Original Article

Changes of proportions of circulating lymphocyte subsets in cancer patients after chemotherapy

Weimin Wang[1], Yun Wang[2], Zong Cao[3]

[1]Medical Oncology, Hefei Cancer Hospital, Chinese Academy of Sciences, Hefei, China; [2]Department of Oncology, Chest Cancer Center, Hefei Cancer Hospital, Chinese Academy of Sciences, Hefei, China; [3]Medical Imaging Center, Hefei Cancer Hospital, Chinese Academy of Sciences, Hefei, China

Contributions: (I) Conception and design: W Wang, Z Cao; (II) Administrative support: W Wang, Z Cao; (III) Provision of study materials or patients: W Wang, Y Wang; (IV) Collection and assembly of data: W Wang, Y Wang; (V) Data analysis and interpretation: W Wang, Y Wang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Zong Cao. Medical Imaging Center, Hefei Cancer Hospital, Chinese Academy of Sciences, No. 350 Road of Shushan Lake, Shushan District, Hefei, China. Email: zongcao76@gmail.com.

Background: It remains unknown how chemotherapy affects circulating lymphocyte subsets and whether the pattern of change is related to prognosis in cancer patients.

Methods: Cancer patients who received chemotherapy between 2018/03/01 and 2019/12/31 were enrolled from the Hefei Cancer Hospital, Chinese Academy of Sciences. Peripheral blood samples were collected before and 3 weeks after the start of chemotherapy, and the proportions of T cells (CD3+), helper T cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+), B cells (CD19+), and Natural killer (NK) cells (CD3−CD56+) were examined by flow cytometry. Multivariable logistic regression analysis was employed to explore risk factors associated with overall survival within 12 months after the start of chemotherapy.

Results: A total of 167 patients with cancer were included in the analysis, including 14 cases of cervical cancer, 18 cases of breast cancer, 33 cases of gastric cancer, 48 cases of lung cancer, 21 cases of colorectal cancer, and 33 cases of esophageal cancer. The proportion of T cells (72.58%±10.44% *vs.* 80.67%±11.63%, P<0.001) and cytotoxic T cells (25.38%±8.87% *vs.* 39.20%±12.26%, P<0.001) significantly increased, while the proportion of helper T cells (45.58%±10.19% *vs.* 41.98%±10.47%, P<0.001), B cells (15.10%±5.23% *vs.* 11.29%±5.60%, P<0.001), and NK cells (19.33%±7.54% *vs.* 18.28%±7.62%, P<0.001) significantly decreased at 3 weeks after chemotherapy when compared to baseline levels. The overall mortality rate was 14.97% (25/167) within 1 year after the start of chemotherapy. Patients who survived showed a significantly less increase in cytotoxic T cells (13.38%±8.28% *vs.* 17.28%±7.97%, P=0.030) and less decrease in B cells (−3.58%±2.81% *vs.* −5.29%±3.03%, P=0.006) when compared to non-survivors. Greater decreases in helper T cells (OR 0.81, 95% CI, 0.68–0.96) and B cells (OR 0.72, 95% CI, 0.59–0.87), and a greater increase in cytotoxic T cells (OR 1.09, 95% CI, 1.03–1.16) were risk factors for poor overall survival.

Conclusions: Circulating lymphocyte subsets of cancer patients presented characteristic changes after chemotherapy. Patients with a greater decrease in helper T cells and B cells, or greater increase in cytotoxic T cells, may have worse survival.

Keywords: Lymphocyte subsets; chemotherapy; cancer; prognosis

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Introduction

Chemotherapy has evolved over the past 100 years to become a standard cancer therapy (1), and is now recommended as treatment for cancers of the cervix (2), breast (3), stomach (4), lung (5), bowel (6), and esophagus (7). Traditionally, cytotoxic drugs are used for chemotherapy, which target rapidly proliferating cells such as cancer cells. Underpinning the theory behind this therapy is the belief that cancer is a cell-autonomous disease driven by the activation of oncogenes or the inactivation of tumor suppressor genes (8). However, the nonselective mode of action of traditional cytotoxic agents also causes toxicity to normal proliferative cells or tissues (9), raising concerns about the adverse effects of chemotherapy (10,11).

