Increased Th17 and Th22 cell levels predict acute lung injury in patients with sepsis

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Abstract

Background: Acute lung injury (ALI) is one of the major complications of severe sepsis. This study was conducted to investigate the levels of Th22 and Th17 cells in the peripheral blood septic patients with ALI and their clinical significance.

Results: A total of 479 septic patients admitted between January 2013 to January 2018 were divided into non-ALI (n = 377) and ALI groups (n = 102) based on the presence or absence of ALI. The levels of Th22 and Th17 cells, interleukin 22 (IL-22), 6 (IL-6) and 17 (IL-17) were determined. Receiver operating characteristic curve (ROC) analysis was performed to assess the early diagnostic value of Th22 and Th17 cells to predict sepsis-induced ALI. The lung injury prediction score (LIPS), IL-6, IL-17, IL-22, and levels of Th17 and Th-22 cells were 9.13, 14.02 ng/L, 13.06 ng/L, 22.90 ng/L, 8.80% and 7.40%, respectively, in the ALI patients and were significantly higher in the ALI group than in non-ALI group ($P < 0.05$). Pearson correlation analysis showed that LIPS, IL-17, IL-22, Th17 cells and Th22 cells were significant factors affecting sepsis-induced ALI ($P < 0.05$). The correlation analysis showed that the levels of Th22 cells in the peripheral blood of septic patients with ALI were positively correlated with LIPS, IL-22 and the levels of Th17 cells ($P < 0.05$), and the levels of Th17 cells were positively correlated with LIPS and IL-17 ($P < 0.01$). Multivariate logistic regression analysis showed that the LIPS (OR = 1.130), IL-17 (OR = 1.982), IL-22 (OR =2.612) and levels of Th17 (OR = 2.211) and Th22 (OR =3.230) cells were independent risk factor for ALI. The area under the curve of Th22 cells was 0.844 with a cutoff value of 6.81% to predict ALI. The sensitivity and specificity for early diagnosis of sepsis-induced ALI by Th22 cells were 78.72% and 89.13% respectively, which were better but statistically similar as compared with Th17 cells ($P > 0.05$).

Conclusions: The levels of Th22 and Th17 cells in peripheral blood are significantly increased in septic patients with induced ALI, and may be used for early diagnose of sepsis-induced ALI.

Background

Acute lung injury (ALI) is one of the major complications of severe sepsis. Although tremendous progress has been made in the treatment of acute lung injury, there are still needs for effective early diagnostic indicators for better control of the disease [1]. Recent studies show that lung injury prediction score (LIPS) is promising to discriminate patient with and without ALI development after severe sepsis [2], with high sensitivity but slightly low specificity. A significant amount of progress has been made in the elucidation of ALI pathophysiology and in predicting patient response, however, currently viable predictive molecular biomarkers for predicting the severity of ALI, or molecular-based ALI therapies is still not available. The proinflammatory cytokines TNF-α (tumor necrosis factor α), interleukin (IL)−1β, IL−6, IL−8, and IL−18 are among the most promising as biomarkers for predicting morbidity and mortality [3] and new and better biomarkers are important to further improve the diagnostic accuracy for better management of the disease.

ALI caused by sepsis is related to the disorder of autoimmunity and different CD4$^+$ T cells are involved in the onset and development of sepsis-induced ALI [4, 5]. In recent years, new helper T cell subsets have been discovered, including Th22 cells [6] that secrete interleukin−22 (IL−22). IL−22 plays very important role in a variety of autoimmune diseases and malignant tumors [7, 8]. However, the role of Th22 cells and IL−22 in sepsis-induced ALI is unclear. Early study shows that Th17 cell and interleukin IL−17 (IL−17) secreted from Th17 cells participate in the occurrence and development of sepsis [9]. However, their role in sepsis-induced ALI is largely unknown.

In this study, we compared the levels of Th22 and Th17 cells in septic patients with and without ALI development and assessed their value for early diagnosis of sepsis-induced ALI and the findings will help develop new biomarkers for diagnosis of sepsis-induced ALI.

