The Prognostic Significance of The Absolute Counts of Peripheral Blood Lymphocyte Subsets In Patients With Advanced Gastric Cancer

Guan Zhang
Tianjin University of Traditional Chinese Medicine
Ying Xia
Tianjin University of Traditional Chinese Medicine
Aqing Liu
Tianjin University of Traditional Chinese Medicine
Yanjie Yang
Tianjin University of Traditional Chinese Medicine
Wentao Li
Tianjin University of Traditional Chinese Medicine
Yunhe Liu
Tianjin University of Traditional Chinese Medicine
Jing Zhang
Tianjin University of Traditional Chinese Medicine
Qian Cui
Tianjin University of Traditional Chinese Medicine
Dong Wang
Tianjin University of Traditional Chinese Medicine
Xu Liu
Tianjin University of Traditional Chinese Medicine
Yongtie Guo
Tianjin University of Traditional Chinese Medicine
Huayu Chen
Tianjin University of Traditional Chinese Medicine
Jianchun Yu (yujianchun2000@163.com)
Tianjin University of Traditional Chinese Medicine

Research Article

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Abstract

Background: At present, the percentages of lymphocyte subsets (PL) which mainly include $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, B and NK cells are widely focused in tumor clinic, but the absolute counts of lymphocyte subsets (ACL) are seldom concerned yet. The clinical significance and value of ACL are not clear. It is still unclear whether the immune status of advanced gastric cancer (AGC) patients, especially the PL and ACL of peripheral blood, are closely related to the disease progression and prognosis.

Methods: The research was a retrospective cohort study, which including 291 patients with untreated AGC and 63 normal controls (NCs). The PL and ACL of peripheral blood were detected by flow cytometry based single-platform method. The end points were progression free survival (PFS) and overall survival (OS).

Results: Compared to NCs, the percentages of $CD3^+$, $CD3^+CD4^+$ and NK cells were no significantly different, but that of $CD3^+CD8^+$ and B cells were decrease in all AGC patients (AGCs) obviously ($p = 0.022$; $p = 0.004$, respectively). The AC of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, B and NK cells were significantly lower than those in the NCs ($p < 0.001$). The percentages of $CD3^+CD8^+$ and B cells in AGCs with stage Ⅲ were lower than those in NCs ($p = 0.032$; $p = 0.036$, respectively). But, the AC of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, B and NK cells in AGCs with stage Ⅲ were all significantly decrease than that of NCs ($p < 0.001$). Compared with normal controls, only the percentage of B cells in AGCs with stage IV was lower ($p = 0.005$), while the ACL in AGCs with stage IV were all significantly lower ($p < 0.001$). There was no significant difference in the percentage between stage Ⅲ and Ⅳ, but the ACL in AGCs with stage Ⅳ were significantly lower than those in stage Ⅲ ($p \leq 0.001$). The results showed that the ACL in AGCs were significantly impaired and were closely related to the progression of the disease. The Binary logistic regression and Kaplan-Meier showed that patients with high ACL had longer PFS and OS than those with low ACL ($p < 0.0001$, respectively). Multivariate analysis showed that when the number of $CD3^+CD4^+$ cells were more than 405 cells/μL, the PFS and OS of AGCs were significantly prolonged (HR 0.192, 95% CI [0.092 to 0.398], $p < 0.001$; HR 0.196, 95% CI [0.093 to 0.411], $p < 0.001$, respectively), and the sensitivity and specificity were the most obvious.

Conclusion: The ACL in AGCs were significantly impaired, and were closely related to PFS and OS. Thereinto, the AC of $CD3^+CD4^+$ cells was the most sensitive and specific parameter for the prognosis of AGCs and have more important clinical value.

Chinese Clinic Trial Registry number: ChiCTR-IOR-17014139; Registry date: 2017/12/25.

Introduction

As the fifth most common cancer and the third leading cause of cancer death in the world, the incidence of gastric cancer (GC) is gradually showing a younger trend, and the mortality rate is very high[1, 2]. Human immune function plays an important role in the occurrence and progression of cancer[3, 4]. In the process of tumor immunity, besides the coordination of many kinds of immune cells[5], a large number of immune cells is needed to fight against cancers. Tumor-infiltrating lymphocytes have great value and attract much attention[6], but it is difficult to obtain and apply in clinical diagnosis and treatment. Peripheral blood

circulating lymphocytes can not only reflect the general immune status and response of patients[7], but also easy to access. According to the biological function and the expression of cell surface antigens[8], human peripheral blood lymphocytes can be divided into three main groups, including T lymphocytes ($CD3^+$), B lymphocytes ($CD19^+$) and natural killer (NK) lymphocytes ($CD16^+CD56^+$). The T lymphocytes can be mainly divided into two subsets[9]: $CD4^+$ and $CD8^+$. Studies have shown that the absolute count (AC) of $CD3^+CD4^+$ lymphocytes is mainly used for HIV virus monitoring, and HIV carriers show a continuous decline in the AC of $CD3^+CD4^+$ lymphocytes during infection[10]. Percentages or AC of $CD3^+CD4^+$ and total T and B lymphocytes are used to determine and monitor certain kinds of immunodeficiency or autoimmune diseases[11]. NK cells ($CD16^+CD56^+$) are cytotoxic to tumor cells and other cells infected by viruses[12]. The cytotoxicity which mediated by NK cells doesn't require the expression of class I or class II major histocompatibility complex (MHC) molecules on the target cells[13]. However, the impact of the absolute counts of lymphocyte subsets (ACL) on the prognosis of patients with AGC is not clear, and less attention has been paid to it. Our previous study suggested that the immune function of patients with non-small cell lung cancer was impaired, showing decrease in the ACL significantly. Moreover, patients with high baseline AC of $CD3^+CD4^+$ cells (> 502 cells/μL) had longer PFS[14]. Hence, accurate assessment of the ACL in patients with AGC may have important reference significance for clinical diagnosis, treatment and prognosis of patients with solid tumors.

Now, chemotherapy, immunotherapy and immune response of vaccine have been studied in AGC patients (AGCs)[15-17]. Nevertheless, the ACL in AGCs in the clinical prognosis evaluation research are unknown[18]. Moreover, some AGCs had not benefit from these treatments[19]. The prognosis of GC is not only related to the pathological characteristics, tumor progression, metastasis and treatment[20], but also related to the percentages and AC of peripheral blood lymphocyte subsets. In this study, the percentages of lymphocyte subsets (PL) and ACL in peripheral blood of AGCs and NCs were detected by flow cytometry using single platform technology, and analyzed the relationship between lymphocyte subsets and prognosis. The report is as follows.