As blood cells in the bone marrow are labile, they are usually greatly affected by chemotherapy. Almost all traditional cytotoxic agents used for chemotherapy can suppress bone marrow and decrease blood cell quality and quantity, leading to depression of the immune system (12). Although some practitioners are of the view that the immunological effects of conventional chemotherapy can be beneficial since they may increase the immunogenicity of malignant cells or inhibit immunosuppressive circuitries that are established by developing neoplasms (13), patients with immunosuppression and myelosuppression are at a significantly increased risk of infection which can be life-threatening (14,15). Despite this, the impact of chemotherapy on the immune function of cancer patients has attracted much attention in recent years (13,16,17).

Lymphocytes play a crucial role in cancer development, either in anti-tumor immunity or pro-tumor immunity (18). Circulating lymphocyte subsets have been observed to be associated with the prognosis of several types of cancer (19-21), and to be affected by various treatments including chemotherapy (17,22). Some research has shown that compared to T cells, the number and function of B cells are more adversely affected by chemotherapy (23), and the alternations of immune function may last for a longer period than the treatment (17). Therefore, it is necessary to investigate potential changes of proportions of circulating lymphocyte subsets in cancer patients after chemotherapy and explore whether these changes are associated with prognosis. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/tcr-21-1688).

Methods

Study participants

Cancer patients who received chemotherapy in Hefei Cancer Hospital, Chinese Academy of Sciences between 2018/03/01 and 2019/12/31 were screened to be included. The inclusion criteria were: (I) patients with a confirmed diagnosis of cervical cancer, breast cancer, gastric cancer, lung cancer, colorectal cancer, or esophageal cancer; and (II) patients who received chemotherapy between 2018/03/01 and 2019/12/31. The exclusion criteria were any of the following: (I) chemotherapy was not recommended by relevant guidelines; (II) patients who could not tolerate chemotherapy or who were contraindicated for chemotherapy, for reasons including short life expectancy or pregnancy; (III) patients with other primary cancers; (IV) patients with other severe acute or chronic diseases which might affect immune function or bone marrow function; (VI) patients receiving other therapies which might affect immune function or bone marrow function, such as high-dose glucocorticoids; and (VII) patients who did not agree to participate in the study. Except for the intervention mentioned below (i.e., determination of proportions of circulating lymphocyte subsets), no other interventions were implemented in the study. The diagnosis of cancer, indications for chemotherapy, strategies for chemotherapy, and other management before and after chemotherapy was determined by the responsible clinicians. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of Hefei Cancer Hospital (No. 2019KY007), Chinese Academy of Sciences, and informed consent was waived.

Determination of proportions of circulating lymphocyte subsets

For each included patient, peripheral blood (5.0 mL) was collected by routine venipuncture and placed into sodium EDTA tubes before chemotherapy (i.e., baseline), and about 3 weeks (i.e., 21±5 days) after the start of chemotherapy respectively. Proportions of circulating lymphocyte subsets were examined by a (DxFLFX flow cytometer, Beckman, USA) as previously reported (22). In brief, a 100 μL blood sample was incubated with 20 μL antibody in a flow cytometry tube for 30 min at room temperature in the

