IL-18 and IL-35 in the serum of patients with sepsis thrombocytopenia and the clinical significance

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Abstract. Expression levels of interleukin-18 (IL-18) and IL-35 in the serum of patients with sepsis and without thrombocytopenia and patients with sepsis thrombocytopenia (TCP) were detected to preliminarily investigate their clinical significance. One hundred and sixty-six patients admitted to Jinan Central Hospital Affiliated to Shandong University from July 2013 to September 2015 were retrospectively analysed. There were 96 patients with sepsis without thrombocytopenia in the sepsis group, and 70 patients with sepsis TCP in the sepsis TCP group. In the same period, 80 healthy subjects were selected as the control group. Fluorescent quantitative PCR was used for the detection the expression of mRNA levels of IL-18 and IL-35, and Enzyme-linked immunosorbent assay for the detection of the protein concentrations of IL-18 and IL-35 in the serum of peripheral blood. The correlation between IL-18, IL-35 and platelets was analyzed. There were significant differences in albumin, creatinine, total bilirubin and platelet count between the sepsis group and the sepsis TCP group ($P<0.05$); the expression levels of mRNA of IL-18 and IL-35 in a karyocyte in peripheral blood in the sepsis group and the sepsis TCP group were higher than those in the control group ($P<0.05$); the expression of mRNA of IL-18 and IL-35 in the sepsis TCP group was higher than those in the sepsis group ($P<0.05$). The concentration of IL-18 and IL-35 in the sepsis TCP group was higher than in the sepsis group ($P<0.05$); IL-18 and IL-35 were negatively correlated with platelets ($r=-0.8749$, -0.6228, $P<0.001$). There was a significant positive correlation between serum IL-18 and IL-35 in the control group, sepsis group, and sepsis TCP group ($r=0.5124$, 0.5718, 0.5511, $P<0.001$). IL-18 and IL-35 were negatively correlated with the reduced degree of platelets in patients with sepsis and are likely to play an important role in the pathogenetic process of sepsis TCP.

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Introduction

Sepsis refers to hosts' uncontrolled immune response to infection, which in turn affects organ functions and causes death of critically ill patients; it is also one of the main problems in the global health care system (1-3). Pathogenesis of sepsis is extremely complex and has not yet been fully clarified (4). Data show that the decrease of platelets occur in 35 to 59% of patients with sepsis. It was found that the symbolic risk factor of the death of patients with sepsis is thrombocytopenia (TCP), which is induced by infections, and the recovery of the minimum value of platelet count is associated with the decrease of mortality (5-7). Studies have shown that when patients are stimulated by endotoxin, platelet activation can be induced by some pathways, which makes platelets play an important role and induce the body to produce an immune response (8,9).

Interleukin-18 (IL-18), a general pro-inflammatory cytokine, is widely distributed in the body, and it can facilitate the production of interferon-γ (IFN-γ) and regulate the initial immune response of infection and inflammation (10). Studies have found that the severity and prognosis of sepsis are closely related to IL-18 (11,12). IL-35 is produced by regulatory T cells and is related to occurrence and progression of a variety of diseases; it is mainly involved in immune response, autoimmune diseases, infections and inflammation, as a new inflammatory factor, IL-35 can be used as a promising therapeutic target (13,14). Recent studies have shown that IL-35 can effectively improve infectious diseases and delay the development of the immune inflammatory response caused by itself, and infections can promote Treg and other cells to secrete more IL-35, which enhances the body's tolerance to inflammatory response caused by infections (15). Therefore, it has been speculated that IL-18 and IL-35 are involved in sepsis TCP. However, there are currently few studies on the expression of IL-18 and IL-35 and the correlation between them and thrombocytopenia in patients with sepsis TCP.

In this study, the expression of IL-18 and IL-35 in the serum and in karyocytes in peripheral blood of patients with sepsis and patients with sepsis TCP were studied, and the correlation between IL-18, IL-35 and platelet and their clinical significance were investigated.

Patients and methods

General data. In total, 166 patients who were admitted to Jinan Central Hospital Affiliated to Shandong University (Jinan, China) from July 2013 to September 2015 were collected, and they conformed to the diagnostic criteria of sepsis developed by the American College of Critical Care Medicine in 2001 (16). Among them, 96 patients who had sepsis without thrombocytopenia were the sepsis group; there were 55 males and 41 females, aged 65.12±8.11 years. Seventy patients with sepsis TCP were the sepsis TCP group; there were 45 males and 25 females, aged 66.21±10.12 years. Eighty healthy subjects were selected as the control group, aged 64.20±6.81 years, including 49 males and 31 females.