Materials And Methods

Study design

All subjects were given the informed consent in accordance with the Declaration of Helsinki and the clinical trial was approved by the Clinical Research Ethics Committee of First Teaching Hospital of Tianjin University of Traditional Chinese Medicine (TYLL2017[K]002) and registered at Chinese Clinic Trial Registry (ChiCTR-IOR-17014139). Total 387 patients with GC were enrolled in the First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China, from January 1, 2010 to October 31, 2020, and followed up for half a year. The date cut-off was April 30, 2021. After screening according to inclusion criteria, 291 AGCs and 63 age-matched NCs were included in the study, the flow diagram of patient inclusion and analysis procedures in the study showed in Fig.1. Peripheral blood was collected from the subjects before the treatment, and the PL and ACL in AGCs at stage □ and stage □ and NCs were assayed by flow cytometry (BD FACS Canto II: U6573380-00541, USA) using a lyse/no-wash procedure based on a

single-platform technique, the known number of fluorescence labelled beads in BD Trucount tube was used as the internal reference for absolute count assay. The PL and ACL in AGCs and NCs, and in AGCs of stage and stage were compared. The primary endpoints of outcome were PFS and OS. The 291 AGCs included 206 male and 85 female with a median age of 66 years (range, 34-80). The characteristics of AGCs were showed in Table 1. The 63 NCs included 44 males and 19 females with a median age of 64 years (range, 39-78). There was no significant difference in gender and age between AGCs cohort and NCs cohort ($p > 0.05$).

Table 1 The baseline characteristics of AGCs

Characteristics	N	%
Age (years)		
≤66	149	51.2
66	142	48.8
Sex		
Male	206	70.8
Female	85	29.2
Family history		
Yes	185	63.6
No	106	36.4
Past medical history		
Yes	128	44
No	163	56
Smoking history		
Yes	188	64.6
No	103	35.4
Drinking history		
Yes	139	47.8
No	152	52.2
Adenocarcinoma		
Yes	236	81.1
No	55	18.9
Differentiated degree		
Low	252	86.6
Medium or High	39	13.4
Clinical stage		
	124	42.6
	167	57.4
Lymph node metastasis		
Yes	196	67.4

No	95	32.6
CD3+ cell proportion (%)		
Low (≤69)	147	50.5
High (>69)	144	49.5
CD4+ cell proportion (%)		
Low (≤40)	150	51.5
High (>40)	141	48.5
CD8+ cell proportion (%)		
Low (≤23)	155	53.3
High (>23)	136	46.7
B cell proportion (%)		
Low (≤9)	162	55.7
High (>9)	129	44.3
NK cell proportion (%)		
Low (≤15)	153	52.6
High (>15)	138	47.4
CD3+ cell absolute count (cells/μL)		
Low (≤625)	129	44.3
High (>625)	162	55.7
CD4+ cell absolute count (cells/μL)		
Low (≤405)	167	57.4
High (>405)	124	42.6
CD8+ cell absolute count (cells/μL)		
Low (≤215)	146	50.2
High (>215)	145	49.8
B cell absolute count (cells/μL)		
Low (≤77)	146	50.2
High (>77)	145	49.8
NK cell absolute count (cells/μL)		

Low (≤140)	148	50.9
High (>140)	143	49.1

NOTE. Date cutoff: April 30, 2021.

All data were collected when subjects were first enrolled in the study. The cutoff points of lymphocyte subsets were in Table 2.

Patients

The AGCs recruited must met the inclusion criteria: 1. had clear pathological and immunohistochemical diagnosis[21]; 2. were not accompanied by other malignant tumors[22]; 3. had complete clinical and laboratory data; 4. had no serious diseases of heart, liver, kidney, brain, hematopoietic system and immune system; 5. The expected survival of AGCs should be more than 6 months. The exclusion criteria were the following conditions: 1. with undetermined AGC diagnosis or GC with stage I or II; 2. with other malignant tumors; 3. with incomplete clinical and laboratory data; 4. the survival time was less than 6 months (Fig.1). All examinations of the NCs enrolled were normal, including physical examination, blood routine, liver function, renal function and blood glucose.

Assay of lymphocyte subsets by Flow cytometry

The PL and ACL in the peripheral blood of the subjects were detected by a ten-color flow cytometer (BD FACS Canto II: U6573380-00541). The reagents were BD Multitest IMK kit (Catalog NO: 662965) containing CD3/CD8/CD45/CD4 (FITC-labeled CD3, PE-labeled CD8, PerCP-labeled CD45, APC-labeled CD4), and CD3/CD16^{+}CD56/CD45/CD19 (FITC-labeled CD3, PE-labeled CD16, PE-labeled CD56, PerCP-labeled CD45, and APC-labeled CD19) and BD Multitest IMK Kit Lysing Solution (Catalog NO: 91-1087). The EDTA blood collecting tubes and BD Trucount tubes (Catalog NO: 340334) were also purchased from BD Biosciences, USA.

Sample collection, cellular staining and analyzing

Two milliliters of fresh peripheral blood of 354 subjects were collected using a BD Vacutainer EDTA blood collection tube, and peripheral blood lymphocyte subsets were stained and analyzed according to the instructions of BD Multitest IMK kit.

1. For each patient sample, labeled A and B to two BD Trucount Tubes;

2. Pipette 20 μL of BD Multitest CD3/CD8/CD45/CD4 and CD3/CD16^{+}CD56/CD45/CD19 reagents into the bottom of each tube labeled A and B without touching the bead pellet, respectively;

3. Reverse pipetting method was used to draw 50 μL of well-mixed, anticoagulated whole blood onto the side of the tube just above the metal retainer;

4. Cap the tubes and gently rotated to mix the antibody and the sample;

5. The mixture was placed in a dark place for incubation for 15 minutes at a room temperature of 20°C-25°C;

6. Add 450 μL of 1× BD Multitest IMK Kit Lysing Solution to each tube, covered the tube and gently shook the tube until the liquid was uniform;

7. Incubated the tube in the dark at a room temperature of 20-25 ℃ for 15 minutes;

8. Analyzed on a ten-color flow cytometer.

A known volume of sample was stained directly in a BD Trucount Tube. The lyophilized pellet in the tube dissolves, releasing a known number of fluorescent beads. During analysis, the absolute number (cells/) of gated cells in the sample can be determined by comparing cellular events to bead events[23, 24]. During the analysis, the absolute number of positive cells (cells per microliter) in the sample can be determined using BD FACS Canto-specific BD clinical software. This is the absolute count formula of cells.