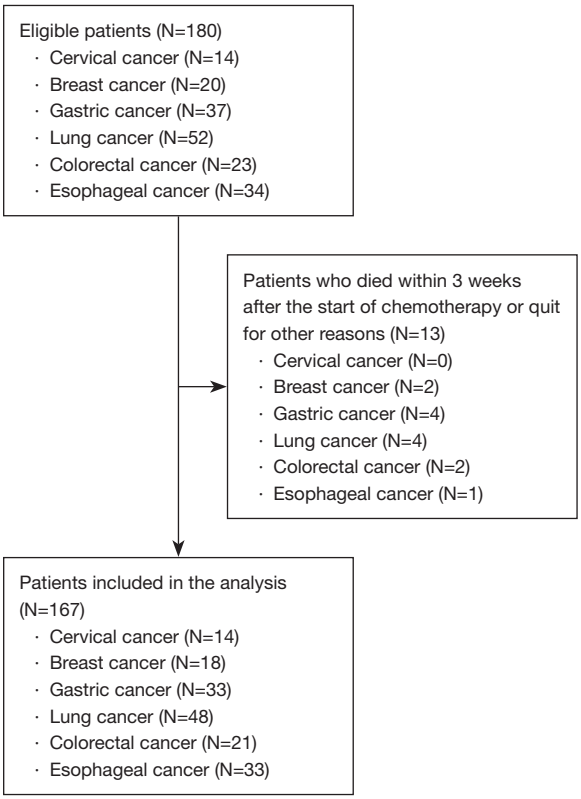

Figure 1 Inclusion of the study participants.

dark. After treatment by a hemolytic agent, the supernatant was removed by centrifugation (600 g for 5 min) and the cell lysate was washed by phosphate buffer saline. The cell lysate was then suspended in 0.5 mL 4% paraformaldehyde and detected by flow cytometry. The following antibodies were used to determine different subsets of circulating lymphocytes: anti-CD3-FITC for T cells ($CD3^+$); anti-CD3-FITC/anti-CD4-PE for helper T cells ($CD3^+CD4^+$); anti-CD3-FITC/anti-CD8-PE for cytotoxic T cells ($CD3^+CD8^+$); anti-CD19-FITC for B cells ($CD19^+$); and anti-CD3-FITC/anti-CD56-PE for natural killer (NK) cells ($CD3^-CD56^+$).

Baseline characteristics and overall survival

Baseline characteristics including age, sex, cancer stage (advanced or not), surgical therapy before chemotherapy, and smoking history were obtained. Overall survival within 12 months after the start of chemotherapy was studied as survival outcome, and survival information was collected via screening hospital data and contact via mobile phone when needed. Patients who failed to survive at 3 weeks after the start of chemotherapy, who quit the study within 3 weeks after the start of chemotherapy, or whose survival information within 12 months after the start of chemotherapy was unavailable, were excluded from the final analysis.

Statistical analysis

Continuous data are presented as mean ± standard deviation and categorical data as count (percentage). Comparisons of proportions of circulating lymphocyte subsets before and after chemotherapy were analyzed by a paired samples *t*-test, and comparisons of absolute changes of proportions of circulating lymphocyte subsets between survivors and non-survivors were examined by an independent *t*-test. Univariable logistic regression analysis was used to evaluate the associations of the studied baseline chrematistics and absolute changes of proportions of circulating lymphocyte subsets with overall survival. Multivariable logistic regression analysis was employed to explore risk factors associated with overall survival according to a backward elimination (conditional) selection method. All statistical analyses were performed using SPSS software (Version 22.0), and a P value less than 0.05 was considered statistically significant.

Results

Baseline characteristics of the study participants

A total of 167 patients with cancer were included in the final analysis, including 14 cases of cervical cancer, 18 cases of breast cancer, 33 cases of gastric cancer, 48 cases of lung cancer, 21 cases of colorectal cancer, and 33 cases of esophageal cancer (*Figure 1*). Among the patients, 64.07% (107/167) were in an advanced stage of cancer, and 53.89% (90/167) had received surgical therapy before chemotherapy. In detail, among patients with cervical cancer, four had early-stage cervical cancer (FIGO stage IA, IB1, and IB2), and 10 patients had locally advanced cervical cancer (FIGO stage II to IVA); among patients with breast cancer, all had positive human epidermal growth factor receptor 2 (HER2), five had node-negative breast cancer <2 cm, and 13 patients had tumours >2 cm and/or were node positive; among the patients with gastric cancer, 12 had stage $T_{3-4}N_0$, and 21 patients had locally advanced unresectable gastric cancer; among the patients with lung cancer, all had non-small cell

