Clinical Correlations of Peripheral Blood Lymphocyte Populations in Sepsis

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Clinical correlations of peripheral blood lymphocyte populations in sepsis

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Abstract

Background Our understanding of sepsis-associated immune impairment is incomplete. The objective of this retrospective study was to investigate correlations of sepsis clinical manifestations with peripheral blood lymphocyte subpopulations in lymphocyte immunity.

Methods Twenty individuals without sepsis and eighteen with sepsis were enrolled. Lymphocyte phenotypes (CD3+, CD4+, CD8+, CD3-CD16+CD56+, CD19+, CD4+CD25+CD127+, CD8+CD28-, and CD8+CD28+) were assessed by flow cytometry. Fresh fecal bacteria cue proportion was measured to determine intestinal dysbacteriosis.

Results Compared with the non-sepsis group, the sepsis patients had clearly lower proportions of CD3+, CD4+ and CD8+CD28+ cells and substantially higher proportions of CD19+ cells ($p<0.05$). Among 38 patients with infection, CD4+ cells and CD8+CD28+ cells in a survivor group had significantly higher presence compared with patients who had died ($p<0.05$), The subgroup analysis results showed that CD4+ cells in the survivor subgroups were higher than those in the deceased subgroups ($p<0.05$). CD8+CD28+ cells in the non-sepsis survivor subgroup were higher than those in the deceased subgroups ($p<0.05$). Bivariate correlation analysis showed that the intestinal dysbacteriosis was significantly correlated with the severity of sepsis and its prognosis ($r^2=0.2788$, $p=0.001$, $r^2=0.1764$, $p=0.009$, respectively). CD4+, CD19+, and CD8+CD28+ cells were significantly correlated with intestinal dysbacteriosis

(r^2=0.1024, p=0.049, r^2=0.1063, p=0.046, r^2=0.1909, p=0.006, respectively).

Conclusions In conclusion, the lymphocyte populations of CD3+, CD4+, CD8+CD28+ and CD19+ cells were accessible for predicting the severity and mortality of sepsis patients. In addition, intestinal dysbacteriosis had a significant impact on the immune system of sepsis patients as revealed by peripheral blood lymphocyte population.

Introduction

Sepsis is a serious condition associated with infection. Sepsis has high morbidity and high mortality (30–70%) in intensive care units [1, 2]. A hyper-enhanced systemic anti-inflammatory response and immune imbalance are the main mechanisms of sepsis pathogenesis [3, 4].

Immunosuppression can arise from decreased lymphocyte reactivity or a nonreactive state [5-7]. Immunosuppression causes sepsis patients to be prone to uncontrolled infections that cause worsening disease and death [8, 9].

In critical care medicine, it is widely acknowledged that the intestinal tract is the central organ of stress and, in multiple organ failure, the first organ to fail [10-12]. The intestinal tract is the source of secondary infection of sepsis patients. Secondary infection caused by displacement of intestinal bacteria can aggravate sepsis.

Intestinal dysbacteriosis is being studied increasingly in the pathogenesis of immune-related diseases such as tumorigenesis, metabolic diseases, and autoimmune disease. Liu et al. [13] reported that enteric dysbiosis was associated with sepsis. Li et al. reported that, after seven days of fecal transplantation, the small intestine CD4+, CD8+, B220+ cells, colon CD4+ and part CD8+ cells were restored from abnormalities induced by long-term use of antibiotics [14]. Kishida et al. [15] reported CD3+ cells decreased and CD19+ cells increased in a murine model of antibiotic-treated intestinal microflora.

Rimmele, et al. [7]have summarized the changes of lymphocyte subsets in sepsis, but different studies are still controversial. Therefore, the aim of this study was to assess correlations between peripheral blood lymphocyte populations in sepsis.