$$\text{cell absolute count } (\text{cells}/\mu\text{L}) = \frac{Acquired\ \ cells\ \ events \times Total\ \ Beads}{Acquired\ \ beads \times Voulme\ \ of\ \ sample}$$

Statistical analysis

The differences in PL and ACL between AGC and NCs, and AGCs between stage □ and □ were analyzed by two independent samples t test. The survival rate was calculated by Kaplan-Meier method. PFS was defined as the time from the onset of study enrollment to the disease progression or death in AGCs. OS was defined as the time from the start of the study to death from any cause. Patients who were still alive were reviewed at the last available follow-up. The cut-off value was calculated by ROC curve. Univariate analysis and multivariate analysis were used to analyzed the factors affecting disease progression. Variables with p□0.05 in univariate analysis were entered for multivariate analysis. Log-rank test was used for univariate analysis and proportional hazards regression model (COX model) was used for multivariate analysis. P□0.05 was considered statistically significant. The data were analyzed by SPSS 25. 0 software and plotted by GraphPad Prism 9. 00 software.

Results

Comparison of lymphocyte subsets between AGCs and NCs

The PL and ACL in AGCs and NCs were detected and compared. Compared with NCs, the PL of AGCs were different only in $CD3^+CD8^+$ and B cells (p= 0.022; p= 0.004, respectively), while the percentage of $CD3^+$, $CD3^+CD4^+$ and NK cells showed no significant difference (p >0.05) (Fig. 2a). Whereas, the ACL in AGCs were all significantly decreased (p□0.001) (Fig. 2b). More importantly, when analyzing relationship between PL, ACL and clinical stage, only the percentage of $CD3^+CD8^+$ and B cells in PL were different in

AGCs with stage III compared with NCs (p= 0.032; p= 0.036, respectively) (Fig. 2c), while the ACL in AGCs with stage III were all significantly different from that of NCs (p□0.001) (Fig. 2d). When compared AGCs with stage □ to NCs, only the percentage of B cells in AGCs was different (p = 0.005) (Fig. 2e), the ACL of AGCs was all significantly different from that of NCs (p□0.001) too (Fig. 2f).

Comparison of PL and ACL in AGCs between stage □ and stage □

The PL had no significant difference in AGCs between stage □ and stage □ (p > 0.05) (Fig. 2g). However, the ACL in AGCs between stage □ and stage □ decreased significantly (p□0.001; p□0.001; p□0.001; p□0.001; p = 0.001, respectively) (Fig. 2h). This results suggested that the decrease of ACL in AGCs were more significant and sensitive to reflect the injury of lymphocyte subsets than PL. Only attention was paid in clinical practice, it could lead to clinical misjudgment and affect treatment. The PL represents the proportion and composition of lymphocyte subsets[25], which direct reflecting their development and differentiation function, while ACL was exact number, direct reflecting their proliferation ability[26]. These results suggested that the impaired proliferation of lymphocyte subsets in peripheral blood of AGCs may be an important reason for the progression of GC.

Prognostic impact of ACL on PFS

The PFS of AGCs with stage III was significantly higher than that of AGCs with stage IV (HR 0.26; 95%CI[0.18-0.38]; p□0.0001)(Fig. 3a). To analyzed the effect of ACL on PFS further, the cut-off points of AC of $CD3^+$ and $CD3^+CD4^+$ cells were analyzed and calculated by ROC curve (Table 2). Univariate, Multivariate analysis were also shown in Table 2. The cut-off points of $CD3^+$ and $CD3^+CD4^+$ cells were 625 cells/μL (sensitivity = 0.754, specificity = 0.769, Youden index = 0.523) and 405 cells/μL (sensitivity = 0.898, specificity = 0.647, Youden index = 0.546), respectively. The cut-off points of AC of $CD3^+CD8^+$, B and NK cells were analyzed and calculated by ROC curve, due to the area under the curve was less than 0.7, so we used the median method to calculated the cut-off points of AC of $CD3^+CD8^+$, B and NK cells: 215 cells/μL, 77cells/μL, 140cells/μL, respectively (Table 2). The AC of each subgroup of lymphocytes was high than the cut-off point, the PFS of AGCs was also significantly increased. The AC was low than the cut-off point, the PFS was also significantly decreased, which showing a significant positive correlation. The related results of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, B and NK cells were shown in Fig. 3b (HR 5.04; 95% CI [3.47-7.31]; p □0.0001), Fig. 3c (HR 9.25; 95% CI [6.44-13.26]; p□0.0001), Fig. 3d (HR 2.16; 95% CI [1.51-3.10]; p□0.0001), Fig. 3e (HR 2.05; 95% CI [1.43-2.95]; p□0.0001) and Fig. 3f (HR 2.39; 95% CI [1.67-3.43]; p□0.0001), respectively.

Thus, the more severe the progression of AGC and the distant metastasis, the lower the PFS. The ACL in peripheral blood was closely related to the prognosis of PFS of AGCs. The higher the ACL was, the better the prognosis was, otherwise, the prognosis was poor. This suggested that the number of lymphocyte subsets was the basis of normal immune function, which showed conducive to improving the PFS of patients.

Table 2 Univariate and Multivariate analysis in PFS and OS

Characteristics	Cutoff point (or median)	PFS				OS			
		Univariate		Multivariate		Univariate		Multivariate	
		P-value	HR	HR and 95% *CI*	P-value	P-value	HR	HR and 95% *CI*	P-value
Age (years)	66	0.271	1.225			0.380	1.176		
Sex		0.308	0.806			0.177	0.751		
Family history		0.025	1.581	1.603 (1.053 to 2.441)	0.028	0.061	1.465		
Past medical history		0.960	1.009			0.429	1.158		
Smoking history		0.726	1.071			0.503	1.141		
Drinking history		0.936	0.985			0.779	0.949		
Adenocarcinoma		0.772	0.935			0.872	1.038		
Differentiated degree		0.100	0.615			0.105	0.618		
Clinical stage		0.001	3.934	2.433 (1.513 to 3.911)	0.001	0.001	4.360	2.288 (1.411 to 3.710)	0.001
Lymph node metastasis		0.289	1.241			0.232	1.280		
CD3+ cell proportion (%)	69	0.375	1.178			0.187	1.279		
CD4+ cell proportion (%)	40	0.189	0.784			0.657	0.920		
CD8+ cell proportion (%)	23	0.004	1.712			0.014	1.578		
B cell proportion (%)	9	0.490	0.879	0.908 (0.560 to 1.474)	0.697	0.421	0.860	0.867 (0.537 to 1.399)	0.559
NK cell proportion (%)	15	0.509	0.885			0.267	0.813		
CD3+ cell absolute count (cells/μL)	625	0.001	0.192	0.659 (0.362 to 1.199)	0.172	0.001	0.190	0.636 (0.348 to 1.161)	0.141