Table 1 Baseline characteristics of the study participants

Variables	All patients (N=167)	Cervical cancer (N=14)	Breast cancer (N=18)	Gastric cancer (N=33)	Lung cancer (N=48)	Colorectal cancer (N=21)	Esophageal cancer (N=33)
Age (years)	55.56±11.94	43.14±8.80	48.61±12.46	58.24±12.63	60.62±8.77	56.81±10.46	53.76±11.80
Sex, n (%)							
Male	88 (52.69)	0 (0.00)	0 (0.00)	17 (51.52)	35 (72.92)	13 (61.90)	23 (69.70)
Female	79 (47.31)	14 (100.00)	18 (100.00)	16 (48.48)	13 (27.08)	8 (38.10)	10 (30.30)
Advanced cancer, n (%)							
No	60 (35.93)	4 (28.57)	5 (27.78)	12 (36.36)	18 (37.50)	6 (28.57)	15 (45.45)
Yes	107 (64.07)	10 (71.43)	13 (72.22)	21 (63.64)	30 (62.50)	15 (71.43)	18 (54.55)
Surgical therapy before chemotherapy, n (%)							
No	77 (46.11)	12 (85.71)	8 (44.44)	11 (33.33)	30 (62.50)	8 (38.10)	8 (24.24)
Yes	90 (53.89)	2 (14.29)	10 (55.56)	22 (66.67)	18 (37.50)	13 (61.90)	25 (75.76)
Smoking history, n (%)							
No	92 (55.09)	10 (71.43)	14 (77.78)	17 (51.52)	20 (41.67)	10 (47.62)	21 (63.64)
Yes	75 (44.91)	4 (28.57)	4 (22.22)	16 (48.48)	28 (58.33)	11 (52.38)	12 (36.36)

lung cancer, 18 had stage I, II, or III, and 30 patients had stage IV disease; among patients with colorectal cancer, all had colon cancer, six had locally advanced, potentially resectable colon cancer, and 15 patients had metastatic disease; among patients with esophageal cancer, 15 had stage IIb to IIIc disease, and 18 patients had stage IV. The mean age of patients was 55.56±11.94 years, and 52.69% (88/167) of the patients were male. Differences were seen in some baseline characteristics among patients with different types of cancer, such as sex proportion, and are shown in *Table 1*.

Changes of proportions of circulating lymphocyte subsets after chemotherapy

At 3 weeks after chemotherapy, the proportion of T cells (72.58%±10.44% *vs.* 80.67%±11.63%, P<0.001) and cytotoxic T cells (25.38%±8.87% *vs.* 39.20%±12.26%, P<0.001) significantly increased, while the proportion of helper T cells (45.58%±10.19% *vs.* 41.98%±10.47%, P<0.001), B cells (15.10%±5.23% *vs.* 11.29%±5.60%, P<0.001), and NK cells (19.33%±7.54% *vs.* 18.28%±7.62%, P<0.001) significantly decreased when compared to that at baseline. The changes were broadly consistent in each subgroup according to the type of cancer (*Table 2*).

Changes of proportions of circulating lymphocyte subsets between survivors and non-survivors

The overall mortality rate was 14.97% (25/167) within 1 year after the start of chemotherapy. Patients who survived showed a significantly less increase in cytotoxic T cells (13.38%±8.28% *vs.* 17.28%±7.97%, P=0.030) and less decrease in B cells (–3.58%±2.81% *vs.* –5.29%±3.03%, P=0.006) when compared to the non-survivors, but there was no significant difference in the changes of proportions of T cells ($CD3^+$), Helper T cells, and NK cells (*Table 3*).