Inclusion criteria: Patients who did not have unhealty habits; patients wgo actively cooperated with the treatment; patients with complete clinicopathological data; patients with sepsis caused by different pathogen infections (G-bacteria, G^+ bacteria, fungi and no-bud anaerobic bacteria) and met the latest diagnostic criteria for sepsis; patients with peripheral blood platelet count $\leq 50x10^9$/l (the criterion for TCP).

Exclusion criteria: Patients in gestation period or puerperium; patients under the age of 18 years; patients had diseases which affected the formation of platelet, such as primary thrombocytosis; patients who had a history of malignancy in blood system; patients with decompensated cirrhosis or failure; patients who had history of chemotherapy; patients who received therapeutic anticoagulation or blood transfusion in the prior four weeks; patients died within 24 h after they were hospitalized.

This study was approved by the Ethics Committee of Jinan Central Hospital Affiliated to Shandong University, and the experimental content relating to the patients was described in detail. The patients and their families agreed and signed an informed consent form.

The collection of samples. Sequential organ failure assessment (SOFA) and acute physiology and chronic health evaluation (APACHE II) were carried out for all the patients within 24 h after they were hospitalized. Peripheral venous blood (5 ml) was taken immediately, and left at room temperature for 20 min. Then it was centrifuged at 1,006.2 x g at 4˚C for 10 min, with a centrifugal radius of 10 cm. The supernatant was collected and placed at -80˚C until testing. At the same time, 5 ml of peripheral venous blood of healthy controls was collected, centrifuged and stored in a refrigerator at -80˚C, and repeated freeze and thaw were avoided.

Experiment steps

Fluorescent quantitative polymerase chain reaction (RT-PCR) was used to detect the expression of mRNA levels of IL-18 and IL-35 in karyocytes in peripheral blood. Expression of mRNA levels of IL-18 and IL-35 in karyocytes in peripheral blood were detected by RT-PCR, and mononuclear cells in peripheral blood were isolated by Ficoll-Hypaque density gradient centrifugation at 400 x g at 4˚C for 40 min. TRIzol extraction reagent was used to extract total RNA; an ultraviolet spectrophotometer was used to detect the purity and concentration; reverse transcription kits were used to transcribe RNA samples into cDNA in strict accordance with the instructions. ABI PRISM-7500 amplification instrument and SYBR Premix Ex Taq kit were used for RT-PCR reactions. For PCR amplification, the reaction system was: at 95˚C for 10 min, at 95˚C for 15 sec, at 60˚C for 1 min, for 40 cycles. The melting curve was analyzed by increasing the temperature from 60 to 95˚C, with a temperature transition rate of 0.1˚C/sec, three parallel reaction wells were set for each sample, and the experiment was repeated at least three times. The primers of the experiment were designed by Primer Premier 5.0 (Premier Biosoft) primer design software, and they were synthesized by Tianjin Saier Biotechnology Co., Ltd. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal reference, and the specific sequences are shown in Table I. In the results, the fluorescence signal is in the process of amplification of the cycle Ct value, and the inflection point which started from the background and entered the exponential growth phase corresponding to the number of cycles; $2^{-\Delta Ct}$ was used to calculate the relative expression levels of mRNA of IL-18 and IL-35 of each sample.

The levels of IL-18 and IL-35 in the serum of the samples were detected by enzyme-linked immunosorbent assay (ELISA). Standard sample (50 μl) was added into the wells coated with enzyme label. First, 40 μl of sample diluent was added into the wells of the sample to be tested, and then 10 μl of the sample (the dilution ratio of the sample was 5 times) was added, avoiding touching the wall of the wells during these steps, then the wells were shaken gently. The reaction wells were sealed with a sealing film, and then incubated in a water bath kettle or an incubator for 30 min. After this, the sealing film was carefully removed, the liquid was discarded; and the wells were dried with absorbent paper, then each well was filled with washing solution. After 30 sec, this step was repeated five times and the wells were dried. Apart from the blank wells (the steps of the blank control wells were identical to the other steps, but enzyme-labeled reagent and sample were not added), 50 μl of enzyme-labeled reagent was added into each well, next they were incubated at 37˚C for 30 min and then they were washed. 50 μl of substrate A and substrate B was added into each well, and the color was developed at 37˚C for 15 min in the dark. Then 50 μl of stop solution was added into each well, and zero setting was made with a blank well, and the absorbance (OD value) of each well was detected at the wavelength of 450 nm in 25 min. The levels of IL-18 and IL-35 in the serum were calculated.