CD4+ cell absolute count (cells/μL)	405	< 0.001	0.100	0.192 (0.092 to 0.398)	< 0.001	< 0.001	0.098	0.196 (0.093 to 0.411)	< 0.001
CD8+ cell absolute count (cells/μL)	215	< 0.001	0.462	0.969 (0.561 to 1.675)	0.910	< 0.001	0.411	0.973 (0.563 to 1.683)	0.922
B cell absolute count (cells/μL)	77	< 0.001	0.484	0.811 (0.539 to 1.220)	0.315	< 0.001	0.447	0.716 (0.475 to 1.079)	0.110
NK cell absolute count (cells/μL)	140	< 0.001	0.415	0.714 (0.461 to 1.106)	0.131	< 0.001	0.385	0.717 (0.465 to 1.107)	0.133

Prognostic impact of ACL on OS

Fig. 4a showed that OS was significantly high in AGCs with stage III than that in AGCs with stage IV (HR 0.25; 95%CI [0.18 to 0.36]; p<0.0001). Similar to PFS, AGCs with $CD3^+$ > 652 cells/μL (Fig. 4b, HR5.13; 95%CI [3.53 to 7.45]; p<0.0001), $CD3^+CD4^+$ > 405 cells/μL (Fig. 4c, HR9.61; 95%CI [6.70 to 13.80]; p< 0.0001), $CD3^+CD8^+$ > 215 cells/μL (Fig. 4d, HR2.41; 95%CI [1.68 to 3.46]; p<0.0001), B > 77 cells/μL (Fig. 4e, HR2.19; 95%CI [1.52 to 3.14]; p<0.0001) and NK > 140 cells/μL (Fig. 4f, HR2.57; 95%CI [1.79 to 3.69]; p< 0.0001), their OS were significantly longer than those of AGCs with lower ACL than cut-off point of their itself, respectively. Prognostic impacts of PL on PFS and OS were no significantly different (Data Supplement, Fig. 6).

The OS of AGCs was closely related to the ACL, so the maintenance of a certain number of lymphocytes could ensure the normal role of immune function.

Evaluation of ACL on the PFS and the OS of AGCs

In order to analyze and evaluate whether ACL and other variables affected the PFS, OS and progression of AGCs. Univariate analysis and Log-rank test were used to analyze the variables, and then the variables of p<0.05 were input to multivariate analysis for COX model analysis, and finally the forest plots were drawn. In Table 2 and Fig. 5a, $CD3^+CD4^+$ cells > 405 cells/μL (HR 0.192; 95%CI[0.092to0.398]; p < 0.001) was an independent protective factor for PFS, while clinical stage IV (HR 2.433; 95%CI[1.513to3.911]; p< 0.001) and family history (HR 1.603; 95%CI [1.053 to 2.441]; p= 0.028) was an independent risk factor for PFS. In Table 2 and Fig. 5b, $CD3^+CD4^+$ cells > 405 cells/μL (HR 0.196; 95%CI [0.093 to 0.411]; p< 0.001) was an independent protective factor for OS, while clinical stage IV (HR 2.288; 95%CI [1.411 to 3.71]; p = 0.001) was an independent risk factor for OS.

Discussion

In this study, the PL and ACL of peripheral blood in AGCs were analyzed by flow cytometry using single platform technique, which provide evidence for the prognostic value of the ACL in AGC. The results showed that the PL was not a sensitive indicator in the progression of AGC and could not reflect the immune damage of AGCs sensitively, while the ACL were significantly damaged in AGCs (Fig. 2h), which was negative correlated with the disease progression and had a good prognosis on PFS and OS. This indicated that both innate and adaptive immunity play an important role in the anti-tumor process[27], and the AC of immune cells plays a key role in the anti-tumor immune response process.

Therefore, detection of the ACL of patients will help clinicians to understand the overall immune function of patients, facilitate clinical decision-making and predict efficacy. The decrease in overall ACL in AGCs, resulting in reduced anti-tumor activity, indicated that impaired immune function could not prevent the progression of tumors[28]. Therefore, increasing the number of lymphocytes in AGCs is the first priority in clinical treatment, which may help to control or even delay the progression of solid tumors and prolong the survival of patients. B cells mediate humoral immune response[29, 30], and involved in regulating the functions of various immune cells, such as macrophages, dendritic cells, NK cells and T cells[31-33]. B cells also act as antigen presenting cells for uptaking, processing and presentation of soluble antigens[34]. NK cells have direct recognition, activation and cytotoxicity on tumor cells[35]. T cell mediate cellular immunity[36]. TCR-$CD3^+$ was a specific marker on the surface of T cells[37]. According to the difference of TCR, T cells can be divided into αβ T cells and γδ T cells[38]. According to the difference of function, αβ T cells can be divided into $CD4^+$ helper T cells (Th) and $CD8^+$ cytotoxic T cells (CTL)[39]. Th1 cells secrete IL-2, IFN-γ, LT-α and other cytokines to mediate cellular immune response[40]. Th2 secretes IL-4, IL-5, IL-6, IL-10, IL-13 and other cytokines to assist humoral immune response[41]. CTL induce target cell lysis and apoptosis by secreting perforin, granzyme, lymphotoxin and expressing Fas ligand[42]. Multivariate analysis showed that $CD3^+CD4^+$ cells > 405 cells/μL was the independent positive factor of PFS. Therefore, we should pay more attention to the absolute count of $CD3^+CD4^+$ cells in the clinical prognosis of AGCs. $CD3^+CD4^+$ cells exert their anti-tumor effects by dominating cellular immune response, assisting humoral immune response and promoting the activation, proliferation and effector function of CTL[43, 44]. $CD3^+CD4^+$ cells can directly proliferate and differentiate into $CD3^+CD4^+$ effector cells[45], and can also eliminate tumor cells by regulating the tumor microenvironment[46]. $CD3^+CD4^+$ cells can help B cells and CTL proliferation in secondary lymphoid organs[47]. Tumor antigens were taken up by dendritic cell (DC) and combined with MHC class I and MHC class II molecules respectively in DC to form complexes, which were expressed on the surface of DC[25]. P-MHC I (peptide-MHC complex 1) combined to $CD3^+CD8^+$ TCR to activate CTL precursor cells, Th cells were activated by P-MHC □ combining to $CD3^+CD4^+$ TCR[48]. CTL precursor cells proliferated and differentiated into CTL cells under the combined action of P-MHC I specific activation signals and cytokines released by Th cells[49]. Therefore, $CD3^+CD4^+$ cells play a commander role in the anti-tumor immune response, and its number may be the key to the anti-tumor immune response.

