Inflammation and Cell Signaling 2022; 9: e1186. doi: 10.14800/ics.1186; © 2022 by Yi-hui Zuo, *et al.*
http://www.smartscitech.com/index.php/ics

RESEARCH ARTICLE

Plasma level of M-CSF was independently related to 30-day survival in patients with suspected sepsis, and correlated to pathogen load: A prospective cohort study

Yi-hui Zuo[1], Zhen-ju Song[2], Zhe Luo[3], Min-jie Ju[3], Jing Zhang[1,*]

[1] *Department of Pulmonary Medicine, Zhongshan hospital, Shanghai Medical College, Fudan University, Shanghai 200000, China*
[2] *Department of Emergency Department, Zhongshan hospital, Shanghai Medical College, Fudan University, Shanghai 200000, China*
[3] *Department of Critical Care Medicine, Zhongshan hospital, Shanghai Medical College, Fudan University, Shanghai 200000, China*

[*] Correspondence: Jing Zhang
E-mail: huxizhangjing@foxmail.com
Received: June 12, 2021
Published: February 15, 2022

The purpose of our study was to screen the plasma cytokines to find possible indicators of disease progression and prognosis of patients with infection. With a prospective cohort study, selected patients were divided into sepsis group and non-sepsis group. Demographic and clinical information were collected. Blood samples were tested for the levels of plasma cytokines and metagenomic next-generation sequencing (mNGS). 30-day follow-up information was recorded, and data was analyzed by SPSS22.0 (SPSS Inc, Chicago, IL). A total of 95 patients were selected. After propensity score matching of age and gender, 36 patients with sepsis and 36 with non-sepsis were enrolled. 30-day follow-up data exhibited that 41 patients died and 31 survived. Patients with sepsis and 30-day death had higher plasma levels of cytokines, including macrophage-stimulating factor (M-CSF), monocyte chemoattractant protein-3 (MCP-3), etc., than patients with non-sepsis and 30-day survival, respectively. M-CSF > 8.21pg/ml was an independent risk factor for 30-day death, and the reads of pathogens in mNGS reports was positively correlated with the plasma concentrations of various cytokines, including M-CSF.

Keywords: infection, sepsis, cytokines, MCP-3, prognosis

Abbreviations: IL, Interleukin; CCL, Chemokine C-C motif ligand; MIP, Macrophage inflammatory protein; MIF, Macrophage migration inhibitor factor; MCP, Monocyte chemoattractant protein; GM-CSF, Granulocyte-macrophage colony stimulating factor; M-CSF, Macrophage-stimulating factor; APACHE, The Acute Physiologic and Chronic Health Evaluation; qSOFA, Quick Sepsis Related Organ Failure Assessment; PCT, Procalcitonin; mNGS, Metagenomic next-generation sequencing; MIG, membrane-bound immunoglobulin; LIF, leukemia inhibitory factor; SCF, stem cell factor; SDF, stromal derived factor; TNF, tumor necrosis factor; IP, interferon inducible protein; TRAIL, TNF-related apoptosis-inducing ligand; CTACK, cutaneous T cell-attracting chemokine.

To cite this article: Yi-hui Zuo, *et al.* Plasma level of M-CSF was independently related to 30-day survival in patients with suspected sepsis, and correlated to pathogen load: A prospective cohort study. Inflamm Cell Signal 2022; 9: e1186. doi: 10.14800/ics.1186.

Introduction

Infection is one of the major causes of death and disability of hospitalized patients, and also a serious threat to the health and life safety of the public [1, 2]. With acute and progressive exacerbation, infection could develop into sepsis and septic shock, of which the mortality rate was reported to be as high as 50% [2]. One of the potentially effective ways to improve the prognosis of patients with infection is the early monitoring of disease progression. Therefore, the effective indicators of infection progression and prognosis were needed, so as to discover the tendency of disease progression in time and carry

out targeted treatment to improve the prognosis [3, 4].