Experimental instruments and reagents. SYBR Premix Ex Taq kit, TRIzol extraction kit, cDNA reverse transcription kit (all from Takara Company), UV spectrophotometer (Shanghai Mapada Instrument Co., Ltd.), ABI PRISM-7500 amplification instrument (Shanghai Bajiu Industrial Co., Ltd.), IL-18 ELISA kit and IL-35 ELISA kit were from Moshake Biology Co., Ltd., BS-1101 enzyme-labeled instrument was from Beijing Linmao Technology Co., Ltd.

Observation indicators. The expression of platelet count (PLT), C-reactive protein (CRP), creatinine, and total bilirubin was detected. APACHE II score, SOFA score, infection site and 28-day mortality were recorded. The expression of mRNA of

Table I. Sequences.

Gene	Upstream	Downstream
IL-18	5'-CTTGAATCTAAATTATCAGTC-3'	5'-GAAGATTCAAATTGCATCTTAT-3'
IL-35	5'-GCTCCCTACGTGCTCAATGTC-3'	5'-AGGGTCGGGCTTGATGATGT-3'
GAPDH	5'-GGAGCGAGATCCCTCCAAAAT-3'	5'-GGCTGTTGTCATACTTCTCATGG-3'

IL, interleukin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Table II. Comparison of the clinical basic data in the three groups (mean ± SD)/[n(%)].

Clinical features	Control group (n=80)	Sepsis group (n=96)	Sepsis TCP group (n=70)	F/χ^2 value	P-value
Sex				0.85	0.65
Male	49 (61.3)	55 (57.3)	45 (64.3)		
Female	31 (38.7)	41 (42.7)	25 (35.7)		
Age (years)	64.20±6.81	65.12±8.11	66.21±10.12	1.08	0.34
Hemoglobin (g/dl)	13.93±3.13	10.12±2.42[a]	10.45±2.33[a]	52.15	<0.01
Albumin (g/dl)	3.65±1.21	3.03±0.78[a]	2.32±0.62[a,b]	40.12	<0.01
Creatinine (mg/dl)	1.22±0.65	1.51±0.87[a]	1.94±1.03[a,b]	13.26	<0.01
Total bilirubin (mg/dl)	0.21±0.12	0.50±0.20[a]	0.65±0.31[a,b]	80.32	<0.01
Platelet count (x10^9/l)	186.11±21.31	178.34±13.21[a]	72.36±20.12[a,b]	654.3	<0.01
Whole blood leukocyte count (x10^9/l)	9.34±1.02	10.45±1.32[a]	10.32±1.41[a]	19.19	<0.01
Fibrinogen (g/l)	3.47±1.00	4.78±1.32[a]	4.98±1.48[a]	32.73	<0.01
CRP (mg/dl)	4.23±3.01	9.32±6.68[a]	10.11±7.21[a]	22.94	<0.01

[a]P<0.05 compared with the control group; [b]P<0.05 compared with the sepsis group. CRP, C-reactive protein; TCP, thrombocytopenia.

IL-18 and IL-35 in karyocytes in peripheral blood and their expressions in the serum were observed. The correlation between IL-18, IL-35 and platelets in the serum of the patients with sepsis TCP was analyzed.

Statistical processing. SPSS 19.0 software system (IBM Corp.) was used to statistically analyze the experiment data. The enumeration data were expressed in the form of [n(%)], Chi-square test was used in comparison between groups in this study. The measurement data were expressed in the form of mean ± SD, t-test was used in the comparison between two groups; variance analysis followed by LSD-t test was used in the comparison between groups, Pearson's correlation coefficient was used in the bivariate normal distribution data. At P<0.05, the difference was statistically significant.