Materials and methods

1. Patients

The criterion of sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection[16]. Thirty-eight patients with infection were admitted to the ICU of Beijing Shijitan Hospital, Capital Medical University from April, 2017 to January, 2019. The patients were divided into a group with sepsis and a nonsepsis group. The nonsepsis group was composed of 20 patients, 11 men and 9 women, with a mean age of 76.06±17.13 years. The sepsis group included 18 patients,

10 men and 8 women, with a mean age of 76.61±18.07 years. Patients with organ transplantation or neoplastic disease were excluded.

2.**Methods of detection**

Peripheral blood mononuclear cells were obtained from heparinized venous blood by Ficoll-Hypaque (Hyclone, USA) density gradient centrifugation. The FACSCalibur flow cytometer (Beckman Coulter, USA) was used for cytometric analysis and to determine the percent of CD3+, CD4+, CD8+, CD3-CD16+CD56+, CD19+, CD4+CD25+CD127+, CD8+CD28-, and CD8+CD28+ lymphocytes. Experimental data were collected and analyzed with FC500 software (Beckman Coulter, USA).

To detect intestinal dysbacteriosis, fresh stool was picked out with a uniform velocity of 30~40 degree angle and an area of 1.5 cm*2 cm. After natural drying, the sample was fixed three times in an alcohol lamp flame and subjected to Gram staining. One thousand bacteria were observed by microscopy (at least 3-5 field views) with a field lens of 100x and an eye lens of 10x [17]. Intestinal dysbacteriosis was confirmed when one of the following standards was met: 1. The number of bacteria was < 200 cfu/field view(FV); 2. Gram negative bacilli were < 50%; 3. Gram positive cocci were > 50%; 4. yeast-like fungi >10%; 5. Bacillus > 10% [18-20].

3. Statistical analysis

Data were expressed as mean ± SD. Analyses were performed using SPSS-19.0 software (SPSS, USA). Analysis of variance between groups was performed by ANOVA or T test. The Mann-Whitney *U* test was used for comparisons by sex, survival, main diagnosis category, and site of infection. A value of $P < 0.05$ was considered statistically significant. Bivariate correlation analysis was used for correlation analysis.

Results

1. Characteristics of participants

Table 1 shows the clinical characteristics for the enrolled patients. There were no significant differences between the sepsis and nonsepsis groups in age, sex, and site of infection (P>0.05).

2. Peripheral lymphocyte subsets of the two groups.

Compared with the non-sepsis group, the sepsis group had lower proportions of CD3+, CD4+ and CD8+CD28+ cells (p<0.05) (Fig.1A,B,I). We did not observe any differences between the two groups in the proportions of CD8+T cells, CD3-CD16+CD56+ cells, CD3+CD16+CD56+ cells, CD4+CD25+ cells, and CD4+CD25+

117 cells ($p>0.05$) (Fig.1C, D, E, G, H). The proportion of CD19+ cells in sepsis group was
118 greater than that of non-sepsis group (Fig.1F; $p<0.05$).

119 From the above results and with the degree of aggravation of disease, the
120 proportions of CD3+, CD4+, and CD8+CD28+ cells in sepsis decreased, and the
121 proportion of CD19+ cells increased. These observations indicated that the immune
122 paralysis of the patients was aggravated.

123 **3. Lymphocyte composition in death and survivor nonsepsis and sepsis group**
124 **patients.**

125 Because the CD4+ and CD8+CD28+ cells between the nonsepsis and the sepsis
126 groups showed statistically significant differences we further analyzed the relationship
127 between death and lymphocyte subsets in sepsis patients. The standard was whether a
128 patient was alive for 28 days after entering the ICU. First, we divided the patients
129 according to whether they were deceased or surviving. Then, we divided the nonsepsis
130 group and sepsis group into the deceased patient subgroup and the survivor subgroup.
131 Finally, we compared the characteristics of lymphocyte subsets between the two groups
132 and the subgroups, respectively.

