Expression of Serum Cytokines Profile in Neonatal Sepsis

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Abstract

Background

Sepsis remains a major cause of neonatal death, but its underlying pathological mechanisms are poorly understood.

Methods

To characterize the serum cytokine/chemokine profile in neonates with sepsis, we enrolled 40 full-term neonates with sepsis and 19 neonates without infection as controls. Forty cytokines/chemokines in serum were analyzed using the Luminex Bead Immunoassay System. Serum IL-17 was measured using an enzyme-linked immunosorbent assay.

Results

Our results showed that serum IL-6, IL-8, TNF-α, IL-1β, MIF, CXCL13, CXCL1, CXCL2, CXCL5, CXCL6, CXCL16, CCL27, CCL2, CCL8, CCL3, CCL20, CCL23, CCL27, and CX3CL1 levels were significantly increased in neonates with sepsis compared to those in the control group (all $p<0.05$). The levels of serum CXCL6, CXCL1, IL-6, CXCL10, CXCL11, CCL20, and IL-17 were higher in late-onset sepsis (LOS) than in early-onset sepsis (EOS) (all $p<0.05$). Conversely, serum IL-16, CXCL16, and CCL22 were lower in LOS than in EOS (all $p<0.05$). The levels of CX3CL1, CXCL2, CCL8, and TNF-α were all positively correlated with SOFA scores.

Conclusion

Our findings revealed that excessive pro-inflammatory cytokines might be involved in neonatal sepsis. In addition, chemokines significantly increased the recruitment of immune cells after infection to participate in the anti-infection defense of neonates, but this could lead to damage.

Background

Neonatal sepsis is a systemic infection by bacteria, viruses, or fungi, which is characterized by life-threatening organ dysfunction [1]. Despite advances in the management of neonates and the new generation of antibiotics, sepsis is still the leading cause of neonatal deaths with more than one million deaths worldwide each year [2-4].

After infection, pathogen-associated molecular patterns are recognized by sentinel immune cells through several classes of pathogen recognition receptors (e.g., toll-like receptors) [5-7]. Activation of these receptors can stimulate the release of inflammatory mediators, including cytokines and chemokines. Therefore, the clinical features of neonatal sepsis include systemic inflammatory response syndrome. Several studies have demonstrated that serum pro-inflammatory TNF-α, IL-1β, IL-6, and IL-8 levels are rapidly and strikingly elevated in neonatal sepsis [8-10]. Moreover, in previous studies, the levels of serum

CXCR4 and CXCL12 in neonatal sepsis were found to be significantly higher than those in controls [11, 12]. Another study showed that the level of CXCL10 is increased in the blood and peritoneum in a murine model of neonatal polymicrobial sepsis [13]. Furthermore, these cytokines serve as valuable biomarkers for the diagnosis of neonatal sepsis [8-12]. However, studies on the relationship between cytokine/chemokine levels and sepsis severity and on the cytokines/chemokines differences between early-onset sepsis (EOS) and late-onset sepsis (LOS) are limited. Moreover, little work has been done to assess the dramatic changes in innate immunity and adaptive immunity during neonatal sepsis. Therefore, in the present study, to better characterize the inflammatory response during neonatal sepsis, we systematically analyzed cytokine/chemokine profiles in neonatal sepsis.

Material And Methods

Subjects and ethics statement

Neonates who were hospitalized for sepsis at the neonatal intensive care unit of the Second Affiliated Hospital of Wenzhou Medical University, between October 2016 and June 2018, were eligible to participate in this study. Written informed consent was obtained from the parents or legal guardians. A total of 40 full-term neonates with sepsis were enrolled in this study, and 19 neonates without clinical manifestations or maternal risk factors for infection were included as the control group. Neonates with congenital malformations, those treated with antibiotics, and those who had undergone surgery were excluded. The study was approved by the Ethical Committee of the Second Affiliated Hospital of Wenzhou Medical University (Registration code: LCKY2018-65).