The PL represents the proportion of lymphocytes[25], and the ACL represents their respective cell numbers. In the long-term anti-tumor stress state of AGCs, it is very important to maintain the corresponding

proportion of different types of immune cells in the body, coordinate with each other and maintain the immune balance, which restricts the development of tumors. On this basis, a sufficient number of immune cells are needed to maintain the continuous killing effect on tumor cells.

Conclusion

The retrospective cohort study showed that the ACL in AGCs was significantly lower than that in NCs, which indicated that the impaired immune function of AGCs was closely related to the decrease in the number of lymphocyte subsets. By comparing the OS and PFS of AGCs between stage III and stage IV, the prognostic value of ACL in AGCs was demonstrated further. Moreover, when the AC of $CD3^+CD4^+$ cells > 405 cells/μL, it was an independent protective factor of AGCs. These results suggested that the AC of $CD3^+CD4^+$ cells is an important clinical predictor of AGCs, which provided an important theoretical basis for clinical diagnosis and immunotherapy of AGCs.

Declarations

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Author's contributions

Guan Zhang drafted the manuscript. Jianchun Yu conceived and designed manuscript. Ying Xia were responsible for data acquisition and interpretation. Aqing Liu performed the statistical analysis. Yanjie Yang, Wentao Li, Yunhe Liu and Jing Zhang verified the contents and revised the manuscript., Qian Cui, Dong Wang, Xu Liu, Yongtie Guo, Huayu Chen critically revised the manuscript. All authors reviewed and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All enrolled participants signed the informed consent. The present study involving human samples was approved by the Ethics Committee of the First Teaching Hospital, Tianjin University of Traditional Chinese Medicine (Tianjin, China) (TYLL2017 [K] 002, 25 December 2017) and was conducted in accordance with the Declaration of Helsinki. The clinical trial was registered at Chinese Clinic Trial Registry (ChiCTR-IOR-17014139).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

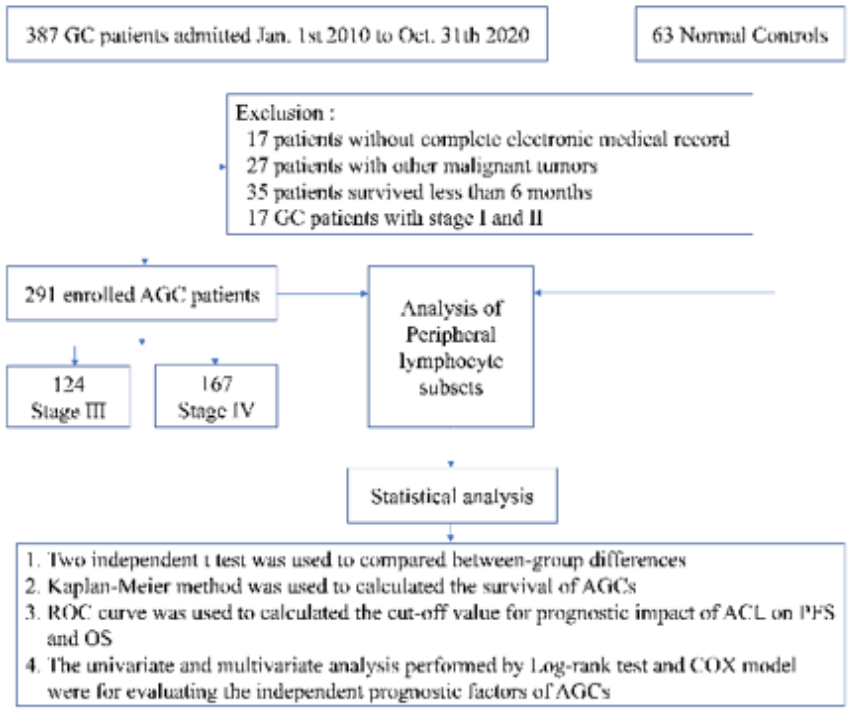

Figure 1

The flow chart of patient inclusion and analysis procedures in the study.