133 Overall analysis showed that the proportions of CD4+ and CD8+CD28+ cells in
134 the survivor group were higher than that in the deceased patient group ($p<0.05$) (Fig.2A,
135 B). The subgroup analysis results showed that CD4+ cells in the survivor subgroups
136 were higher than those in the deceased subgroups, respectively ($p<0.05$, respectively).

In addition, CD8+CD28+ cells in the nonsepsis survivor subgroup were higher than those in the nonsepsis and sepsis deceased subgroups, respectively ($p<0.05$, respectively). However, the number of CD8+CD28+ cells in the sepsis survivor subgroup was not statistically different from those in the nonsepsis and sepsis deceased subgroups, respectively ($p>0.05$, respectively).

4. Relationship between intestinal dysbacteriosis and lymphocyte subsets in sepsis

Bivariate correlation analysis showed that the intestinal dysbacteriosis was correlated with the severity and prognosis of sepsis patients (Fig.3A, $r^2=0.2788$, $p=0.001$, $r^2=0.11764$, $p=0.009$, respectively). Bivariate correlation analysis between the lymphocyte subsets and intestinal dysbacteriosis in sepsis patients showed that CD4+ cells, CD19+ cells and CD8+CD28+ cells were correlated with intestinal dysbacteriosis ($r^2=0.1024$, $p=0.049$, $r^2=0.1063$, $p=0.046$, $r^2=0.1909$, $p=0.006$, respectively).

Discussion

Sepsis, as a complex systemic disease, is a principal cause of death of ICU patients [1, 2]. We know little about the pathogenesis of sepsis. The study of sepsis has focused mainly on the interactions of various inflammatory factors, and the immune status reflected by lymphocyte subsets in sepsis has not received sufficient attention [21, 22]. In addition, studies on intestinal dysbacteriosis in sepsis pathogenesis are not

156 conclusive [20, 23], and there is relatively little literature on the effect of intestinal
157 dysbacteriosis in lymphocyte immunity in sepsis. Therefore, we retrospectively
158 collected recent clinical data and conducted this study to address those deficiencies.

159 We found that the values of variation in lymphocyte subset proportions predicted
160 sepsis severity and prognosis. The proportions of CD4+ and CD8+CD28+ cells in the
161 survivor group were higher than in the group of patients who died of sepsis. These
162 results corroborated the findings of many other studies [24, 25]. Decline in the number
163 of CD3+, CD4+ and CD8+CD28+ cells represents impairment of immune function,
164 which may result from massive apoptosis of lymphocytes in sepsis [26]. We also found
165 that the proportion of CD19+ cells increased during sepsis. This increase was contrary
166 to findings of Chen et al. [24], who observed that CD19+ lymphocytes were clearly less
167 abundant in patients with septic shock. A possible explanation for this difference is that
168 the patients in the two studies were different. Our study included elderly patients who
169 had mainly pulmonary infections, whereas the patients in Chen's study had mainly
170 abdominal infections after surgery. Further study is required to clarify these disparate
171 findings.

172 Our subgroup analysis result showed that CD4+ cells in the survivor subgroups
173 were higher than those in the deceased subgroups. The result were consistent with
174 previous reports[24, 25]. But, the CD8+CD28+ cell results and our subgroup results
175 were not entirely consistent. However, because the extraction times for peripheral blood

were not the same, and the methods of grouping were also not the same, our findings need confirmation [27]. Diminution of CD8+CD28+ cells in the nonsepsis and sepsis group suggested depletion of T cells.