Results

Comparison of the clinical basic data in the three groups. There were no statistically significant differences in sex and age between the three groups (P>0.05). The differences in hemoglobin, albumin, creatinine, total bilirubin, platelet count, whole blood leukocyte count, fibrinogen and C-reactive protein (CRP) concentration between the three groups were statistically significant (P<0.05). There were significant differences in albumin, creatinine, total bilirubin and platelet count between the sepsis group and the sepsis TCP group (P<0.05) (Table II).

Comparison of infection site and organ damage and other baseline data of the patients in the sepsis group and the sepsis TCP group. There was no difference in APACHE II score and SOFA score between the patients in the sepsis group and the sepsis TCP group (P>0.05); there was no significant difference in the source of infection, the site of infection, the number of organ damage and the type of organ damage between the two groups (P>0.05); there were significant differences in presence and absence of shock, ICU mortality, and 28-day mortality between the two groups (P<0.05); the rate of patients with shock, ICU mortality and 28-day mortality in the sepsis group were lower than those in the sepsis TCP group (Table III).

Expression of mRNA of IL-18 and IL-35 in karyocytes in peripheral blood in the three groups. As shown in Fig. 1, the expression of mRNA of IL-18 in karyocytes in peripheral blood in the sepsis group and the sepsis TCP group was higher than that in the control group (P<0.05), and the expression of mRNA of IL-18 in the sepsis TCP group was higher than that in the sepsis group (P<0.05). Expression of mRNA of IL-35 in a karyocyte in peripheral blood in the sepsis group and the sepsis TCP group was higher than that in the control group

Table III. Comparison of infection site and organ damage and other baseline data of the patients in the sepsis group and the sepsis TCP group (mean ± SD)/[n(%)].

Baseline data	Sepsis group (n=96)	Sepsis TCP group (n=70)	χ^2/t value	P-value
APACHE II score	24.12±7.21	22.79±8.99	1.06	0.29
SOFA score	12.76±4.27	11.56±4.12	1.82	0.07
Source of infection			0.45	0.80
Gram negative bacteria	39 (40.6)	27 (38.6)		
Gram positive bacteria	34 (35.4)	23 (32.8)		
Others	23 (24.0)	20 (28.6)		
Site of infection			0.40	1.00
Pulmonary infection	31 (32.3)	21 (30.0)		
Abdominal infection	11 (11.4)	9 (12.9)		
Blood infection	10 (10.4)	8 (11.4)		
Urinary tract infection	12 (12.5)	10 (14.3)		
Soft tissue infection	9 (9.4)	7 (10.0)		
Others	23 (24.0)	15 (21.4)		
Presence or absence of shock			13.84	<0.01
Yes	14 (14.6)	28 (40.0)		
No	82 (85.4)	42 (60.0)		
The number of organ damage			0.06	0.80
≤2	54 (56.2)	38 (54.3)		
>2	42 (43.8)	32 (45.7)		
Type of organ damage			0.16	1.00
Arterial hypotension	13 (13.5)	9 (12.9)		
Arterial hypoxemia	17 (17.7)	12 (17.1)		
Acute lung injury	12 (12.5)	8 (11.4)		
Acute renal failure	24 (25.0)	18 (25.7)		
Acute respiratory failure	16 (16.7)	13 (18.6)		
Nerve injury	14 (14.6)	10 (14.3)		
ICU mortality	5 (5.2)	14 (20.0)	8.74	0.003
28-day mortality	31 (32.3)	45 (64.3)	16.69	<0.01

TCP, thrombocytopenia; SOFA, sequential organ failure assessment; APACHE II, acute physiology and chronic health evaluation.

(P<0.05), the expression of mRNA of IL-35 in the sepsis TCP group was higher than that in the sepsis group (P<0.05).

Concentration of IL-18 and IL-35 in the serum in the three groups. The concentration of IL-18 and IL-35 in the serum in the three groups was detected and the results showed that the concentration of IL-18 in the serum in the sepsis group and the sepsis TCP group was higher than that in the control group (P<0.05); the concentration of IL-18 in the sepsis TCP group was higher than that in the sepsis group (P<0.05). The concentration of IL-35 in the serum in the sepsis group and the sepsis TCP group were higher than that in the control group (P<0.05); the concentration of IL-35 in the sepsis TCP group was higher than that in the sepsis group (P<0.05) (Fig. 2).

Correlation analysis between platelets and IL-18 and IL-35. Correlation between platelets and IL-18 and IL-35 was studied. Correlation analysis of the protein concentrations of IL-18 and IL-35 in the serum in the sepsis TCP group and the platelet count of the patients was performed. Figs. 3 and 4 show that IL-18 and IL-35 are negatively correlated with platelets (r=-0.8749, -0.6228, P<0.001).