Risk factors associated with overall survival

According to results of the univariable analysis, not receiving surgical therapy before chemotherapy tended to be associated with an increased risk of death, but the association was not statistically significant (*Table 4*). The absolute changes of cytotoxic T cells [odds ratio (OR) 1.06 per 1% increase, 95% confidence interval (CI) 1.00–1.12, P=0.033] and B cells (OR 0.80 per 1% increase, 95% CI, 0.68–0.94, P=0.008) were significantly associated with survival. Results of multivariable analysis (*Table 5*) suggested a greater decrease in helper T cells (OR 0.81, 95% CI, 0.68–0.96) and B cells (OR 0.72, 95% CI, 0.59–0.87), and greater increase in cytotoxic T cells (OR 1.09, 95% CI,

Table 2 Changes of proportions of circulating lymphocyte subsets after chemotherapy

Circulating lymphocyte subsets	Before chemotherapy, %	At 3 weeks after chemotherapy, %	Absolute change, %	P value
All patients				
T cells ($CD3^+$)	72.58±10.44	80.67±11.63	8.18±6.42	<0.001
Helper T cells ($CD3^+CD4^+$)	45.58±10.19	41.98±10.47	–3.52±2.80	<0.001
Cytotoxic T cells ($CD3^+CD8^+$)	25.38±8.87	39.20±12.26	13.96±8.32	<0.001
B cells ($CD19^+$)	15.10±5.23	11.29±5.60	–3.84±2.90	<0.001
Natural killer cells ($CD3^-CD56^+$)	19.33±7.54	18.28±7.62	–1.00±0.93	<0.001
Cervical cancer				
T cells ($CD3^+$)	74.02±10.06	84.63±10.12	10.61±7.22	<0.001
Helper T cells ($CD3^+CD4^+$)	48.86±12.67	44.49±13.81	–4.37±2.83	<0.001
Cytotoxic T cells ($CD3^+CD8^+$)	30.66±8.99	46.06±15.11	15.40±9.58	<0.001
B cells ($CD19^+$)	15.45±6.31	10.94±6.24	–4.51±3.03	<0.001
Natural killer cells ($CD3^-CD56^+$)	18.70±7.63	17.76±7.73	–0.94±0.89	0.002
Breast cancer				
T cells ($CD3^+$)	70.61±11.10	78.33±11.36	7.72±7.12	<0.001
Helper T cells ($CD3^+CD4^+$)	41.22±10.32	37.34±9.99	–3.88±3.09	<0.001
Cytotoxic T cells ($CD3^+CD8^+$)	22.76±8.95	34.96±12.68	12.21±9.76	<0.001
B cells ($CD19^+$)	14.84±5.35	11.68±4.97	–3.16±3.68	0.002
Natural killer cells ($CD3^-CD56^+$)	19.58±9.11	18.69±9.25	–0.89±1.07	0.003
Gastric cancer				
T cells ($CD3^+$)	73.42±10.06	82.27±11.53	8.85±6.83	<0.001
Helper T cells ($CD3^+CD4^+$)	45.12±11.36	41.25±10.87	–3.87±3.21	<0.001
Cytotoxic T cells ($CD3^+CD8^+$)	25.79±7.97	38.78±12.65	12.99±9.80	<0.001
B cells ($CD19^+$)	15.14±5.79	11.25±7.29	–3.89±3.74	<0.001
Natural killer cells ($CD3^-CD56^+$)	17.20±8.38	16.04±8.54	–1.16±1.07	<0.001
Lung cancer				
T cells ($CD3^+$)	74.08±8.48	81.44±10.79	7.35±6.97	<0.001
Helper T cells ($CD3^+CD4^+$)	48.80±8.66	45.23±9.69	–3.57±2.82	<0.001
Cytotoxic T cells ($CD3^+CD8^+$)	25.26±10.07	41.05±12.02	15.79±8.49	<0.001
B cells ($CD19^+$)	14.99±5.28	11.77±5.51	–3.22±2.13	<0.001
Natural killer cells ($CD3^-CD56^+$)	17.77±6.14	16.91±6.06	–0.85±0.90	<0.001
Colorectal cancer				
T cells ($CD3^+$)	74.46±12.34	82.48±14.59	8.02±6.95	<0.001
Helper T cells ($CD3^+CD4^+$)	47.93±11.89	45.20±11.48	–2.73±3.22	0.001
Cytotoxic T cells ($CD3^+CD8^+$)	24.90±9.19	38.14±14.53	13.24±9.52	<0.001
B cells ($CD19^+$)	13.81±6.30	9.68±5.91	–4.14±3.29	<0.001
Natural killer cells ($CD3^-CD56^+$)	21.83±9.44	20.78±9.43	–1.04±1.25	0.001