In ICU patients, the intestinal tract is the central organ of stress and, in multiple organ failure, the first organ to fail [28]. The intestinal tract is the location of secondary infection in sepsis patients. Liu's study confirmed that sepsis is associated with disruption of gut microbial compositional [13]. Generally, the intestinal microbiota is inevitably affected by antibiotic treatment. In our study, all the patients from both groups accepted antibiotics when stool samples were examined. But the types and dosage of antibiotics were not different between the sepsis group and non-sepsis group (data not shown). Our data also prove that patients with intestinal dysbacteriosis had a higher mortality rate than those with non-intestinal dysbacteriosis. At the same time, intestinal microbes play an important role in the development of human immune system. For example, in a mouse model, intestinal TH17 cells contributed to development of a microbiota that maintained metabolic homeostasis [29]. Mice treated with penicillin demonstrated a elevated percent of B cells,regulatory T cells, T helper 1 (Th1) and T helper 2 (Th2) cells, but a decreased percent of total CD4+ cell[30]. Treg cell numbers are normalized when the microbiota is reconstituted in germ-free mice, and their development appears to be driven by a mixture of clostridial species [31]. The microbiome of the critically ill patient: Critically ill patients undergo dysbiosis at several organ sites, such as the skin, gastrointestinal system, and the lungs, with loss of

197 microbial diversity and a propensity for potentially pathogenic organisms to dominate
198 a microbiome. These microbiome changes appear to be predictive of clinical outcome
199 [20]. The peripheral CD8+ cell repertoire is largely independent of the presence of
200 intestinal flora [32]. However, the activity of intestinal microbes in the immunization
201 of patients with sepsis is unclear; thus, we made an observation about the relationship
202 between intestinal dysbacteriosis and lymphocyte subsets in sepsis patients .

203 In summary, our study suggests the changes in lymphocyte subset proportion are
204 significantly related to severity, prognosis, and interestingly, intestinal dysbacteriosis, in
205 sepsis.

206 Our study was still preliminary. Further study on the role of lymphocyte subsets
207 in sepsis, especially the mechanism of their interaction with intestinal dysbacteriosis, is
208 still needed.

209

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216 **Availability of data and materials**

The datasets for the present study are available from web of ScienceDB (http://www.dx.doi.org/10.11922/sciencedb.00325.).

Authors' contributions

WC and JR contributed to conception and design and gave final approval of the version of the manuscript to be published. LZ, GQ, SW and DY were responsible for analysis and interpretation of data. LZ, XZ wrote the original draft of the manuscript. JW preformed flow cytometry. LZ, DY, XW and XZ were involved in acquisition of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of Beijing Shijitan Hospital approved this study. Participants or their guardians provided informed consent. All participants had complete clinical data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table 1. The demographic and clinical characteristics for the non-sepsis, sepsis groups

	Non-sepsis	**sepsis**
n	20	18

Age	76.06±17.13	76.61±18.07	P=0.918
Gender			P=0.988
Male	11	10	
Female	9	8	
Survivor	16	4	**P=0.002**
Dead	4	14	
Site of infection			P=0.718
pulmonary	18	15	
Abdominal	1	1	
others	1	2	

Figure legends:

Figure 1. Differences in peripheral lymphocyte subsets between sepsis and non-sepsisgroup. **A.** Percent of CD3+ lymphocytes, **B.** Percent of CD4+ lymphocytes, **C.** Percent of CD8+ lymphocytes, **D.** Percent of CD3-CD16+CD56+ lymphocytes, **E.** Percent of CD3+CD16+CD56+ lymphocytes, **F.** Percent of CD19+ lymphocytes, **G.**

Percent of CD4+CD25+CD127+ lymphocytes, **H.** Percent of CD8+CD28- lymphocytes, and **I.** Percent of CD8+CD28+ lymphocytes.

Figure 2. Differences in lymphocyte composition in deceased and survivor groups between nonsepsis and sepsis group **A.** Percent of CD3+CD4+ lymphocytes, **B.** Percent of CD8+CD28+ lymphocytes, and those of subgroups **C.** Percent of CD3+CD4+ lymphocytes, **D.** Percent of CD8+CD28+ lymphocytes.

Figure 3. A. Relationship between intestinal dysbacteriosis and the severity of disease (red dashed line), prognosis (green dashed line) in sepsis. **B.** Relationship between intestinal dysbacteriosis and the percent of CD3+CD4+(blue dashed line) , CD19+(green dashed line), CD8+CD28+(red dashed line) lymphocytes in sepsis.