The diagnostic criteria of neonatal sepsis

Confirmed neonatal sepsis was defined as a positive blood culture accompanied by the presenting signs and symptoms. Suspected neonatal sepsis was defined as the presence of laboratory findings suggestive of infection (neutrophilia/neutropenia, thrombocytopenia, elevated C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR)) in combination with at least three of the following symptoms and signs without other causes: temperature instability (core temperature $\geq$ 38.5 or $\leq$ 36°C); respiratory symptoms (apnea, tachypnea with respiratory rate > 60 per minute, cyanosis, need for high ventilator settings or oxygen); cardiovascular symptoms including hypotension (blood pressure < fifth percentile for age), tachycardia (heart rate > 160 beats per minute), bradycardia (heart rate < 80 beats per minute), or poor perfusion; neurological symptoms (hypotonia, hyporeflexia, irritability, lethargy, and seizures); gastrointestinal symptoms (poor feeding, abdominal distension, green or bloody residuals, and vomiting). EOS was defined as onset in the first 72 hours after birth, and LOS was defined as onset on or after the first 72 hours of life. Organ dysfunction and severity was scored according to the Sequential Organ Failure Assessment (SOFA) [14].

Quantification of serum cytokines and chemokines

A total of 2 mL of venous blood was collected from all participants and centrifuged at 3000 rpm for 15 min. The sera were stored at -70 °C until analysis. Forty cytokines and chemokines, including CCL21, CXCL13, CCL27, CXCL5, CCL11, CCL24, CCL26, CX3CL1, CXCL6, GM-CSF, CXCL1, CXCL2, CCL1, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-16, CXCL10, CXCL11, CCL2, CCL8, CCL7, CCL13, CCL22, MIF, CXCL9, CCL3, CCL15, CCL20, CCL19, CCL23, CXCL16, CXCL12, CCL17, CCL25, and TNF-α, were analyzed using the Luminex Bead Immunoassay System (Bio-Rad Laboratories, Hercules, CA) following the manufacturer's instructions. Serum IL-17 levels were measured using human IL-17 Quantikine ELISA kits (eBioscience, San Diego, CA) according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using SPSS 23.0 (SPSS Inc., Chicago, IL). Categorical variables were expressed as frequencies and percentages. Continuous variables are presented as median (interquartile range, IQR). Statistical significance of differences in serum cytokine and chemokine levels between different groups was assessed using the Mann-Whitney U test. Differences before and after treatment were analyzed using the paired *t* test. For correlation analysis, Spearman's correlation coefficients were calculated. Statistical significance was set at $p < 0.05$ and all reported *p*-values were 2-sided.

Results

1. Clinical characteristics of the enrolled neonates

Fifty-nine full-term neonates were enrolled in this study, including 40 neonates with sepsis (30 EOS and 10 LOS) and 19 uninfected neonates as controls. The body temperature was 38.1℃ (IQR 37.13℃-38.65℃) and 36.8℃ (IQR 36.5℃-37.2℃) in neonates with sepsis and those in the control group, respectively (*p<0.0001*). In 13 neonates with positive blood culture, the following pathogens were isolated: *Escherichia coli* (seven cases), *Streptococcus lactis* (four cases), *Klebsiella* (one case), and *Enterococcus faecium* (one case). The levels of serum CRP and serum amyloid protein A (SAA) in neonates with sepsis were 9.11 mg/mL (IQR 6.54 mg/mL-10.13 mg/mL) and 29.38 mg/mL (IQR 4.83 mg/mL-36.24 mg/mL), respectively, which were significantly higher than those in the control group (both *p<0.0001*). The neutrophil counts of the neonates with sepsis and those in the control group were 9.06×10^9/L (IQR 5.34 $\times 10^9$/L-14.84 $\times 10^9$/L) and 6.14×10^9/L (IQR 4.29 $\times 10^9$/L-7.76 $\times 10^9$/L) (*p=0.018*), respectively. There were no significant differences in the monocyte, lymphocyte, and platelet counts between the two groups (all *p>0.05*). The duration of disease was 4.5h (IQR 1h-12h) and 24h (IQR 5.75h-30h) in EOS and LOS, respectively (*p=0.0026*). Detailed information on the clinical characteristics is shown in **Table 1.**