Correlation between serum IL-18 and IL-35. Correlation between IL-18 and IL-35 in serum was analyzed using Pearson's correlation coefficient (Figs. 5-7). There was a significant positive correlation between serum IL-18 and IL-35 in the control group, sepsis group, and sepsis TCP group (r=0.5124, 0.5718, 0.5511, P<0.001).

Discussion

Sepsis is one of the serious complications of acute and critically ill patients with shock, burns and severe trauma in clinic, often leading to septic shock and multiple organ dysfunction syndrome, which poses a serious threat to human health (17).

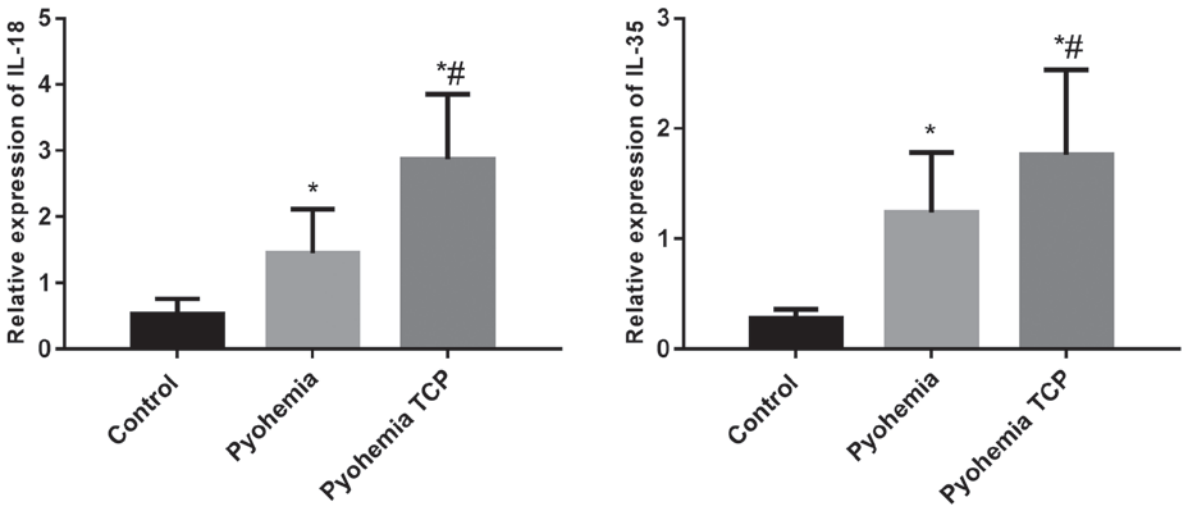

Figure 1. Expression of IL-18 and IL-35 mRNA in three groups of peripheral blood mononuclear cells. Expression of IL-18 mRNA in peripheral blood mononuclear cells of the sepsis and sepsis TCP groups was higher than that of the control group (P<0.05), and the mRNA expression of IL-18 in the sepsis TCP group was higher than that in the sepsis group (P<0.05). Expression of IL-35 mRNA in peripheral blood mononuclear cells of the sepsis and sepsis TCP groups was higher than that of the control group (P<0.05), and the mRNA expression of IL-35 in the sepsis TCP group was higher than that in the sepsis group (P<0.05). *P<0.05 compared with the control group; #P<0.05 compared with the sepsis group. TCP, thrombocytopenia; IL, interleukin.