Figures

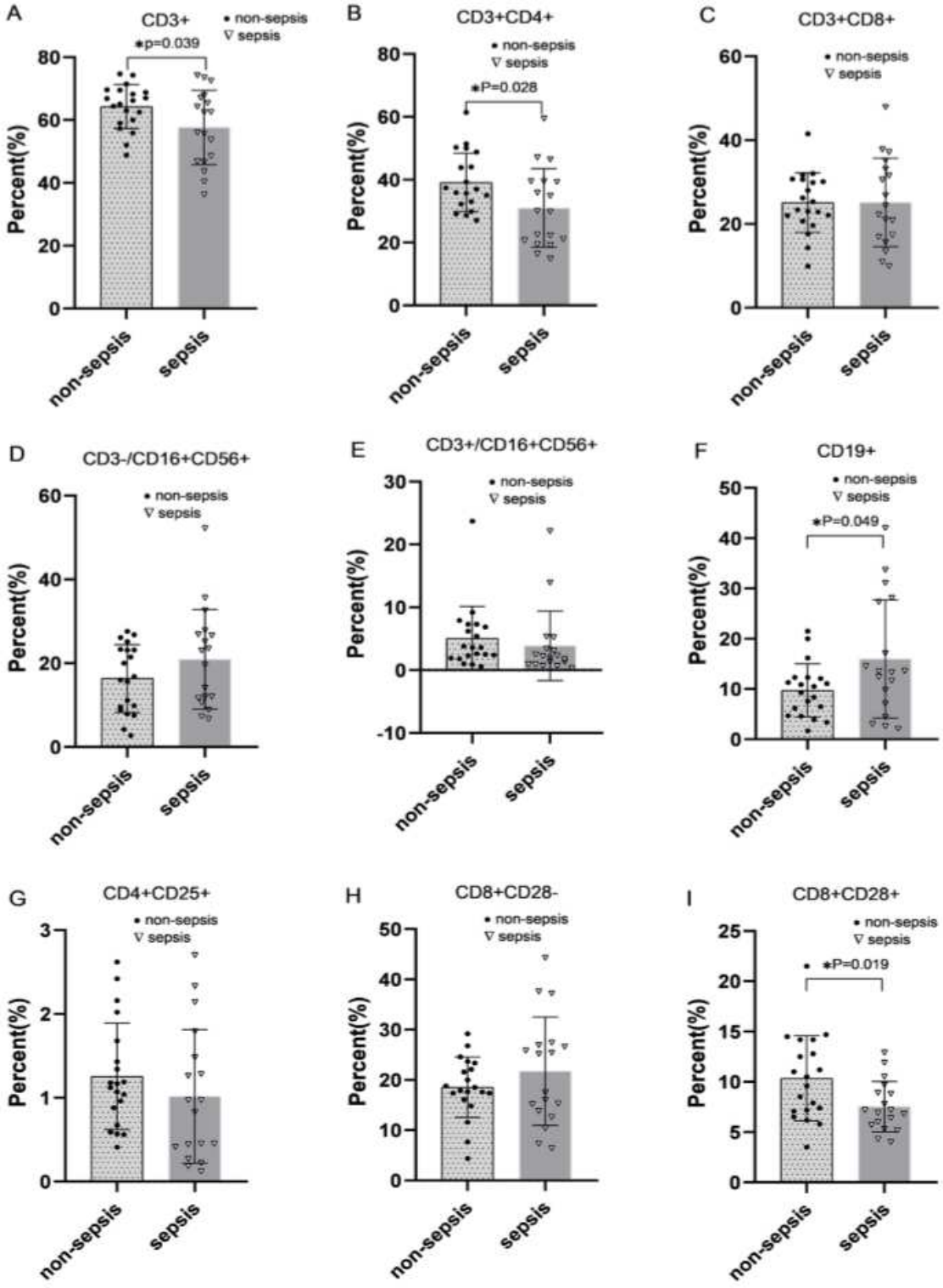

Figure 1

Differences in peripheral lymphocyte subsets between sepsis and non-340 sepsisgroup. A. Percent of CD3+ lymphocytes, B. Percent of CD4+ lymphocytes, C. Percent of CD8+ lymphocytes, D. Percent of CD3-CD16+CD56+ lymphocytes, E. Percent of CD3+CD16+CD56+ lymphocytes, F. Percent of CD19+