Table 1 The clinical characteristics of the enrolled neonates

Variable	Neonate sepsis (n=40)	Control (n=19)	*p* value
Age (Median, IQR), days	12(3.25-19.75)	10(7-16.25)	0.672
Male gender, n (%)	25(62.5)	13(68.4)	0.775
Temperature (℃)	38.1(37.13-38.65)	36.8(36.5-37.2)	<0.0001
Blood culture, n (%)	13(32.5)	nd	nd
Early of sepsis, n (%)	30(75)	0	nd
WBC($x10^9$/L)	17(11.68-21.13)	12.1(9.9-14.7)	0.005
Neutrophils($x10^9$/L)	9.06(5.34-14.84)	6.14(4.29-7.76)	0.018
Monocytes($x10^9$/L)	1.49(0.90-2.05)	1.26(0.96-51.46)	0.189
lymphocytes($x10^9$/L)	4.07(3.14-5.53)	4.22(3.43-5.02)	0.942
Platelets($x10^9$/L)	316.5（249-436）	262（227-345）	0.076
CRP (mg/ml)	9.11（6.54-10.13）	0.35（0.18-0.69）	<0.0001
SAA (mg/ml)	29.38（4.83-36.24）	0.38（0.04-3.86）	<0.0001

Note: nd, not done; WBC, White Blood Cell; CRP, C reactive protein; SAA, serum amyloid protein A. Values are given as median (interquartile range).

2. Levels of serum cytokines and chemokines in neonatal sepsis

We compared serum cytokine and chemokine levels between neonates with sepsis and those in the control group. Since the level of CCL15 was not in the quantitative range of the assay and exceeded the highest calculated concentration, we did not analyze it further. As shown in **Table 2**, the levels of the pro-inflammatory cytokines, IL-6, IL-8, TNF-α, and IL-1β, were significantly higher in neonates with sepsis than those in the control group (all *p<0.05*). There was no difference in the levels of IL-4, IFN-γ, IL-17, IL-2, IL-10, GM-CSF, and IL-16 between the two groups (all *p>0.05*). Serum MIF level was remarkably increased in neonates with sepsis, and was 6,270 pg/mL (IQR 4,615 pg/mL-11,820 pg/mL) and 4,947 pg/mL (IQR 3,204 pg/mL-6,767 pg/mL) in the sepsis and control groups, respectively (*p=0.0223*). As shown in **Table 3**, serum CXCL13, CXCL1, CXCL2, CXCL5, CXCL6, CXCL16, CCL27, CCL2, CCL8, CCL3, CCL20, CCL23, CCL27, and CX3CL1 levels were significantly increased in neonates with sepsis compared to those in the control group (all *p<0.05*).

Table 2 The serum levels of cytokines in control group and neonatal sepsis group.

Cytokine	Control group	Sepsis group	*p* value
GM-CSF (pg/ml)	125.2（87.8-234.3）	149.4（96.62-228.9）	0.4533
IFN-γ(pg/ml)	105.5（78.75-167.2）	107.8（76.35-160.6）	0.6977
IL-1β (pg/ml)	**10.93（5.955-11.22）**	**12.02（10.68-18.19）**	**0.0071**
IL-2 (pg/ml)	30.52（14.01-46.2）	36.81（24.28-45.02）	0.2794
IL-4 (pg/ml)	38.02（25.45-42.63）	36.1（30.99-43.43）	0.418
IL-6 (pg/ml)	**24.25（12.9-39.01）**	**58.57（37.03-236.6）**	**< 0.0001**
IL-8 (pg/ml)	**38.5（17.45-64.08）**	**262.1（130.1-630）**	**< 0.0001**
IL-10 (pg/ml)	89.27（42.78-134.7）	95.2（67.34-137.3）	0.2871
IL-16 (pg/ml)	1176(617.8-1832)	1005(727.2-1331)	0.3417
MIF (pg/ml)	**4947(3204-6767)**	**6270(4615-11820)**	**0.0223**
TNF-α (pg/ml)	**45.72(26.27-55.92)**	**53.14(44.26-79.38)**	**0.0213**
IL-17 (pg/ml)	1.13(0-2.525)	1.792(0.3479-5.423)	0.1723

Table 3 The chemokine levels of serum in control group and neonatal sepsis group.