Results

Factors affecting the onset of ALI in septic patients

Univariate analysis showed that LIPS, IL−6, IL−17, IL−22, the levels of Th22 and 17 cells were associated with the development of ALI in the septic patients, while gender, age and infection site were not (Table 1, Figure 1). In addition, PCT, CPR and WBC were also similar between the non-ALI and ALI groups. Multivariate logistic regression analysis using ALI as a dependent variable showed that LIPS, IL−17, IL−22, levels of Th22 and Th17 cells were significant factors leading to the development of ALI in the septic patients, after excluding confounding factors such as gender, age, length of stay and underlying diseases (Table 2).

Correlation analysis

The levels of Th22 cells in the peripheral blood of septic patients with ALI were significantly correlated with LIPS (r = 0.861, P < 0.01), IL−22 (r = 0.811, P < 0.01) and levels of Th17 cells (r = 0.791, P < 0.01). The levels of Th22 cells were significantly correlated with LIPS (r = 0.821, P < 0.01) and IL−17 (r = 0.881, P < 0.01).

ROC curve analysis

ROC curve analysis showed that the levels of circulating Th22 and Th17 cells had significantly value for early diagnosis of ALI in septic patients. With Th22 cells, the AUC was 0.844 with a sensitivity of 78.72% and specificity of 89.13%, which are slightly higher than those obtained with Th17 cells, although they were not statistically different (Figure 2 and Table 3, X^2 = 0.911, *P* > 0.05).

Discussion

The mortality of ALI caused by sepsis may be as high as 80% if it is not diagnosed and treated early [12]. Therefore, early diagnosis and effective evaluation of sepsis-induced ALI are important to reduce the risk

of mortality. The pathogenesis and development of sepsis involve tissue damage and infection by microorganisms and toxins, and imbalance of immune function in the patients.

In normal conditions, the ratio of T helper cell subsets is in a balanced state. When the body is stimulated by infection, cancer and other factors, the ratio may be changed [13]. Th22 cell is a newly discovered T cell helper cell subset and is shown to be associated with the occurrence, development and outcome of malignant tumors and autoimmune diseases [6, 14, 15]. For example, the Th22 levels in peripheral blood and cancer tissues of patients with gastric cancer increased significantly, resulting in poor prognosis [16]. Th22 cells are mainly derived from CD4 T cells and they specifically secrete IL−22 when stimulated by IL−6 or TNF-α [7], resulting in increased signal transduction by TNF-α that promotes inflammation [17, 18]. Sepsis is a T lymphocyte mediated acute inflammatory diseases and Th17 cell is shown to be involved in its occurrence and development [19, 20]. Since Th17 and Th22 both belong to T helper cell subsets, it is likely that Th22 cells play regulatory role in sepsis-induced ALI.

In this study, septic patients with and without ALI development were compared for the levels of Th22 cells and IL−22. It was found that the levels of Th22 cells and IL−22 are significantly higher in ALI patients than in non-ALI patients, suggesting that Th22 cells and IL−22 may be involved in the occurrence of sepsis-induced ALI. The mechanism underlying the involvement of IL−22 in ALI is unclear. It is likely that when IL−22 binds to its receptor, Janus Kinase 1 (JAK1)- signal transducer and signal transducer and activator of transcription 3 (STAT3) pathways are *a*ctivated, resulting in the production of chemokines and inflammatory factors that promote the recruitment of neutrophils and macrophages into the lungs [21]. IL−22 is also shown to upregulate the expression of pro-inflammatory factor S100 calcium binding protein that induces the production of acute phase proteins via the STAT3 pathway to generate liver injury as a result of acute-phase response [22−24]. In addition, IL−22 may enhance the signal transduction of tumor necrosis factor-α (TNF-α) to amplify the inflammatory response that leads to ALI.[25]. Nevertheless, more studies are needed to elucidate how Th22 cells and IL−22 are involved in the pathogenesis of sepsis-induced ALI.

IL−22 is mainly produced in three T cell subsets Th22, Th17 and Th1 cells [26]. Of which Th22 cells are the major producer of IL−22 [7]. Our analysis showed that the levels of Th22 cells and IL−22 are positively correlated, confirming that Th22 cells are important source of IL−22. Therefore, Th22 cells may involve in ALI development via IL−22.