lymphocytes, G. Percent of CD4+CD25+CD127+ lymphocytes, H. Percent of CD8+CD28- lymphocytes, and I. Percent of CD8+CD28+ lymphocytes.

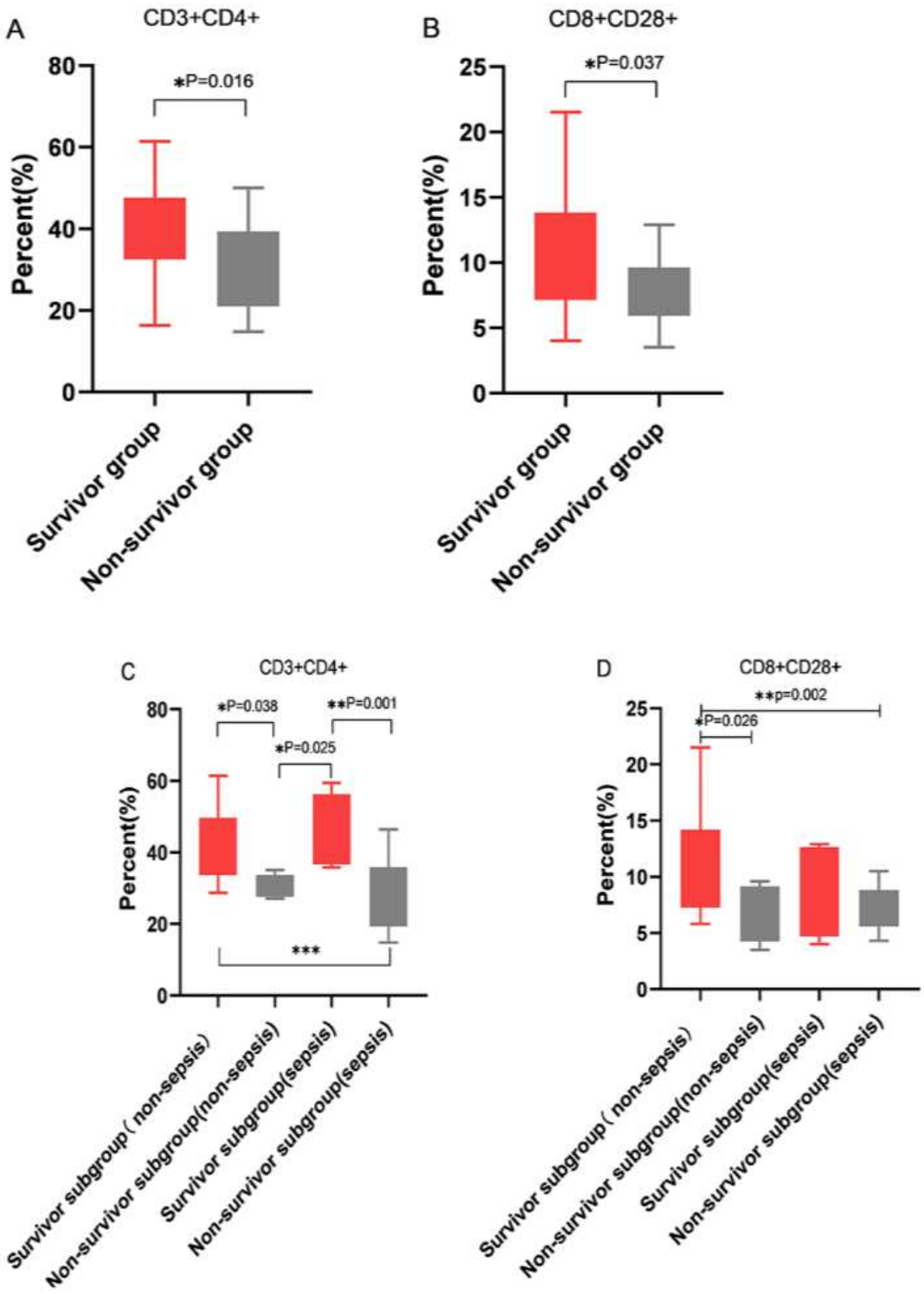

Figure 2

Differences in lymphocyte composition in deceased and survivor groups between nonsepsis and sepsis group A. Percent of CD3+CD4+ lymphocytes, B. Percent of CD8+CD28+ lymphocytes, and those of subgroups C. Percent of CD3+CD4+ lymphocytes, D. Percent of CD8+CD28+ lymphocytes.