Chemokine	Control group (n=19)	Sepsis group (n=40)	*p* value
CCL21(pg/ml)	7380（4948-15970）	6356（3958-9688）	0.1434
CXCL13(pg/ml)	**53.38（41.35-72.76）**	**86.9（51.3-142.8）**	**0.003**
CCL27(pg/ml)	**1565（945-2621）**	**2041（1385-2895）**	**0.0361**
CXCL5(pg/ml)	**969.7（550.4-1366）**	**1187（929.4-1947）**	**0.0321**
CCL11(pg/ml)	63.4（50.19-89.31）	77（58.22-89.35）	0.4947
CCL24(pg/ml)	263（122.4-485.8）	231.1（124.8-356.8）	0.4493
CCL26(pg/ml)	70.31（46.26-102.6）	77.38（58.56-98.76）	0.3283
CX3CL1(pg/ml)	**365.9（223.1-584.3）**	**609.7（529.9-857.1）**	**< 0.0001**
CXCL6(pg/ml)	**48.4（30.76-95.48）**	**88.18（54.28-108.4）**	**0.005**
CXCL1(pg/ml)	**277.9（244.3-332.5）**	**436.8（359.2-763）**	**< 0.0001**
CXCL2(pg/ml)	**143.3（54.57-315.1）**	**331.3（186.3-700.9）**	**0.0027**
CCL1(pg/ml)	112.6（96.16-145.3）	116.7（88.1-153.7）	0.7378
CXCL10(pg/ml)	171.4(87.76-262.7)	210(97.66-593.5)	0.185
CXCL11(pg/ml)	23.95(7.658-36.5)	20.28(13.38-53.64)	0.8251
CCL2(pg/ml)	**77.22(28.18-166.8)**	**168(89.07-363.4)**	**0.0011**
CCL8(pg/ml)	**32.67(21.28-39.7)**	**61.6(40.28-101.8)**	**< 0.0001**
CCL7(pg/ml)	204(145.8-377.9)	238.5(151.5-370.6)	0.5557
CCL13(pg/ml)	53.52(12.8-87.72)	47.06(22.71-91.9)	0.7478
CCL22(pg/ml)	1694(1061-3421)	1234(774.3-1615)	0.0829
CXCL9(pg/ml)	658.3(251.5-960.4)	604.7(394.3-1069)	0.4413
CCL3(pg/ml)	**16.28(9.845-30.3)**	**58.16(32.58-136.5)**	**< 0.0001**
CCL20(pg/ml)	**25.24(13.16-42.4)**	**50.93(20.63-97.86)**	**0.0074**
CCL19(pg/ml)	902.2(274.9-1714)	772.5(466.5-1381)	0.8356
CCL23(pg/ml)	**273.6(107.9-609.3)**	**1092(554.5-2366)**	**< 0.0001**
CXCL16(pg/ml)	**965.4(774.3-1152)**	**1218(1105-1364)**	**0.0004**
CXCL12(pg/ml)	1559(423-1790)	1215(410.4-1607)	0.279
CCL17(pg/ml)	269.7(100.5-999.8)	417.5(122.4-1024)	0.705
CCL25(pg/ml)	1156(698.6-1498)	1285(895.3-1792)	0.3123

Subsequently, we further analyzed the differences in these cytokines and chemokines between the EOS and LOS groups. As shown in **Table 4**, the levels of serum CXCL6, CXCL1, IL-6, CXCL10, CXCL11, CCL20, and IL-17 were higher in LOS than those in EOS (all $p<0.05$). Conversely, the levels of serum IL-16, CXCL16, and CCL22 were lower in LOS than those in EOS (all $p<0.05$).

Table 4 The levels of serum cytokines and chemokines between EOS and LOS.