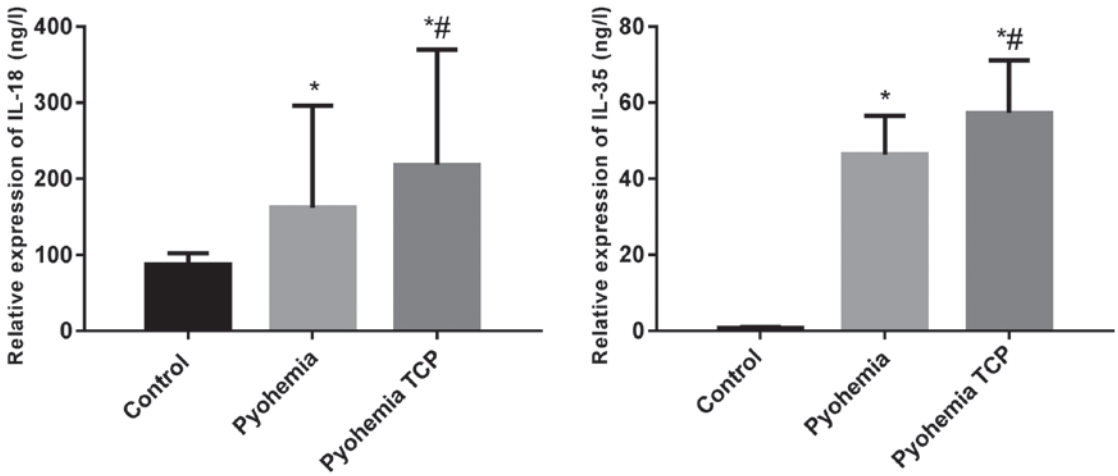

Figure 2. Concentration of IL-18 and IL-35 in the serum of three groups The serum IL-18 concentration in sepsis and sepsis TCP groups was higher than that in the control group (P<0.05). Concentration of IL-18 in the sepsis TCP group was higher than that in the sepsis group (P<0.05). Serum IL-35 concentration in the sepsis and sepsis TCP groups was higher than that in the control group (P<0.05), and the concentration of IL-35 in the sepsis TCP group was higher than that in the sepsis group (P<0.05). *P<0.05 compared with the control group; #P<0.05 compared with the sepsis group. TCP, thrombocytopenia; IL, interleukin.

Bone marrow is the most vulnerable. Inflammatory factors and bacterial toxins in the body inhibit hematopoietic cells in bone marrow (including megakaryocytes), causing thrombocytopenia by inhibiting megakaryocytes from producing platelets (18). Moreover, many ways facilitate platelet activation, thus activating the coagulation pathway, forming local microthrombus and increasing platelet consumption, which can cause thrombocytopenia; the severity of sepsis is closely related to the above processes (19,20).

IL-18, a gene multi-dominant polypeptide regulator, is involved in growth and differentiation of cells and regulates the body's immune response (21). Study by Cui *et al* (22) reported that the expression level of IL-18 increases, and the expression of miR-130a decreases in plasma and miRNA of patients with severe sepsis and thrombocytopenia, suggesting that IL-18 and miR-130a may be involved in the pathophysiological process of severe sepsis accompanied by thrombocytopenia. As a member of the family of IL-12, IL-35 belongs to heterodimeric proteins and is made up of Ebi3 (β-chain) and p35 (α-chain) (23). Studies have found that IL-35 can facilitate the secretion of IL-10 in rheumatoid arthritis and can inhibit the production of IL-17 and IFN-γ, thereby effectively inhibiting the inflammation in antigen-specificity immune response state; IL-35 plays an immunomodulatory role in a variety of inflammatory diseases (24). A study by Du *et al* (25) suggests that IL-35 can be used as a novel candidate biomarker for the diagnosis of early neonatal sepsis, and it is superior to PCT in the diagnosis.

This study found that when hemoglobin, albumin, creatinine, total bilirubin, platelet count, whole blood leukocyte count, fibrinogen and CRP were compared between the three groups, the differences were statistically significant (P<0.05).

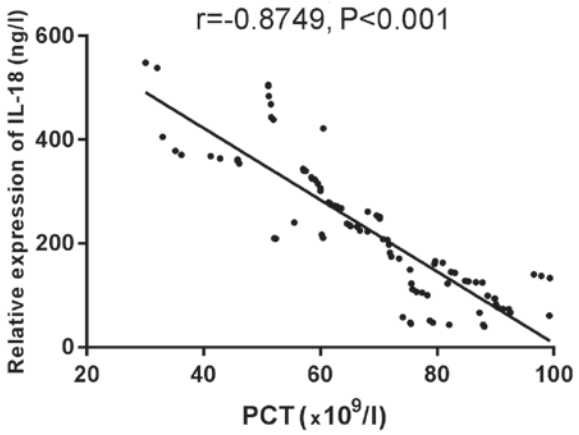

Figure 3. Correlation analysis between platelets and IL-18. Serum IL-18 in the sepsis TCP group was negatively correlated with platelets (r=-0.8749, P<0.001). TCP, thrombocytopenia; IL, interleukin.