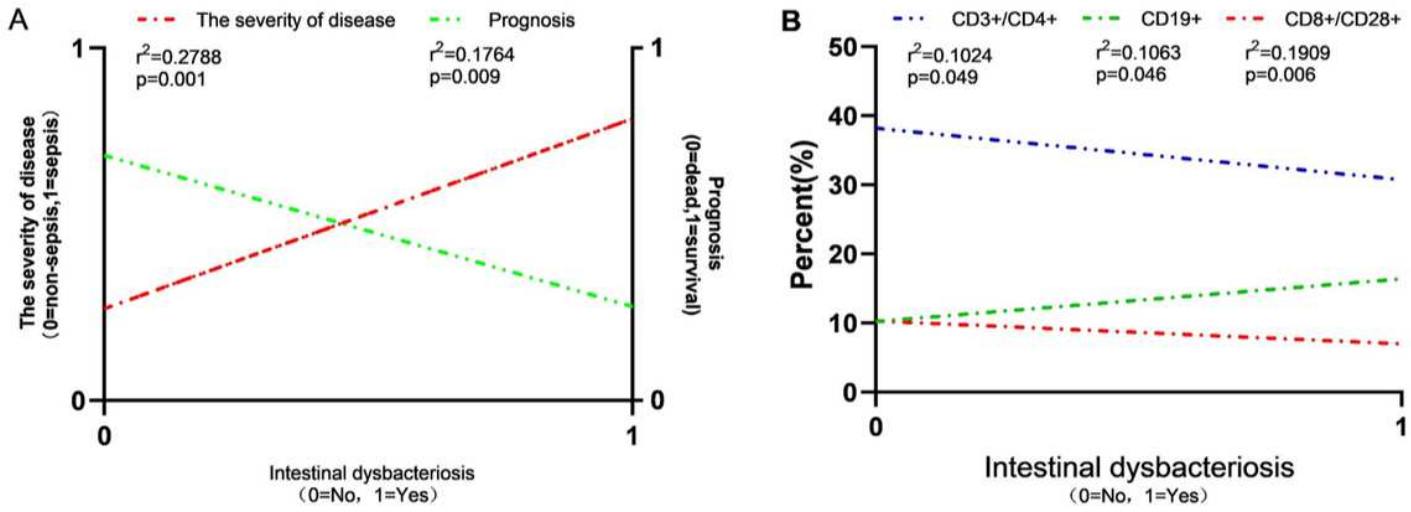

Figure 3

A. Relationship between intestinal dysbacteriosis and the severity of disease (red dashed line), prognosis (green dashed line) in sepsis. B. Relationship between intestinal dysbacteriosis and the percent of CD3+CD4+(blue dashed line) , CD19+(green dashed line), CD8+CD28+(red dashed line) lymphocytes in sepsis.