Cytokine/ Chemokine	EOS (n=10)	LOS (n=30)	*p* value
CXCL6(pg/ml)	66.55（37.7-97.69）	93.27（61.68-121.5）	0.0299
CXCL1(pg/ml)	370.1（266-449.8）	528.6（393.9-957.2）	0.0408
IL-6(pg/ml)	44.18（32.08-57.81）	87.68（44.9-354.5）	0.0479
IL-16(pg/ml)	1449（1099-1948）	885（607.4-1211）	0.0015
CXCL10(pg/ml)	110.9（62.27-268.6）	253.3（136.2-705.1）	0.0415
CXCL11(pg/ml)	11.81（5.853-20.38）	22.48（16.25-62.85）	0.0152
CCL22(pg/ml)	1485（1179-3912）	1164（619.3-1440）	0.0276
CCL20(pg/ml)	22.86（14.3-40.77）	62.76（32.03-124.9）	0.0093
CXCL16(pg/ml)	1403（1169-1532）	1207（1007-1334）	0.0351
IL-17(pg/ml)	0.43（0-0.97）	2.89（1.16-6.47）	0.003

Among the 40 neonates with sepsis, there were 13 neonates with positive blood culture and 27 neonates with negative blood culture. We compared the levels of serum cytokines and chemokines between neonates with positive and negative blood cultures. The levels of CXCL6, TNF-α, CCL8, and CCL23 in neonates with positive blood culture were significantly higher than those with negative blood culture (all *p<0.05*) (**Table 5**). There was no difference in the levels of other cytokines and chemokines between the two groups (data not shown).

Table 5 The chemokine and cytokine levels of serum in blood culture positive group and blood culture negative group.

Cytokine/ Chemokine	Positive (n=13)	Negative (n=27)	*p* value
CXCL6(pg/ml)	99.88(65.67-157.9)	82.05(51.1-102.2)	0.0444
CCL8 (pg/ml)	92.06(49.62-375.7)	43.81(38.66-76.02)	0.0373
CCL23(pg/ml)	1954(528-3292)	746.2(366.1-1223)	0.0311

3. Levels of cytokines and chemokines after treatment

The sera of 15 neonates with sepsis were collected before and after treatment. A total of 41 cytokines and chemokines were measured. As shown in **Supplemental Digital Content (Figure 1**), IL-6, IL-8, TNF-α, IL-1β, CXCL13, CXCL16, CCL27, CCL3, CCL23, and CX3CL1 were significantly decreased after treatment (all *p<0.005*).

4. The association between the levels of cytokines and chemokines and the severity of organ function

We further analyzed the association between the levels of cytokines and chemokines and the SOFA score. As shown in **the Supplemental Digital Content (Figure 2**), the levels of CX3CL1, CXCL2, CCL8, and TNF-α were all positively correlated with SOFA scores (all *p<0.05*).

Discussion

Neonatal sepsis is a major risk factor for neonatal mortality [15]. Furthermore, the underlying pathological mechanisms remain unclear. In the present study, we systematically investigated the dynamic changes in cytokine and chemokine profiles in neonatal sepsis. In accordance with previous studies [8-10], the levels of the pro-inflammatory cytokines IL-6, IL-8, TNF-α, and IL-1β were significantly increased in neonates with sepsis. A moderate increase in cytokines plays a protective role and promotes antimicrobial immune responses, whereas excessive upregulation of pro-inflammatory cytokines is commonly associated with a severe and often fatal outcome due to multiple organ failure [16]. Our results showed that the level of TNF-α was positively correlated with SOFA scores, suggesting its involvement in organ damage in neonates with sepsis.

Chemokines are a family of cytokines that have the capacity to recruit leukocytes to pathogen invasion sites, which is essential for the host to defend against infections [17]. Our results showed that serum CXCL13, CXCL1, CXCL2, CXCL5, CXCL6, CXCL8, CXCL16, CCL27, CCL2, CCL8, CCL3, CCL20, CCL23, CCL27, and CX3CL1 levels were significantly increased in neonates with sepsis compared to those in the control group. *Manoura* et al also found that the levels of serum CXCL1 and CXCL5 were higher in neonates with sepsis [18]. Among these chemokines, CXCL1, CXCL2, CXCL5, CXCL6, and CXCL8 are potent chemoattractants for neutrophils. Neutrophils are the most abundant cells of innate immunity and play an important role in responding to bacterial, viral, and fungal infections [19]. Previous studies have shown that elastase and nitric oxide are upregulated in neonates with sepsis [20, 21]. In our study, neutrophil counts in neonates with sepsis were higher than those in controls. These results suggested that neutrophil-related chemokines were immediately synthesized and released after infection, which might have subsequently led to the recruitment of neutrophils as a defense mechanism against infections in the neonates.