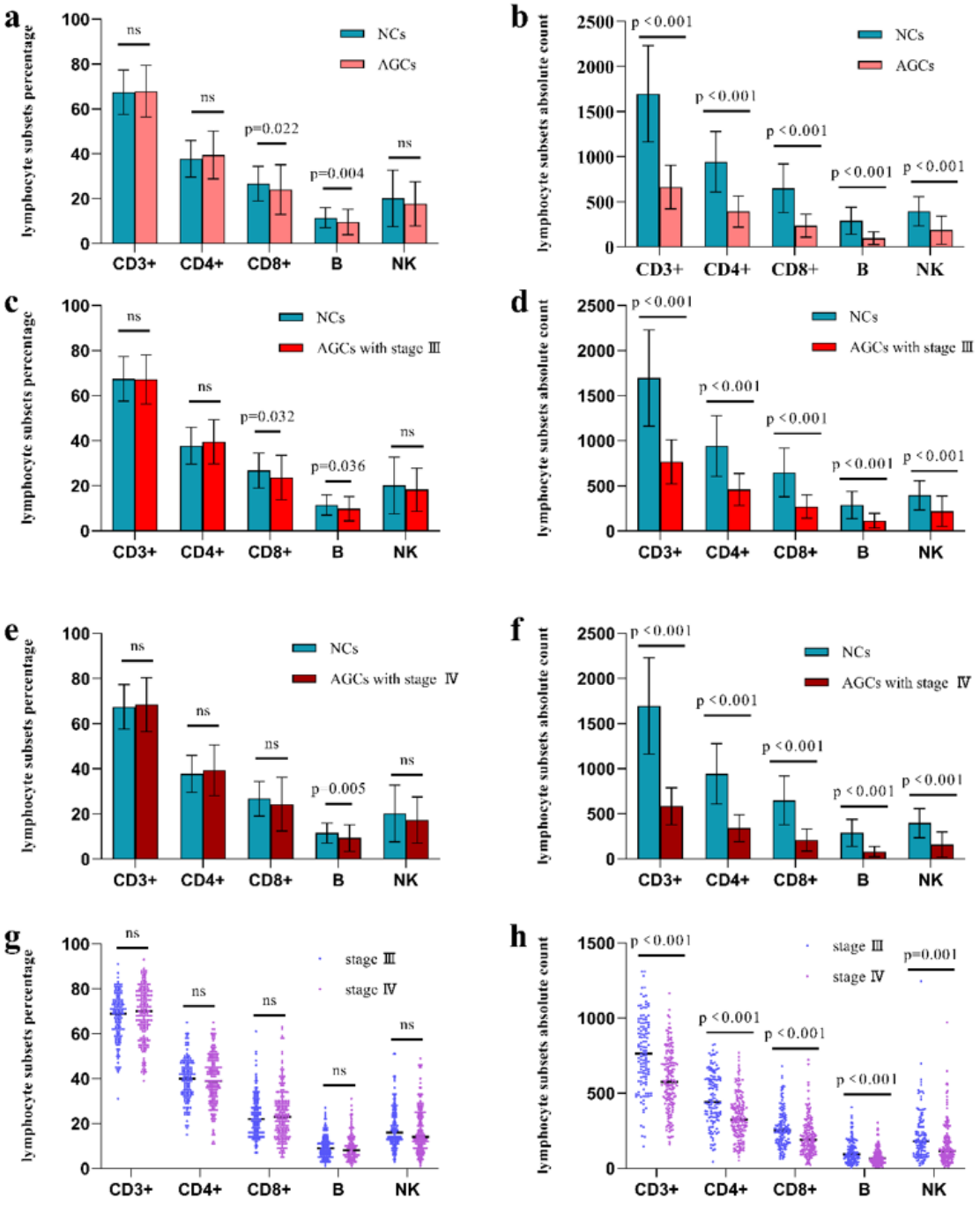

Figure 2

Comparison of PL and ACL between AGCs and NCs. The PL and ACL of peripheral blood in NCs and AGCs were analyzed by flow cytometry: a. The comparison of PL between NCs and AGCs; b. The comparison of ACL between NCs and AGCs; c. The comparison of PL between NCs and AGCs with stage Ⅲ; d. The comparison of ACL between NCs and AGCs with stage Ⅲ; e. The comparison of PL between NCs and AGCs

with stage Ⅲ; f. The comparison of ACL between NCs and AGCs with stage Ⅲ; g. The comparison of PL in AGCs between stage Ⅲ and stage Ⅳ; h. The comparison of ACL in AGCs between stage Ⅲ and stage Ⅳ.

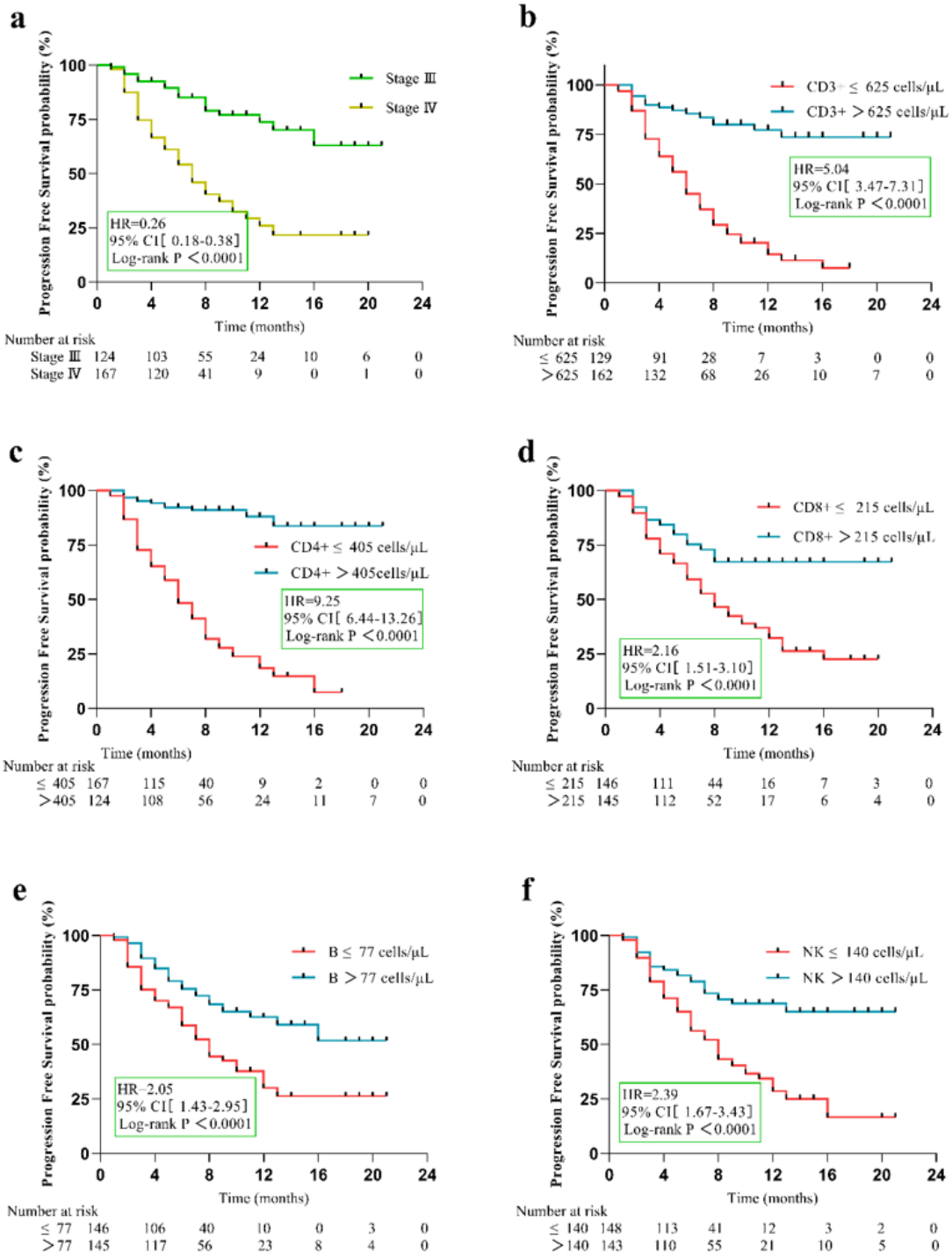

Figure 3

Prognostic impact of ACL on PFS: a. Impact of clinical stage on PFS; b. Impact of AC of CD3+ cells on PFS; c. Impact of AC of CD3+CD4+ cells on PFS; d. Impact of AC of CD3+CD8+ cells on PFS; e. Impact of AC of B cells on PFS; f. Impact of AC of NK cells on PFS.

