, has a lower risk of infection, and better preserves postoperative immune function better [36]. In patients with benign uterine tumors who underwent hysterectomy, laparoscopic surgery had less influence on systemic immune function [37]. Currently, there are few reports on the immune effects of the surgical approaches for treating EC. This study revealed that more kinds of lymphocytes were significantly affected after laparoscopic surgery than after laparotomy. This suggests that in the short term, patients who undergo laparoscopic surgery may experience a greater impact on immune function. Our group previously analyzed the effect of surgical approaches on immune function in patients with cervical cancer and found that laparoscopy had a greater impact on immune function. Moreover, the use of a uterine lifting apparatus and CO_2 pneumoperitoneum might affect immune function in patients with cervical cancer [38]. Endometrial stimulation by the uterine lifting rod and CO_2 pneumoperitoneum might be why the immune system of patients with EC experiences stronger effects during laparoscopic surgery. Surgery can affect the immune function, and tumors may take the opportunity to burst after this immune attack. According to the results of this study, it could be considered that open surgery was more beneficial to the patients with EC, and it was more beneficial to the prognosis of those patients to minimize the impact on the patient's immune function as much as possible. However, further studies are needed to determine why immune cells, such as CD19+B and NK cells, were more likely to be inhibited during laparoscopic surgery [39, 40]. The immune function of malignant tumor patients is affected by many distinct factors, which may be the reason why this study produced different results from the previous results based on benign diseases. Moreover, the above-mentioned differences may also result from the experimental and lymphocyte detection methods. Assessing the correlation between different surgical approaches, immune function, and disease prognosis is expected to provide clinical data to support the need for a more appropriate surgical approach for treating EC.

In addition to the surgery, lesion reduction may also have an impact on immune function. Some studies have shown that tumor burden reduction may reduce or eliminate cancer-associated inhibitory factors and cells, thereby reducing immune suppression [41]. Changes in cytokine levels in the tumor microenvironment lead to a Th1-to-Th2 shift, resulting in Th1/Th2 imbalance, which is one of the determinants of tumor development

[42], but reduced tumor burden reduction helps improve this imbalance. In 2008, Zhang Aiwen et al. showed that in patients with EC, the Th1/Th2 ratio balance was gradually restored after tumor resection [31]. We found that in patients with EC, the Th1/Th2 ratio increased after surgery, which is consistent with the above-mentioned study, although the difference was not statistically significant. Th1 and Th2 cell-secreted cytokines, such as IL-2, tumor necrosis factor-α, interleukin-4, and interleukin-6, are also inflammatory factors. Therefore, the change in the Th1/Th2 ratio shown in this study also proves the role of inflammation in the occurrence and development of EC. The Th1/Th2 balance recovery also indicates enhanced cellular immunity and antitumor capacity recovery, which indicates cancer stability. Moreover, several cancer studies indicate that promoting Th1/Th2 balance recovery is also a potential therapeutic strategy [42–44]. Therefore, PBL levels could be used to monitor patient immune function and predict disease development, and this may also be a good entry point for therapeutic targets.

Conclusion

As a quick and easy way of assessing immune function, PBLs analysis is increasingly used in clinical practice. This study investigated the characteristics of immune function in patients with EC by comparing their PBLs with those of healthy individuals and patients with uterine fibroids. Our findings indicate that lymphocyte levels, such as BMI, lipid levels, and pathological classification, are significantly different between subgroups before and after treatment, which highlights the potential of using PBL detection for EC screening and treatment follow-up. In future studies, we will increase the sample size and follow-up duration to characterize patient PBL changes and provide clinical references for treatment and follow-up plans. Because this was a retrospective single-center study, there may be selection bias. Hence, prospective multicenter studies are needed to validate the conclusions of this study.

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Authors' contributions
T.Y. collected and analyzed the data collection, and was a major contributor in writing the manuscript. Z.Y., T.X., L.J., H.Q., and C.Y. collected and organized the data. Z.Q. was the consultant for peripheral blood lymphocyte detection. W.H. supervised the study and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All the data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (approval number K2023-578). This study was a retrospective study, the main study data were relevant clinical data, and written informed consent was exempted after evaluation by the ethics committee.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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