The defense system in neonates is initially dependent on their innate immune system because adaptive immunity develops later in life [22]. Interestingly, in our study, the levels of Th17 cell-related cytokine/chemokines, IL-17 and CCL20, and Th1 cell-related chemokines, CXCL10 and CXCL11, were significantly higher in LOS than those in EOS. Our results indicated that the pro-inflammatory cytokines were initially released in neonates at the onset of infection, but adaptive immunity gradually developed in the later stage of infection.

The serum MIF level in neonatal sepsis was significantly higher than that in the control group. After treatment, MIF significantly decreased. The MIF of newborns is 10-fold higher than that of children and adults. *E. coli* and *Group B Streptococcus* induce MIF secretion by neonatal monocytes [23]. MIF plays an important role in promoting the production of inflammatory cytokines by monocytes [24]. Previous studies have indicated that MIF is correlated with the expression of pro-inflammatory markers and the dysregulation of pituitary and adrenal function, severity scores, and disease outcomes [25-28]. Therefore, MIF might play a critical role in the immune regulation of neonatal sepsis.

In our study, serum CX3CL1 levels were increased in neonates with sepsis. The level of CX3CL1 positively correlated with SOFA scores. A previous study reported that the level of plasma CX3CL1 was elevated in a mouse model of CLP-induced sepsis [29]. The level of CX3CL1 in adult sepsis patients increased with the severity and number of organ dysfunction [30]. Non-survivors had sustained elevated CX3CL1 levels compared to survivors [30]. Taken together, these data suggest that CX3CL1 is a risk factor for sepsis outcomes. CX3CL1 acts through the CX3CR1 receptor and is a unique member of the CX3C chemokine family. CX3CR1 is mainly expressed on $CD14^{++}CD16^{+}$ and $CD14^{+}CD16^{++}$ monocytes, which represent an activated and a more mature “macrophage-like” subset. Furthermore, the cell population is greatly expanded in various infectious and inflammatory diseases and can be more than 50% of total monocytes during sepsis [31]. These data suggest that CX3CL1 recruits activated monocytes and can be involved in neonatal sepsis damage. However, the roles of CX3CL1 in neonatal sepsis need to be further studied.

Our study has some limitations. First, the subjects enrolled were relatively few; therefore, our findings need to be proven in future studies with a larger cohort. Second, gestational age is an essential factor for these cytokines and chemokines, whereas we only enrolled full-term neonates in our study. Third, our date indicated that the levels of CX3CL1, CXCL2, CCL8, and TNF-α were positively correlated with SOFA scores. Regrettably, there was specific data (for example SOFA score=10) which likely effected the result, therefore, it needed more samples and further confirmed it.

In conclusion, our study suggested that excessive inflammation in neonatal sepsis might be involved in the damage associated with neonatal sepsis. Neutrophils, monocytes, and lymphocyte-associated chemokines increased significantly after infection. To summarize, while the recruited immune cells participate in the anti-infection defense in neonates, they might also cause damage.

Abbreviations

PAMP, pathogen-associated molecular patterns; SIRS, systemic inflammatory response syndrome; EOS, early-onset sepsis; LOS, late-onset sepsis; NICU, neonatal intensive care unit; TLR, Toll-like receptors; SOFA, Sequential Organ Failure Assessment;

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Committee of the Second Affiliated Hospital of Wenzhou Medical University (Registration code: LCKY2018-65). Written informed consent was obtained from the parents or legal guardians

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

Neither this paper nor any similar paper has been or will be submitted to or published in any other scientific journal. All authors are aware and agree with the content of the paper and agree to be listed as the author of the manuscript. There is no conflict of interest or competing financial interests for all authors.