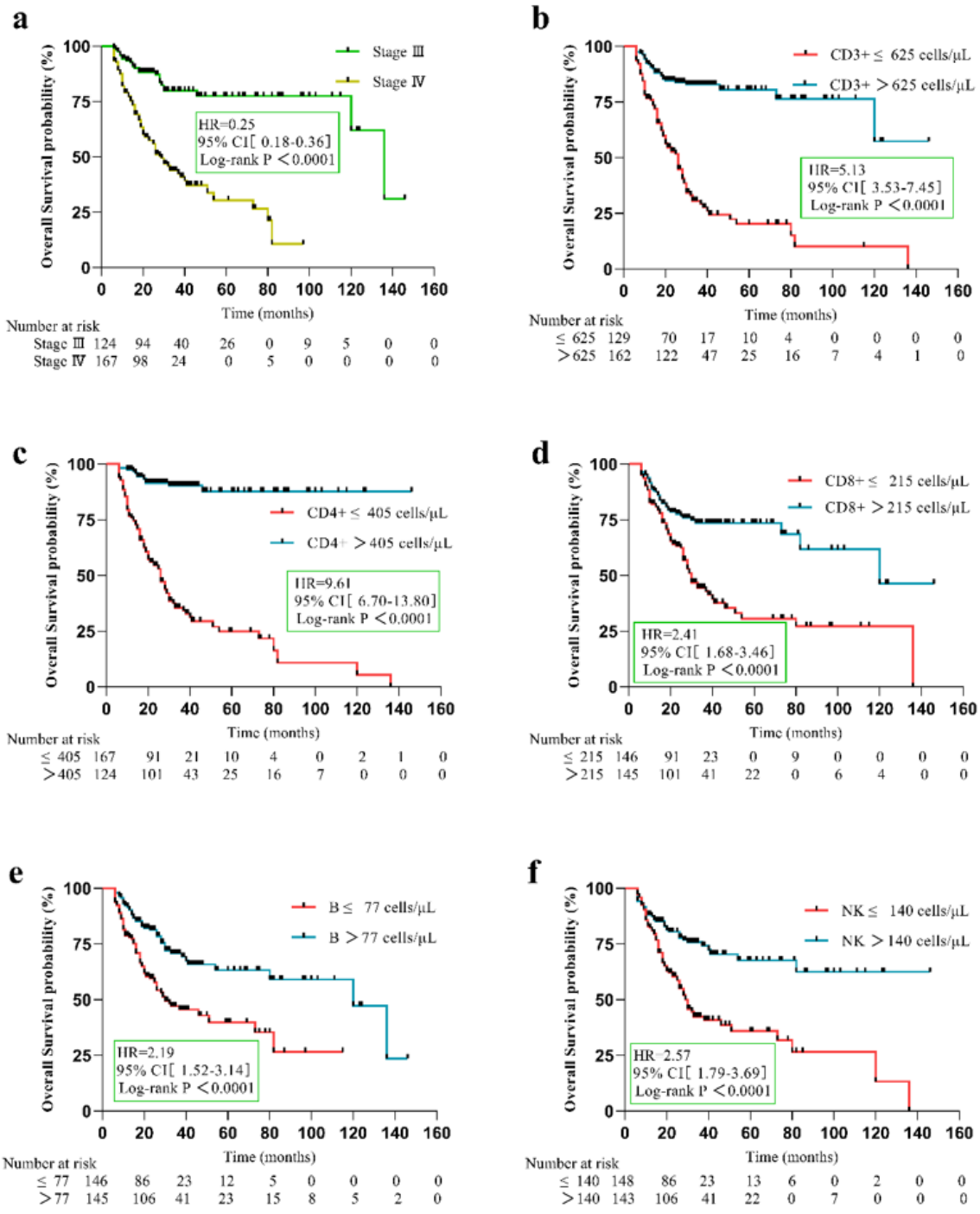

Figure 4

Prognostic impact of ACL on OS: a. Impact of clinical stage on OS; b. Impact of AC of CD3+ cells on OS; c. Impact of AC of CD3+CD4+ cells on OS; d. Impact of AC of CD3+CD8+ cells on OS; e. Impact of AC of B cells on OS; f. Impact of AC of NK cells on OS.