Table 2 (*continued*)

Table 2 (*continued*)

Circulating lymphocyte subsets	Before chemotherapy, %	At 3 weeks after chemotherapy, %	Absolute change, %	P value
Esophageal cancer				
T cells ($CD3^+$)	68.84±11.51	76.41±10.96	8.03±3.66	0.016
Helper T cells ($CD3^+CD4^+$)	40.84±5.61	37.43±6.32	–3.02±1.64	0.064
Cytotoxic T cells ($CD3^+CD8^+$)	24.64±7.12	37.03±7.50	13.09±1.80	<0.001
B cells ($CD19^+$)	16.06±3.05	11.62±3.40	–4.59±1.90	<0.001
Natural killer cells ($CD3^-CD56^+$)	22.26±4.95	20.90±5.41	–1.12±0.41	0.255

Table 3 Absolute changes of proportions of circulating lymphocyte subsets according to overall survival

Circulating lymphocyte subsets	Alive, %	Death, %	P value
All patients			
T cells ($CD3^+$)	8.11±6.42	8.58±6.55	0.739
Helper T cells ($CD3^+CD4^+$)	–3.35±2.74	–4.47±3.01	0.065
Cytotoxic T cells ($CD3^+CD8^+$)	13.38±8.28	17.28±7.97	0.030
B cells ($CD19^+$)	–3.58±2.81	–5.29±3.03	0.006
Natural killer cells ($CD3^-CD56^+$)	–1.02±0.94	–0.87±0.90	0.461
Cervical cancer			
T cells ($CD3^+$)	9.19±7.55	15.80±1.65	0.168
Helper T cells ($CD3^+CD4^+$)	–4.40±2.92	–4.27±3.04	0.946
Cytotoxic T cells ($CD3^+CD8^+$)	11.85±7.34	28.43±1.50	0.003
B cells ($CD19^+$)	–3.77±2.72	–7.20±2.98	0.081
Natural killer cells ($CD3^-CD56^+$)	–1.03±0.93	–0.60±0.81	0.478
Breast cancer			
T cells ($CD3^+$)	7.87±7.43	6.97±6.60	0.847
Helper T cells ($CD3^+CD4^+$)	–4.15±2.85	–2.53±4.61	0.426
Cytotoxic T cells ($CD3^+CD8^+$)	10.74±9.84	19.53±6.03	0.160
B cells ($CD19^+$)	–2.23±3.27	–7.83±1.03	0.011
Natural killer cells ($CD3^-CD56^+$)	–0.83±1.13	–1.16±0.83	0.642
Gastric cancer			
T cells ($CD3^+$)	8.63±6.89	9.69±7.08	0.722
Helper T cells ($CD3^+CD4^+$)	–3.81±3.18	–4.09±3.58	0.842
Cytotoxic T cells ($CD3^+CD8^+$)	13.80±10.57	9.99±5.82	0.369
B cells ($CD19^+$)	–3.65±3.59	–4.77±4.47	0.492
Natural killer cells ($CD3^-CD56^+$)	–1.11±1.10	–1.35±0.97	0.601

Table 3 (*continued*)

Table 3 (*continued*)