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Authors' Contributions

Qipeng Xie and Minghua Jiang conceived and designed the study; Mengjiao Kuang, Suipeng Chen and Shirui Huang detected the cytokines and chemokines, Suipeng Chen, Binbin Gong, Suzhen Lin and Huiyan Wang collected the patient's serum and saves it, Guiye Wang, Hongqun Tao and Zuqin Yang collected and organized clinical information of patients. Shirui Huang and Jian Yu conducted the statistical analyses. Mengjiao Kuang, Minghua Jiang and Qipeng Xie drafted the manuscript. All authors read and approved the final version of the manuscript.

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Figures

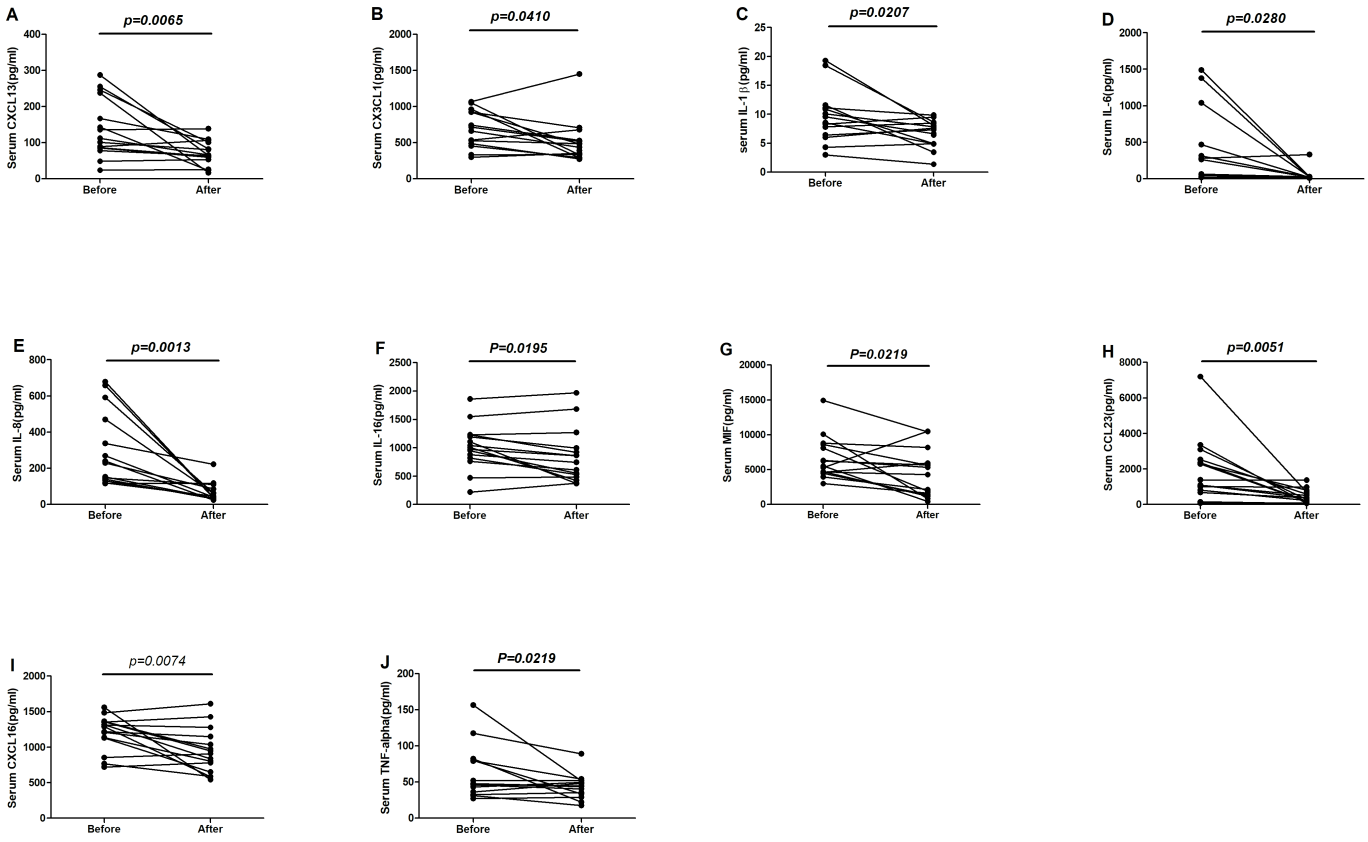

Figure 1

Levels of cytokines and chemokines before and after treatment in 15 neonates with sepsis.