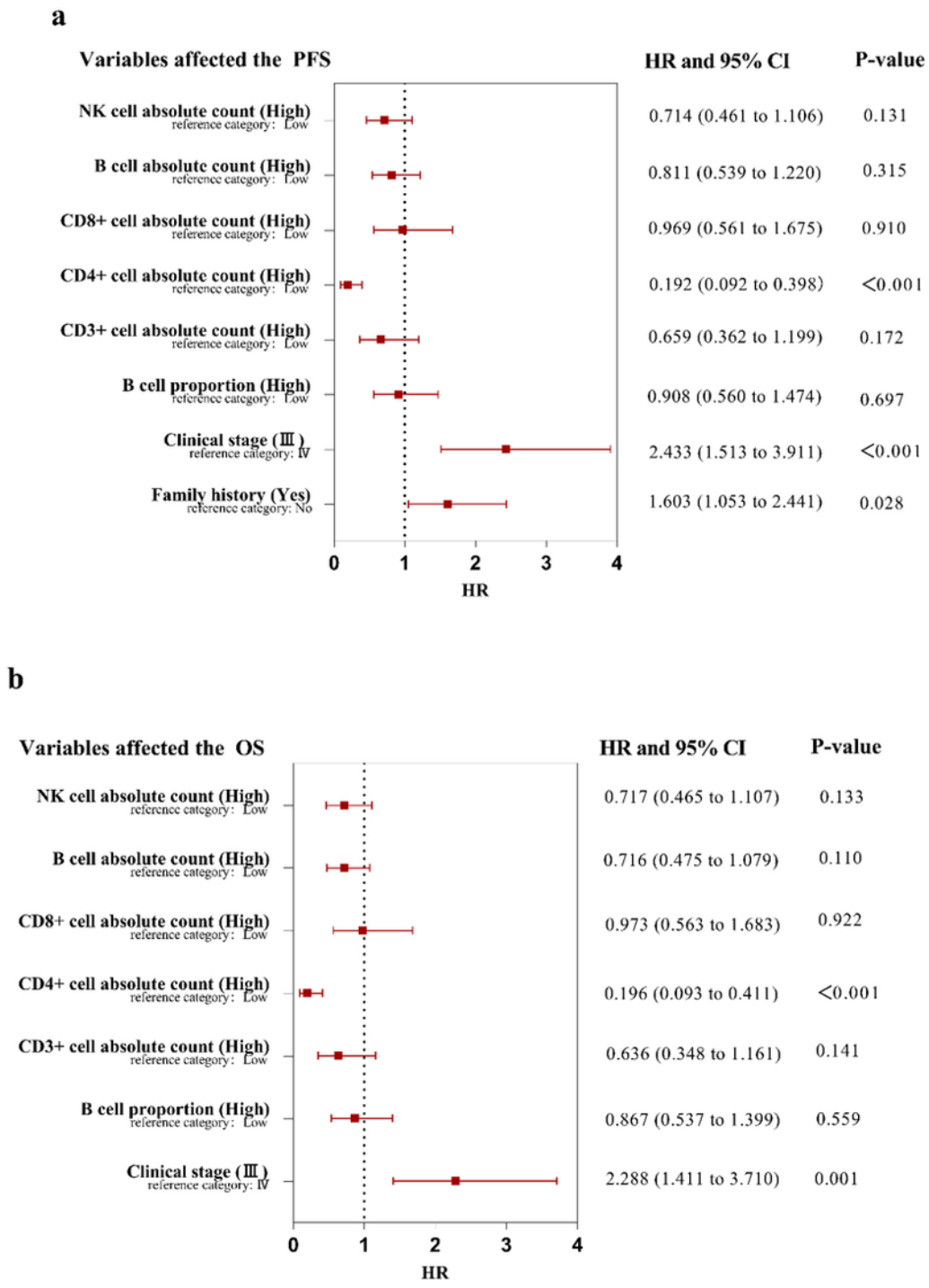

Figure 5

The forest plots of factors affected the PFS (a) and OS (b). HR□1 represented variable was considered a negative factor; OR□1 indicated variable was considered a positive factor.

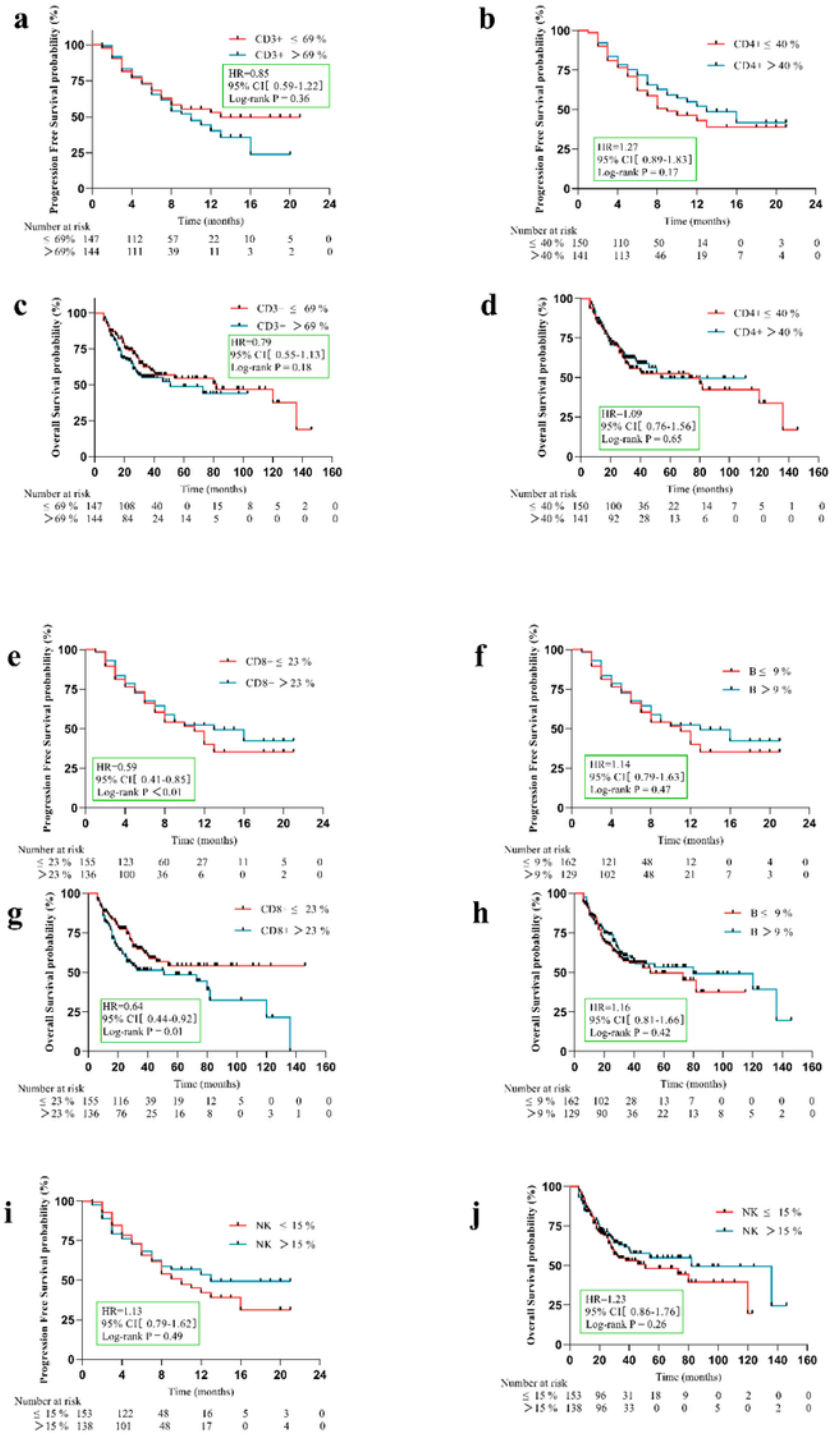

Figure 6

Data Supplement Prognostic impact of PL on PFS and OS: a. Impact of percentages of CD3+ cells on PFS; b. Impact of percentages of CD3+CD4+ cells on PFS; c. Impact of percentages of CD3+ cells on OS; d. Impact of percentages of CD3+CD4+ cells on OS; e. Impact of percentages of CD3+CD8+ cells on PFS; f. Impact of percentages of B cells on PFS; g. Impact of percentages of CD3+CD8+ cells on OS; h. Impact of

percentages of B cells on PFS; i. Impact of percentages of NK cells on PFS; j. Impact of percentages of NK cells on OS.