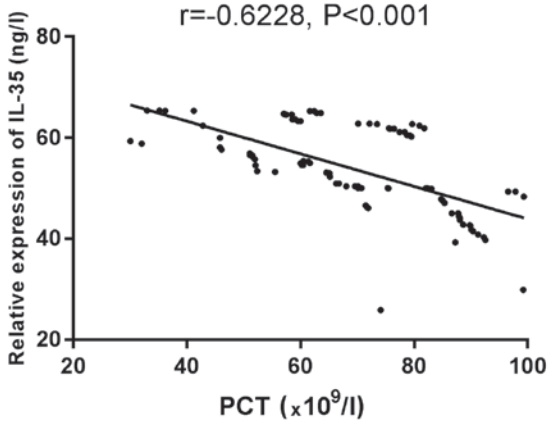

Figure 4. Correlation analysis between platelets and IL-35. Serum IL-35 in the sepsis TCP group was negatively correlated with platelets (r=-0.6228, P<0.001). TCP, thrombocytopenia; IL, interleukin.

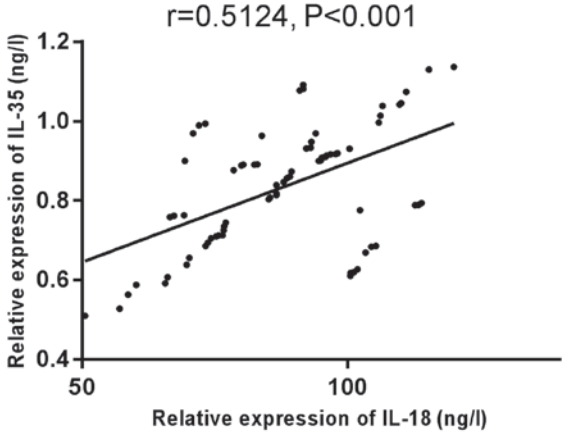

Figure 5. Correlation between serum IL-18 and serum IL-35 in the control group. According to Pearson's correlation coefficient analysis, serum IL-18 was significantly positively correlated with serum IL-35 (r=0.5124, P<0.001). IL, interleukin.

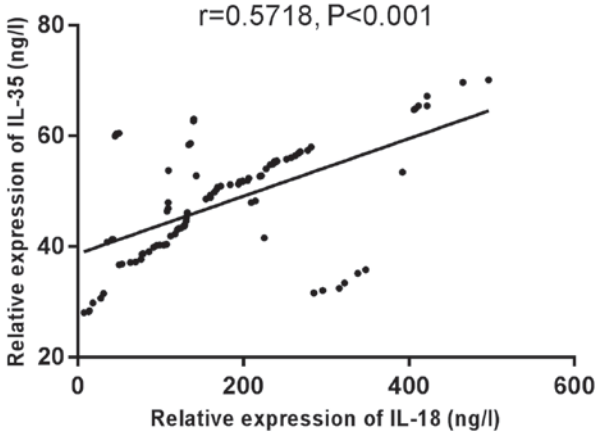

Figure 6. Correlation between serum IL-18 and serum IL-35 in the sepsis group. According to Pearson's correlation coefficient analysis, serum IL-18 was significantly positively correlated with serum IL-35 in the sepsis group (r=0.5718, P<0.001). IL, interleukin.

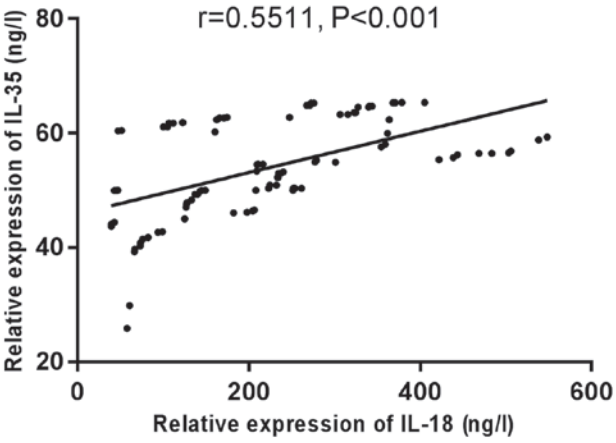

Figure 7. Correlation between serum IL-18 and serum IL-35 in sepsis TCP group. According to Pearson's correlation coefficient analysis, serum IL-18 was significantly positively correlated with serum IL-35 in the sepsis TCP group (r=0.6159, P<0.001). TCP, thrombocytopenia; IL, interleukin.