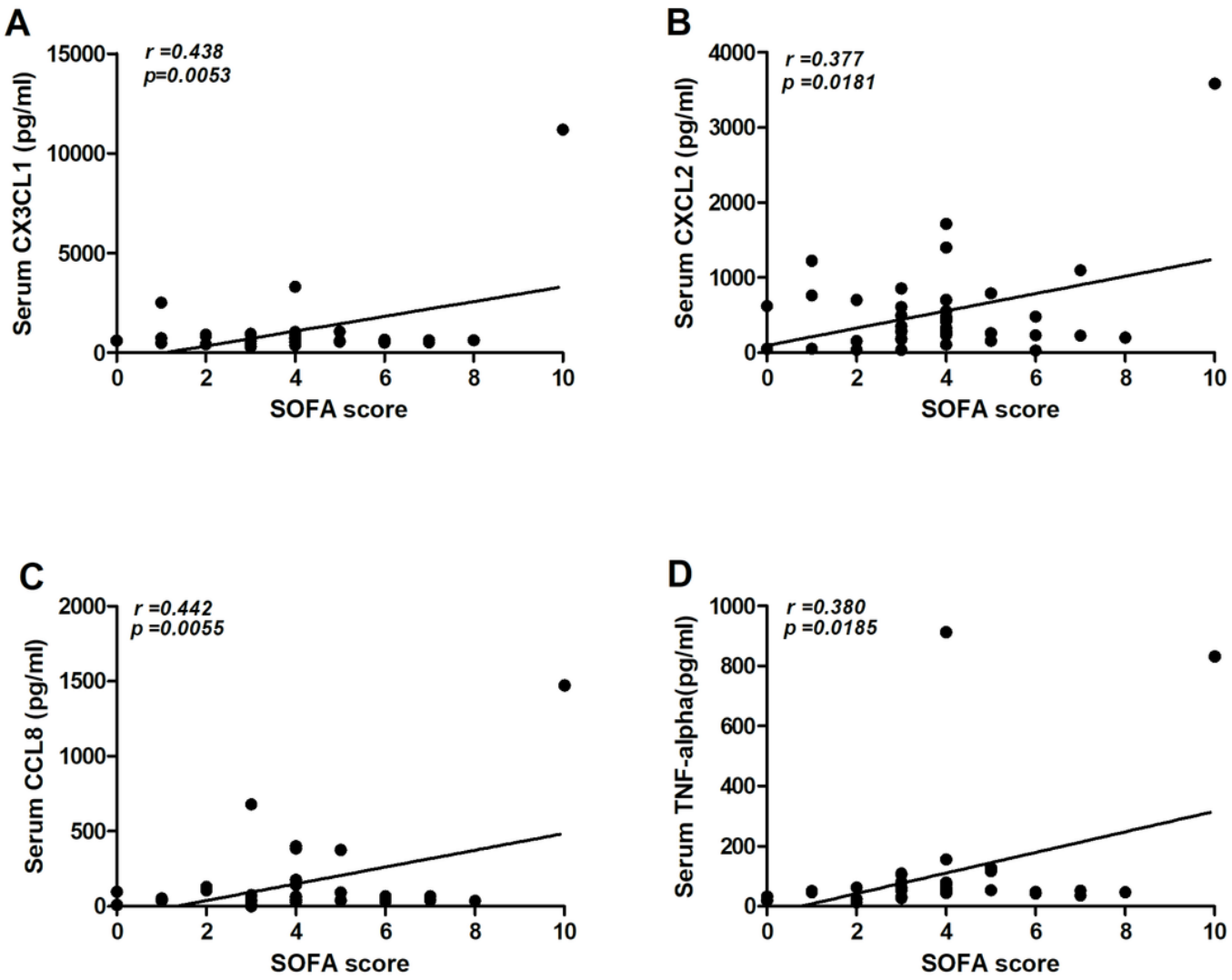

Figure 2

Association between the levels of cytokines and chemokines and SOFA score.