As known, the main procedure of infection lies in local and systemic inflammatory reaction induced by bacteria, fungi, viruses and other kinds of pathogens invading the human body, while sepsis is a life-threatening acute progressive syndrome caused by the maladjusted immune response, resulting in tissue and organ damage. In the process of infection progression and sepsis development, a variety of cytokines interact with human body, affecting the severity of the disease and the final outcome.

Studies have found that various pro-inflammatory cytokines elevated in patients with sepsis. Classic pro-inflammatory cytokines such as interleukin-6 (IL-6) and the Chemokine C-C motif ligand (CCL) family of chemokines such as macrophage inflammatory protein-1α (MIP-1α, also known as CCL-3) were both found to be markedly up-regulated septic patients and useful in diagnosing and early monitoring of infectious diseases [5, 6].

The excessive secretion of pro-inflammatory cytokines feeds back to various immune cells and immunomodulatory centers, causing the secretion of anti-inflammatory factors such as macrophage migration inhibitor factor (MIF) and IL-10 to increase [7]. It was found that MIF was significantly up-regulated in patients with sepsis, and also correlated with disease severity and prognosis [8].

Moreover, it was reported that some cytokines may also play a role in ameliorating the prognosis of infected patients. The increase of monocyte chemoattractant protein-1 (MCP-1) was related to the occurrence and development of sepsis, blocking the production of MCP-1 can significantly reduce the incidence of sepsis [9]. Cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage-stimulating factor (M-CSF) could be used in the treatment of sepsis, the latter can significantly reduce the incidence of sepsis and improve the prognosis of sepsis [10]. It can be inferred that the concentration of certain cytokines in infected patients may be related to the severity of infection, and help with making early responses to reduce the occurrence of sepsis and ameliorating the prognosis.

Therefore, our research was aimed to compare the plasma concentrations of various cytokines in infected patients with different severity and prognosis, so as to explore the possible prognostic risk factors of infected patients and screen out possible markers. The results of our research concluded that plasma levels of cytokines were higher in patients with sepsis and 30-day death group, and M-CSF > 8.21pg/ml was an independent risk factor for 30-day death. A variety of cytokines were positively correlated with the reads of responsible pathogens, which indirectly proved the indicative effect of cytokines on the severity and prognosis of infected patients.

Patients and Methods

1. Study design and patients' population

A prospective cohort study was conducted. Eligible patients were enrolled from December 2018 to November 2019 at Zhongshan Hospital affiliated to Fudan University. The inclusion criteria in our research were as follows: (1) Patients who were suspected with bloodstream infection and needed to be hospitalized; (2) Patients who signed the informed consent; (3) Age is > 18 years old and ≤ 90 years old.

The exclusion criteria were the existence of any conditions listed as follows: (1) Patients aged < 18 or > 90 years old; (2) Those who were not able to cooperate with the observation or with cognitive impairment. The criteria for midway withdrawal were the request of revoking informed consent.

95 patients were enrolled and divided into sepsis and non-sepsis group according to the latest definition of sepsis in 2016 [2]. After the propensity score matching of age and gender, 36 patients in sepsis group and 36 patients in non-sepsis group were remained. Plasma levels of cytokines were detected in those 72 patients.

2. Data collection

After the eligible patients signed the informed consent form, the demographic statistics and clinical information were collected, and 30-day follow-up of survival information was recorded. Demographic statistics include age, gender and age; clinical information includes: (1) Previous and current medical history; (2) The Acute Physiologic and Chronic Health Evaluation (APACHE) II score and quick Sepsis Related Organ Failure Assessment (qSOFA) score, for the evaluation of disease severity; (3) Results of peripheral blood examination on the day of enrollment, including blood routine, procalcitonin (PCT) levels and biochemical indicators covering liver and renal function; (4) blood culture results and results of metagenomic next-generation sequencing (mNGS) test. The mNGS of blood sample was performed by Beijing Genomics institution (BGI) to detect potential pathogens.

3. Etiological diagnosis

The final etiology results were decided by the principal investigator leading physician group in each hospital, and made modification based on clinical features, blood culture and mNGS reports.