Th17 cells are also CD_4 T cell subsets that promote the secretion of inflammatory factors by neutrophils and macrophages. Th17 cells may release IL−17 to promote the aggregation of neutrophils in the lung, resulting in acute exacerbation of chronic obstructive pulmonary disease [27, 28]. This is in line with our results that there is an increased levels of Th17 cells in patients with ALI as compared to those without ALI, suggesting that Th17 may be involved in the development of ALI. Since ALI is correlated with the levels of both Th22 and Th17 cells, it might be possible that during ALI development, T lymphocytes are promoted to differentiate into Th22 and Th17 cells that function synergistically in ALI. However, the mechanism and interactions remain to be further studied.

ROC curve analysis showed that both Th22 and Th17 cells may be used as early predictors of ALI after sepsis. The AUC areas were 0.844 and 0.813 for Th22 and Th17, indicating that they are reliable predictors for ALI. At the cutoff point of 6.18% and 8.04%, the diagnostic sensitivity and specificity were 78.72%, 71.40% and 89.13%, 86.41%. Although a number of biomarkers have been proposed and identified for ALI prediction [3], our findings add new diagnostic tools for ALI after sepsis and would help management of this important disease. However, since this was a single-center study, large scale, multiple-centers studies are needed to further validate our findings.

In conclusion, we have found that the levels of Th22 and Th17 cells in peripheral blood are significantly increased in septic patients with induced ALI in addition to IL−6, IL−17 and IL−22 and may be used for early diagnose of sepsis-induced ALI.

Subjects And Methods

Subjects

A total of 479 septic patients treated between January 2013 and January 2018 at our hospital were enrolled in the study. They were separated into non-ALI (n = 377) and ALI (n = 102) groups based on the presence or absence of ALI at admission. Informed consent was obtained from every patients and the study protocols were approved by the Research Ethic Committee of the hospital.

Patients were included if they were 18 years old or older, and diagnosed severe and septic shock [10]. Diagnostic criteria for ALI includes having a history of primary disease, clinical symptoms of dyspnea or distress, hypoxemia with a PaO2/FIO2 ratio of < 300, and chest radiography showed bilateral lung infiltration. Patients with autoimmune diseases or severe immunodeficiency, malignant tumors and other severe chronic diseases were excluded. Patients were also excluded if they had used immunosuppressive agents with three months.

Data collection and measurements

Baseline demographic characteristics, clinical and laboratory data including the etiology of sepsis infection were collected. The LIPS was calculated using the worst values within the 24 hours after admission using the model validated by the Lung Injury Prevention Study Investigators 2011 [11].

For determination of TH22 and Th17 cells, 4 ml of fasting venous blood was drawn from the patients in the morning within 24 hour of admission. The blood was added with heparin as anticoagulant and diluted with equal volume of RPMI−1640 medium with phorbol ester (1 μg/ml), ionomycin (50 μg/ml) and monensin (100 μg/ml) and incubated at room temperature for 4 h. The cell suspensions were then added with anti-human CD4 monoclonal antibody, fixed and pelleted. The cells were incubated with monoclonal antibodies against IL−22 and IL−17, and loaded onto a flow cytometer (ThermoFisher, USA) for analysis. IL−22, IL−17 and IL−6 were measured using ELISA kits from Jiachen Biotech, Nanjing,

China, according to the supplier's instructions. Procalcitonin (PCT) was assayed using a kit from Roche, USA and C reactive protein (CRP) was determined using immunoturbidimetry method.

Statistical analysis

The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean ± s.d. (standard derivation), and means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Non-normal variables were reported as median (interquartile range [IQR]) and tested using nonparametric rank sum test. The frequencies of categorical variables were compared using Pearson χ^2. The Pearson analysis was performed to calculate the correlation. Receiver operating characteristic (ROC) curves were drawn to calculate the area under curve (AUC), sensitivity and specificity. The data were analyzed by SPSS version 11.5 for Windows (SPSS Inc., Chicago, IL, USA), and A value of $P < 0.05$ was considered significant.