Circulating lymphocyte subsets	Alive, %	Death, %	P value
Lung cancer			
T cells ($CD3^+$)	7.57±6.70	5.44±9.73	0.524
Helper T cells ($CD3^+CD4^+$)	−3.22±2.67	−6.57±2.49	0.011
Cytotoxic T cells ($CD3^+CD8^+$)	15.07±8.43	21.92±6.96	0.088
B cells ($CD19^+$)	−3.22±2.23	−3.20±0.93	0.984
Natural killer cells ($CD3^-CD56^+$)	−0.88±0.90	−0.62±1.05	0.550
Colorectal cancer			
T cells ($CD3^+$)	8.20±7.08	4.60	0.626
Helper T cells ($CD3^+CD4^+$)	−2.50±3.11	−7.50	0.133
Cytotoxic T cells ($CD3^+CD8^+$)	12.61±9.30	25.90	0.179
B cells ($CD19^+$)	−3.90±3.18	−8.90	0.142
Natural killer cells ($CD3^-CD56^+$)	−1.10±1.25	0.17	0.330
Esophageal cancer			
T cells ($CD3^+$)	8.09±3.99	7.75±1.65	0.837
Helper T cells ($CD3^+CD4^+$)	−2.87±1.70	−3.73±1.20	0.251
Cytotoxic T cells ($CD3^+CD8^+$)	12.93±1.62	13.79±2.55	0.300
B cells ($CD19^+$)	−4.54±2.02	−4.82±1.30	0.751
Natural killer cells ($CD3^-CD56^+$)	−1.21±0.19	−0.70±0.79	0.004

1.03–1.16) were risk factors for overall survival.

Discussion

The current study included patients with six types of cancer (i.e., cervical cancer, breast cancer, gastric cancer, lung cancer, colon cancer, and esophageal cancer), and investigated changes of proportions of five subsets of circulating lymphocyte [i.e., T cells ($CD3^+$), helper T cells ($CD3^+CD4^+$), cytotoxic T cells ($CD3^+CD8^+$), B cells ($CD19^+$), and NK cells ($CD3^-CD56^+$)] at 3 weeks after chemotherapy. The results of our study indicated (I) the proportions of T cells and cytotoxic T cells significantly increased, while the proportions of helper T cells, B cells, and NK cells significantly decreased at 3 weeks after chemotherapy when compared to that at baseline, and was broadly consistent between different types of cancer; and (II) a greater decrease in helper T cells and B cells, and greater increase in cytotoxic T cells were associated with poor overall survival. These results suggest that chemotherapy is involved in the regulation of peripheral immune cell composition, and the magnitude of changes might provide information about the prognosis of cancer patients after chemotherapy.

Several types of therapies have been reported to affect circulating lymphocyte subsets. For example, patients after renal transplantation were found to have decreased total B-cell counts and a more differentiated circulating B-cell pool than healthy individuals, which might reflect the chronic antigenic stimulation (24). The number of peripheral T cells was found to decrease at 4 hours after the administration of prednisone, which returned to baseline at 24 hours (25). Similarly, patients with esophageal cancer after radiotherapy also showed a decreased circulating lymphocyte count with an increased proportion of T cells but a decreased proportion of NK cells and B cells (26). The impact of chemotherapy on circulating lymphocyte subsets has been examined previously. Liu *et al.* (21) investigated more than 200 patients with unresectable pancreatic ductal adenocarcinoma who received gemcitabine-based

Table 4 Univariable analysis of overall survival

Variables	Odds ratio	95% CI	P value
Age, per year increase	0.99	0.96–1.03	0.730
Sex			
Male	1.00		
Female	2.23	0.92–5.38	0.075
Advanced cancer			
No	1.00		
Yes	1.00	0.41–2.42	0.994
Surgical therapy before chemotherapy			
No	1.00		
Yes	0.42	0.18–1.02	0.056
Smoking history			
No	1.00		
Yes	0.96	0.41–2.25	0.921
Type of cancer			
Cervical cancer	1.00		
Breast cancer	0.73	0.12–4.35	0.733
Gastric cancer	0.99	0.21–4.54	0.987
Lung cancer	0.43	0.09–2.06	0.289
Colorectal cancer	0.18	0.02–1.98	0.162
Esophageal cancer	0.81	0.17–3.85	0.796
Absolute change, per 1% increase			
T cells ($CD3^+$)	1.01	0.95–1.08	0.737
Helper T cells ($CD3^+CD4^+$)	0.87	0.75–1.01	0.068
Cytotoxic T cells ($CD3^+CD8^+$)	1.06	1.00–1.12	0.033
B cells ($CD19^+$)	0.80	0.68–0.94	0.008
Natural killer cells ($CD3^-CD56^+$)	1.19	0.75–1.88	0.459

CI, confidence interval.