The reads of responsible pathogenic microbes were taken when comparing the reads of pathogens between different groups.

4. Determination of plasma cytokines

The remaining plasma samples were taken for the detection of cytokines using Human Cytokine Screening 48-Plex Services detection kit (BIORAD, Catalog number: 12007283, California, America). The operations were conducted according to the manufacturers' introduction. Cytokines

included interleukin-2 receptor (IL-2R), membrane-bound immunoglobulin (MIG), MIP-1α, MIP-1β, leukemia inhibitory factor (LIF), RANTES, stem cell factor (SCF), stromal derived factor (SDF)-1α, IL-1Rα, tumor necrosis factor (TNF)-β, MIF, interferon inducible protein (IP)-10, MCP-1, TNF-related apoptosis-inducing ligand (TRAIL), M-CSF, cutaneous T cell-attracting chemokine (CTACK). Other cytokines were excluded in statistical analysis due to due to their low plasma levels and could not be detected in more than 20% of the patients.

Statistical Analysis

SPSS 22.0 (SPSS Inc, Chicago, IL) and Medcalc 19.6.0 (MedCalc Software Ltd, Ostend, Belgium) were used for data analysis.

The patients were divided into sepsis group and non-sepsis group. Chi-square analysis was used for categorical variables. One-sample Kolmogorov-Smirnoff was used to test the normality of continuous variables, and t-test was used when compare the continuous variables which fits normal distribution. For the non-normally distributed continuous variables, the Mann-Whitney U test was applied.

The correlation between the levels of cytokines and the severity of the disease was tested by univariate logistic regression, and the correlation between the levels of cytokines and the reads of pathogens was detected by linear regression. The receiver operating characteristic (ROC) curve was applied to determine the cut-off value of M-CSF. Variables in accordance with normal distribution were represented by the mean ±standard deviation, and medians with interquartile ranges were represented for non-normal variables.

Results

1. Basic clinical information

A total of 72 patients were enrolled and divided into sepsis group (n = 36) and non-sepsis group (n = 36). There were 47 males and 25 females. The 30-day follow-up data exhibited that 41 patients died and 31 survived. Other information was shown in Table 1. There was no significant difference in age and gender between the two groups. In terms of disease severity, the shock index and APACHE II score of sepsis group were significantly higher than those of non-sepsis group. The percentage of neutrophils and the level of PCT in patients of sepsis group were higher than those in non-sepsis group, along with increased alanine aminotransferase (ALT) and decreased albumin.

2. Various of pro-inflammatory and anti-inflammatory factors elevated in plasma of patients with sepsis.

As data shown in Table 2, the plasma concentrations of various cytokines in sepsis group were significantly higher than that of non-sepsis group. The concentration of IL-1Rα was in septic patients was about 11 times higher than that in non-sepsis patients. Other pro-inflammatory cytokines, including MCP-1, M-CSF, etc., and anti-inflammatory factors such as MIF in sepsis group were also higher than those in non-sepsis group. These results suggested that the pro-inflammatory and anti-inflammatory processes in septic patients were more active than those in non-septic patients. However, independent correlation between cytokines and sepsis was not found in the multivariate logistic regression (data not provided).

3. Elevated plasma concentration of M-CSF was an independent risk factor for 30-day death in infected patients.

The plasma concentrations of cytokines in patients with different prognosis were also compared. According to the 30-day follow-up data, the patients were divided into 30-day survival group (n = 31) and 30-day death group (n = 41). Data was shown in Table 3. The plasma concentrations of various cytokines in the 30-day death group were significantly higher than those in the 30-day survival group, including IL-1Rα, M-CSF and CTACK, etc., which was similar to the comparison between sepsis and non-sepsis group.

Cytokines with $P < 0.05$ were included into multivariate logistic regression. The results concluded that M-CSF was an independent risk factor affecting the prognosis of patients. The higher M-CSF indicated the higher risk of the 30-day death (shown in Table 4).