Abbreviations

AUC: area under curve

ALI: acute lung injury

IL: interleukin

ROC: Receiver operating characteristic curve

LIPS: lung injury prediction score

TNF-α: tumor necrosis factor α

PCT: procalcitonin

CRP: C reactive protein

IQR: interquartile range

Declarations

Ethics approval and consent to participate⊠This study was approved by the ethical committee of Zhejiang Provincial People's Hospital. Informed consent was obtained every patient.

Consent for publication⊠Not applicable

Availability of data and material⊠The datasets used during the current study are available from the corresponding author on reasonable request.

Authors' contributions GL and LZ designed the study. LZ, NH and KZ collected the data and performed analysis GL, LZ and HL drafted the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Univariate analysis of factors affecting acute lung injury in patients with sepsis

Variables		Septic patients (n = 377)	Septic patients with acute lung injury (n = 102)	Statistics	Pvalue
Gender	Male	215	60	1.180	0.622
	Female	162	42		
Age (year)		52.92 ± 10.21	53.12 ± 11.53	- 0.941	0.594
Infection site (n)	Lung	95	24	0.810	0.708
	Abdominal cavity	79	19		
	Urinary system	58	17		
	Intracranial	56	17		
	Hematogenous	49	12		
	Other	41	13		
Lung injury prediction score		4.16 ± 0.19	9.13 ± 0.18	- 8.251	0.016
Procalcitonin (ng/ml)		0.50 (0.34, 0.71)	0.51 (0.34, 0.75)	1.922	0.138
C reactive protein P (µg/ml)		10.31 (8.85, 12.89)	10.54 (8.42, 13.17)	1.424	0.213
White blood cell ($\times 10^9$/L)		9.78 ± 0.46	9.98 ± 0.51	- 0.967	0.557
IL - 6 (ng/L)		8.41 (6.85, 10.54)	14.02 (11.55, 15.17)	7.472	0.032
IL - 17 (ng/L)		14.72 (11.22, 18.09)	23.06 (18.92, 26.89)	7.954	0.021
IL - 22 (ng/L)		12.99 (10.77, 15.42)	22.90 (19.75, 25.04)	8.511	0.021
Thl7 (%)		3.10 ± 0.83	8.80 ± 1.18	- 8.560	0.020
Th22 (%)		2.10 ± 0.56	7.40 ± 0.83	- 9.123	0.016

Table 2. Multivariate logistic regression analysis of acute lung injury in patients with sepsis

Varuable	β	SE	Wald value	OR	95% CI	Pvalue
Lung injury prediction score	0.531	0.203	8.646	1.130	1.140 ~ 1.962	0.035
IL - 17	0.526	0.212	11.779	1.982	1.221 ~ 2.390	0.021
IL - 22	0.795	0.185	17.238	2.612	1.182 ~ 2.991	0.012
Thl7 cell	0.672	0.199	14.127	2.211	1.133 ~ 2.572	0.021
Th22 cell	0.813	0.174	21.926	3.230	1.814 ~ 4.180	0.011

Table 3. Analysis of ROC curve of predicting sepsis-induced acute lung injury by the levels of Th22 and Th17 cells