Table 5 Multivariable analysis of overall survival

Variables	Odds ratio	95% CI	P value
Helper T cells ($CD3^+CD4^+$)	0.81	0.68–0.96	0.017
Cytotoxic T cells ($CD3^+CD8^+$)	1.09	1.03–1.16	0.006
B cells ($CD19^+$)	0.72	0.59–0.87	0.001

CI, confidence interval.

chemotherapy as the primary treatment, and found the level of $CD3^+$ T cells elevated after two cycles of chemotherapy, and a decreased T regulatory or CD4/CD8 ratio after chemotherapy was associated with a longer overall survival. Wang *et al.* (19) found untreated cancer patients had different levels of NK cells and some subsets of T cells from that of healthy individuals, suggesting circulating activated lymphocyte subsets might serve as potential blood biomarkers of cancer progression. Yang *et al.* (20) investigated more than 400 patients with metastatic breast cancer and found higher circulating levels of $CD4^+$ and $CD3^+$ at first diagnosis were significantly associated with worse survival outcomes. Considering the ease of obtaining peripheral blood samples and general availability of flow cytometry, these results suggest circulating lymphocyte subsets may serve as a circulating signature of cancer patients in terms of their response to chemotherapy and prognosis.

Our results are broadly consistent with those of the studies listed above. However, it should be noted that we included six types of cancers, and our results are more likely to reflect their average impact of chemotherapy on alternations of circulating lymphocyte subsets. Although patients with different types of tumors may have different proportions of circulating lymphocyte subsets at baseline, in the present study we focused on the changes after receiving chemotherapy, which means the proportion of circulating lymphocyte subsets of each individual patient after chemotherapy were always compared to his/her own baseline proportion. We observed that changes in the investigated subsets of circulating lymphocytes were broadly consistent between different types of cancer, but the differences in absolute changes between survivors and non-survivors were not. This may be related to the limited sample size after stratification by types of cancer, which made us unable to observe a significant difference. In addition, in the results of univariable analysis, we found not receiving surgical therapy before chemotherapy tended to be associated with an increased risk of death, but the association was not statistically significant. This may also be due to the limited sample size, and more studies are required to confirm our findings. Nevertheless, the investigation of different types of cancer at the same time provides our study with innovation when compared to other studies, which suggests the changing pattern of subsets of circulating lymphocytes after chemotherapy may be common and consistent between different types of cancer.

There are some limitations to our study. First, we only investigated the mentioned subsets of circulating lymphocyte, and did not include some other subsets that were investigated in other studies (19,20). Second, we only investigated two time points (i.e., before, and at 3 weeks after the start of chemotherapy), while other studies have suggested changes to the immune response including lymphocytes may take more than 12 months to recover (17). Future studies should use a repeated-measures design to further reveal the information behind changes to circulating lymphocyte subsets. Third, our findings are at risk of bias. We did not perform or control other interventions except for the determination of proportions of circulating lymphocyte subsets, while patients with different types of cancer received different types of chemotherapy and may have received other therapy during the follow-up. Meanwhile, other factors that may cause changes in circulating lymphocyte subsets could also bias the association we observed. Fourth, we only studied overall survival as an outcome of prognosis, while the changes in circulating lymphocyte subsets may be also related to other relevant clinical outcomes (such as co-infection after chemotherapy), which warrants more investigations. Lastly, we only included a limited sample size for each type of cancer, which made subgroup analysis based on the types of cancer impossible. With a larger sample size, further studies may also investigate the changes in circulating lymphocyte subsets in patients with malignant tumors after chemotherapy and additional therapies (e.g., immunotherapy).

Conclusions

Circulating lymphocyte subsets in cancer patients presented characteristic changes after chemotherapy. Patients with greater decreases in helper T cells and B cells, or a greater increase in cytotoxic T cells, may have worse survival.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of Hefei Cancer Hospital (No. 2019KY007), Chinese Academy of Sciences, and informed consent was waived.

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