In order to determine the cutoff value of M-CSF, we analyzed the plasma level of M-CSF by ROC curve. The results showed that the plasma concentration of M-CSF exceeding 8.21pg/ml was an independent risk factor for 30-day death, with a sensitivity of 70% and a specificity of 73.2%, and the area under the curve was 0.714.

4. The reads of responsible pathogens in mNGS reports and the plasma levels of inflammatory factors were positively correlated.

We further analyzed the correlation between plasma concentration of cytokines and the reads of pathogens (Data shown in Table 5). The results of linear regression illustrated that plasma concentrations of various kinds of cytokines, including IL-2Rα, MIP-1α, etc., were positively correlated with the reads of pathogens. This was also an indirect proof of the correlation between the level of plasma cytokines and the progression or prognosis of infected patients.

Discussion

The results of cytokine detection showed that the levels of cytokines in patients with sepsis and 30-day death group were higher than those with non-sepsis and 30-day survival group, respectively. M-CSF was independently related to the prognosis of infected patients, and M-CSF > 8.21pg/ml was an independent risk factor for 30-day death. In addition, linear regression suggested that plasma concentrations of a variety of cytokines were positively correlated with the reads of pathogens, which indirectly approved the indicative effect of cytokines on the severity and prognosis of infected patients.

Table 1. The basic information of 72 patients with infection.

Basic information	Total (n=72)	Sepsis group (n=36)	Non-sepsis group (n=36)	P value
Age (years)	62.0 (44.3, 69.8)	64.0(55.0, 69.0)	59.5(37.5, 72.3)	0.093
Gender (Male/Female)	47/25	23/13	24/12	0.805
Fever （n, %）	54, 75.0%	29, 80.6%	25, 69.4%	0.280
Shock index	0.72 (0.86, 1.11)	1.10(0.75, 1.40)	0.82(0.69, 0.89)	0.001
APACHE II score	17 (10, 25)	25.00(19.00, 29.50)	11.00(5.75, 16.00)	0.001
Leukocyte (×10^9)	9.93 (5.73, 15.49)	11.50(5.73, 15.49)	9.55(5.68, 15.58)	0.990
Neutrophils (×10^9)	9.16 (5.30, 13.42)	10.32(5.40, 13.43)	7.70(5.23, 13.10)	0.654
Neutrophils/Lymphocytes	13.04(7.14, 28.51)	16.03(10.49, 38.46)	9.19(4.76, 21.69)	0.277
Neutrophils%	86.85(79.00, 94.05)	89.85(84.40, 94.78)	82.90(74.53, 91.03)	0.008
PCT (ug/L)	1.34(0.27, 7.21)	3.76(1.14, 20.97)	0.32(0.18, 1.44)	0.015
ALT	29.00(15.00, 47.25)	36.00(18.00, 100.00)	20.00(14.00, 39.00)	0.039
AST	34.65(20.95, 55.35)	51.00(27.00, 115.00)	24.00(18.00, 41.00)	0.108
BUN	11.70(7.30, 20.00)	13.40(8.20, 20.10)	9.06(5.35, 19.93)	0.983
Albumin	32.10(27.10, 36.00)	31.50(25.60, 35.00)	33.00(30.00, 37.00)	0.038

Abbreviations: APACHE II, The Acute Physiologic and Chronic Health Evaluation II; PCT, procalcitonin; ALT, alanine aminotransferase; AST, aspartate transaminase; BUN, blood urea nitrogen; Cr, creatinine.

Table 2. Plasma levels of cytokines in sepsis group and non-sepsis group.