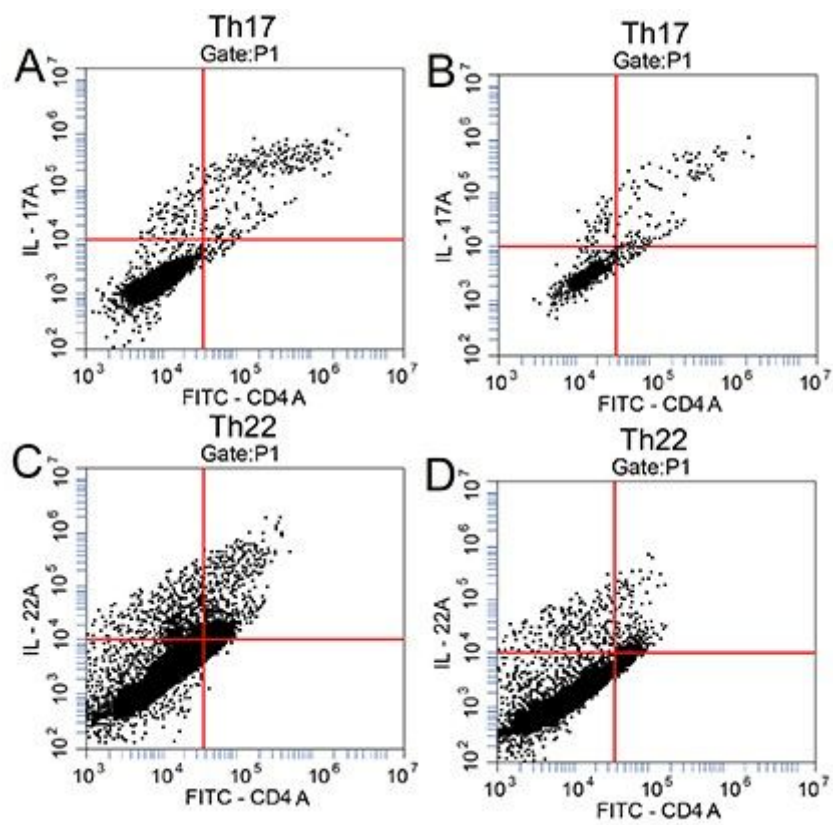| Predictor	AUC (95%CI)	P value	Cutoff (%)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Thl7	0.813 (0.750 ~ 0.896)	< 0.001	8.04	71.40	86.41	86.20	65.13
Th22	0.844 (0.798 ~ 0.941)	< 0.001	6.81	78.72	89.13	90.82	62.62

Figures

Figure 1

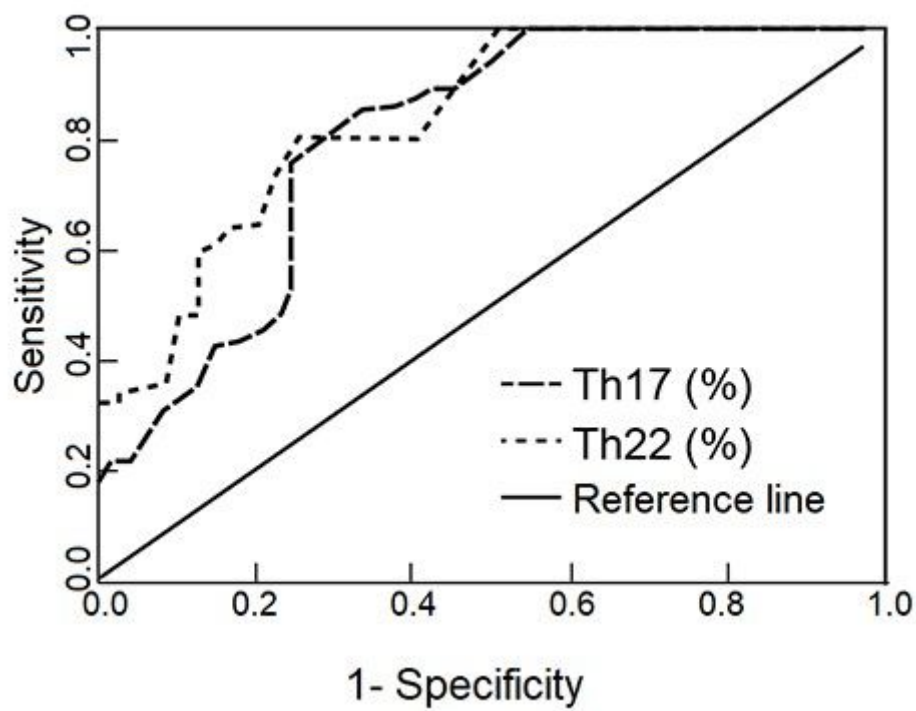Flow cytometric analysis of Th22 and 17 cells.

Figure 2

The ROC curves of Th22 and Th17 cells for predicting sepsis-induced acute lung injury.