There was a significant difference in albumin, creatinine, total bilirubin and platelet count between the sepsis group and the sepsis TCP group. There was no difference in APACHE II score and SOFA score between the sepsis group and the sepsis TCP group; there was no significant difference in the source of infection, the infection site, the number of organ damage and the type of organ damage between the two groups (P>0.05); the number of patients with shock, ICU mortality and 28-day mortality in the sepsis group were lower than those in the sepsis TCP group. Oberholzer *et al* (26) found that the level of IL-18 in patients with sepsis was significantly higher than that in healthy people; the levels of IL-18 in patients with septic shock and patients with sepsis who died were higher than those in patients without shock and patients with sepsis who survived. Sun and Zhang (27) detected the level of IL-18 in patients with ICU sepsis in the first 3 days, and it was found that the severity and prognosis of patients with sepsis were closely related to the increase of level of IL-18. It is reported that the high expression of IL-18 in the serum may be an early predictive factor of death (28). IL-35 in plasma is associated with the severity of sepsis and can also be used as a warning factor of sepsis to predict illness condition (29). This study

showed that the expression levels of mRNA of IL-18 and IL-35 in karyocytes in peripheral blood in the sepsis group and the sepsis TCP group were higher than those in the control group, and the expression levels of mRNA of IL-18 and IL-35 in the sepsis TCP group were higher than those in the sepsis group ($P<0.05$). By detecting the concentrations of IL-18 and IL-35 in the serum in the three groups, the results showed that the concentrations of IL-18 and IL-35 in the serum in the sepsis group and the sepsis TCP group were higher than those in the control group, and the concentrations of IL-18 and IL-35 in the sepsis TCP group were higher than those in the sepsis group. Related studies have found that the concentration of IL-35 in the serum of patients with sepsis is significantly higher than that in healthy people, suggesting that the expression level of IL-35 increases in patients with sepsis, and the concentration of IL-35 in patients with sepsis who die is significantly higher than that in survivors (30). Thrombocytopenia is one of the most common abnormalities in patients with severe sepsis (24). In this study, it is found that the concentration of IL-35 in patients with sepsis and thrombocytopenia is higher than that in patients with sepsis, which provides reference for future clinical research. Related literature reports that platelet counts in children with immune thrombocytopenic purpura have a negative correlation with serum IL-18 (31). Little is known about the correlation between IL-35 and platelet count. In the present study, the correlation analysis was on the concentrations of IL-18 and IL-35 in the serum in the sepsis TCP group and the platelets of patients. It was found that IL-18 and IL-35 are negatively correlated with platelets ($r=-0.8749$, -0.6228, $P<0.001$). This was possibly caused by the death ligand in platelets being highly expressed due to inflammatory factors, which enhance the killing effect of lymphocytes and cause apoptosis of platelets, thereby causing a decrease in platelet count. The specific mechanism remains to be further investigated. It has been reported that elevated serum IL-35 levels in patients with sepsis are associated with logistic organ dysfunction (LOD) or simplified acute physiology score (SAPS II) and are associated with inflammatory markers (32). In this study, the correlation between serum IL-18 and IL-35 was analyzed, and serum IL-18 was found to be significantly positively correlated with IL-35. This indicates that IL-35 may be involved in the regulation of IL-18. However, there is currently no research in this direction, and more investigations are needed.

This study investigated the expression and clinical significance of IL-18 and IL-35 in the serum of patients with sepsis TCP, and confirmed that there is a correlation between the expression of IL-18 and IL-35 and platelets in the serum of patients with sepsis TCP, which has certain clinical significance. However, since this is a retrospective study, all confounding factors that may exist in critically ill patients in different time cannot be controlled.

In conclusion, IL-18 and IL-35 are negatively correlated with the degree of thrombocytopenia in patients with sepsis, which indicates that these two factors play an important role in the pathogenetic process of sepsis.

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Availability of data and materials

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

Authors' contributions

MZ conceived the study and drafted the manuscript. MZ and XR detected CRP and NPY concentrations. ML and SW performed some of the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Jinan Central Hospital Affiliated to Shandong University (Jinan, China). Patients who participated in this research had complete clinical data. Patients and their family signed an informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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