Cytokines	Sepsis group	Non-sepsis group	P value	Exp(B) 95%CI
IL-2Rα (pg/ml)	59.09(40.12, 157.32)	44.89(23.46, 120.96)	0.230	1.364(0.821, 2.263)
MIG (pg/ml)	848.56(221.20, 5423.50)	693.03(329.44, 1743.75)	0.166	3.635(0.586, 22.560)
MIP-1β (pg/ml)	74.20(52.46, 130.61)	113.14(41.21, 130.70)	0.160	1.622(0.826, 3.185)
LIF (pg/ml)	22.71(15.53, 34.41)	11.65(8.66, 16.63)	0.001	2.898E+18(4.58E+7, 1.836E+29)
RANTES (pg/ml)	654.73(269.92, 1440.00)	1886.50(394.64, 3669.0)	0.052	0.405(0.163, 1.009)
SCF (pg/ml)	69.53(43.22, 119.65)	28.05(20.93, 55.16)	0.001	4.657(1.811, 11.970)
SDF-1α(pg/ml)	546.07(374.50, 690.78)	486.62±181.69	0.157	1.446(0.868, 2.411)
IL-1Rα(pg/ml)	898.62(258.63, 5107.50)	73.04(34.99, 235.92)	0.007	137690.51(24.00, 789841430.70)
TNF-β (pg/ml)	61.72(37.01, 115.43)	108.27(39.76, 133.5)	0.176	0.719(0.445, 1.160)
MIF (pg/ml)	173.60(76.56, 287.57)	115.39(50.11, 238.39)	0.026	2.125(1.092, 4.135)
IP-10 (pg/ml)	1259.50(432.65, 6894.50)	880.23(348.71, 1992.00)	0.046	15.631(1.048, 233.187)
MCP-1 (pg/ml)	68.02(37.90, 280.27)	12.80(7.01, 21.44)	0.005	16477.445(20.283, 13385646.710)
MIP-1α (pg/ml)	2.30(1.04, 9.41)	0.98(0.56, 1.70)	0.143	6.290(0.537, 73.670)
TRAIL (pg/ml)	12.79(7.77, 25.53)	12.78(7.28, 19.86)	0.250	1.499(0.752, 2.989)
M-CSF (pg/ml)	17.51(6.89, 34.60)	7.45(2.11, 11.13)	0.010	5.003(1.463, 17.107)
CTACK (pg/ml)	241.57(178.42, 383.30)	170.39±80.73	0.001	11.145(2.626, 47.298)

Abbreviations: IL-2R, interleukin-2 receptor; MIG, membrane-bound immunoglobulin; MIP, macrophage inflammatory protein; LIF, leukemia inhibitory factor; RANTES, regulated on activation normal T cell expressed and secreted; SCF, stem cell factor; SDF, stromal derived factor; TNF, tumor necrosis factor; MIF, macrophage migration inhibitor factor; IP, interferon inducible protein; MCP, monocyte chemoattractant protein; TRAIL, TNF-related apoptosis-inducing ligand; M-CSF, macrophage-stimulating factor; CTACK, cutaneous T cell-attracting chemokine.

Table 3. Plasma levels of cytokines in 30-day death group and 30-day survival group (univariate logistic regression).

Cytokines	30-day death	30-day survival	P value	Exp(B) 95%CI
IL-2Rα (pg/ml)	53.99(37.38, 178.68)	47.91(22.26, 114.43)	0.140	0.653(0.370, 1.151)
MIG (pg/ml)	937.59(210.28, 3606.00)	676.00(377.55, 1800.00)	0.223	0.347(0.063, 1.906)
MIP-1β (pg/ml)	72.43(45.51, 128.92)	114.23(44.72, 131.08)	0.271	0.705(0.379, 1.313)
LIF (pg/ml)	19.63(13.73, 29.89)	13.57±5.24	0.004	0.876(0.801, 0.958)
RANTES (pg/ml)	648.25(199.40, 1629.50)	1982.00(443.49, 3815.0)	0.033	2.690(1.083, 6.680)
SCF (pg/ml)	65.47(34.44, 117.89)	28.96(21.74, 53.51)	0.002	0.211(0.077, 0.578)
SDF-1α(pg/ml)	533.86(365.74, 687.06)	427.96(348.20, 664.83)	0.300	0.766(0.462, 1.268)
IL-1Rα(pg/ml)	527.52(221.30, 3346.5)	75.2(34.98, 238.44)	0.020	0.000(0.000, 0.224)

TNF-β (pg/ml)	58.55(34.84, 117.75)	110.74(49.79, 133.96)	0.057	1.616(0.987, 2.647)
MIF (pg/ml)	154.76(74.49, 284.19)	118.28(56.05, 241.79)	0.061	0.538(0.281, 1.029)
IP-10 (pg/ml)	1172.00(450.30, 4753.00)	947.54(346.14, 2053.00)	0.097	0.107(0.008, 1.499)
MCP-1 (pg/ml)	59.47(23.93, 181.64)	11.52(5.72, 22.2)	0.013	0.980(0.965, 0.996)
MIP-1α (pg/ml)	1.76(1.07, 7.35)	0.82(0.56, 1.73)	0.217	0.268(0.033, 2.163)
TRAIL (pg/ml)	11.95(7.65, 22.52)	13.20(7.72, 21.97)	0.364	0.738(0.383, 1.422)
M-CSF (pg/ml)	15.22(5.83, 33.57)	7.23(2.80, 10.62)	0.019	0.218(0.061, 0.779)
CTACK (pg/ml)	222.43(161.24, 342.43)	171.86(110.29, 231.01)	0.009	0.185(0.052, 0.652)

Abbreviations: IL-2R, interleukin-2 receptor; MIG, membrane-bound immunoglobulin; MIP, macrophage inflammatory protein; LIF, leukemia inhibitory factor; RANTES, regulated on activation normal T cell expressed and secreted; SCF, stem cell factor; SDF, stromal derived factor; TNF, tumor necrosis factor; MIF, macrophage migration inhibitor factor; IP, interferon inducible protein; MCP, monocyte chemoattractant protein; TRAIL, TNF-related apoptosis-inducing ligand; M-CSF, macrophage-stimulating factor; CTACK, cutaneous T cell-attracting chemokine.

Table 4. Multivariate logistic regression of cytokines in 30-day death group and 30-day survival group.

Cytokines	P value	Exp(B) 95%CI
LIF	0.056	0.000(0.000, 2.636)
RANTES	0.066	3.678(0.918, 14.730)
SCF	0.364	0.436(0.073, 2.615)
IL-1Rα	0.247	0.004(0.000, 47.738)
MCP-1	0.125	0.001(0.000, 6.433)
M-CSF	0.047	19.103(1.038, 351.576)
CTACK	0.289	0.312(0.036, 2.679)

Abbreviations: LIF, leukemia inhibitory factor; RANTES, regulated on activation normal T cell expressed and secreted; SCF, stem cell factor; MCP, monocyte chemoattractant protein; CTACK, cutaneous T cell-attracting chemokine; FGF, fibroblast growth factor.

Table 5. Correlation between plasma concentrations of cytokines and reads of responsible pathogens in mNGS reports.

Cytokines	P value	OR (95%CI)	R square
IL-2Rα	＜0.001	0.660(0.416, 0.904)	0.505
MIG	0.902	-0.019(-0.336, 0.298)	0.001
MIP-1-β	＜0.001	0.688(0.504, 0.871)	0.662
LIF	0.011	0.298(0.072, 0.523)	0.195
SCF	＜0.001	0.622(0.426, 0.818)	0.583
SDF-1-α	0.435	-0.119(-0.427, 0.188)	0.020
IL-1Rα	＜0.001	0.732(0.498, 0.965)	0.605
TNF-β	0.121	0.273(-0.076, 0.623)	0.078
MIF	0.105	0.268(-0.059, 0.595)	0.085
RANTES	0.464	-0.216(-0.809, 0.378)	0.018
IP-10	0.547	0.147(-0.347, 0.641)	0.012
MCP-1	＜0.001	0.531(0.283, 0.779)	0.390
MIP-1α	＜0.001	0.762(0.660, 0.863)	0.887
TRAIL	＜0.001	0.735(0.454, 1.015)	0.527
M-CSF	＜0.001	0.551(0.336, 0.766)	0.477
CTACK	＜0.001	0.608(0.467, 0.749)	0.721

Abbreviations: IL-2R, interleukin-2 receptor; MIG, membrane-bound immunoglobulin; MIP, macrophage inflammatory protein; LIF, leukemia inhibitory factor; RANTES, regulated on activation normal T cell expressed and secreted; SCF, stem cell factor; SDF, stromal derived factor; TNF, tumor necrosis factor; MIF, macrophage migration inhibitor factor; IP, interferon inducible protein; MCP, monocyte chemoattractant protein; TRAIL, TNF-related apoptosis-inducing ligand; M-CSF, macrophage-stimulating factor; CTACK, cutaneous T cell-attracting chemokine.

Cytokines elevated in sepsis group included M-CSF, CTACK, MCP-1, *et al.* In previous M-CSF-related studies, Takao Hidaka, *et al.* established a mouse model of severe myelosuppression. They concluded that M-CSF treatment could significantly reduce the incidence of infection and improve the survival rate of mice [10]. However, M-CSF exceeding 8.21pg/ml was an independent risk factor for 30-day death in infected patients in our study, which was contrary to the results of Takao Hidaka, *et al.* The discrepancy may be due to the difference in metabolism between the mouse model

and the human body, as well as the different basic state. Varieties in stages of infection may also be one of the reasons for the opposite effect of M-CSF. Increased responsiveness to M-CSF may cause organ damage in infected patients. Besides, the level of M-CSF was positively correlated with the reads of pathogens, and the patients with higher reads had a higher risk of 30-day death, which indirectly proved that high level of M-CSF was an independent risk factor for 30-day death of infected patients. The specific functioning mechanism of M-CSF and its impact on the prognosis of infected patients are worthy of further study.

Cutaneous T cell-attracting chemokine (CTACK) is a T cell chemokine secreted by skin keratinocytes. There were few studies on the relationship between CTACK and infectious diseases. Nevertheless, a large number of studies have confirmed that CTACK could promote skin inflammation, which was related to many skin diseases such as Stevens-Johnson syndrome. It could be up-regulated by TNF- α and IL-1β and down-regulated by IL-10 [11-13]. In our study, the plasma concentration of CTACK in patients with sepsis and 30-day death group was higher, and it was also positively correlated with the reads of pathogens. Although CTACK did not show an independent correlation with the prognosis of patients, its role in skin and soft tissue infections may be worthy of further study.

Monocyte chemoattractant protein (MCP) was one of the important inflammatory factors in the pathogenesis of sepsis. Raina Devi Ramnath, *et al.* established a mouse model of septicemia and proved that MCP-1 synthesis blockers could significantly reduce the content of MCP-1 in lung and liver of mice, thus reducing the incidence of septicemia [9]. In current study, the plasma concentration of MCP-1 in patients with sepsis was higher than those in patients with non-sepsis. The specific role and mechanism of MCP-1 in patients with sepsis requires further study.

As a member of the CCL family of chemokines, the main function of MIP-1 is to promote the chemotaxis and phagocytosis of effector cells. It was reported that MIP-1β (also known as CCL4) was helpful in distinguishing Gram-negative bacillus bloodstream infection and Gram-positive bacillus bloodstream infection [14]. While previous studies demonstrated that higher levels of CCL4 was related to poor prognosis, the conclusion was not obtained in our research. In current research, CCL4 was downregulated in sepsis group and 30-day death group.

The pathological mechanism of sepsis was mainly determined by the disordered regulation of inflammatory and anti-inflammatory response. Excessive production of pro-inflammatory factors led to an uncontrollable, undifferentiated systemic inflammatory response in human body and caused broad organ damage, while the anti-inflammatory system was also evoked and resulted in extensive production of anti-inflammatory factors. Therefore, sepsis is actually a two-way process, in which anti-inflammatory and inflammatory reactions coexist and antagonize each other [15]. The constantly changing immune status of patients with sepsis at different stages causing levels of cytokines also fluctuate accordingly, which may be the main reason of contradictory conclusions between different studies.

Previous studies suggested that combination of different cytokines may better explain the complex status of sepsis. The research conducted by Sandquist M, *et al.* suggested that through combining PCT, CD64 and soluble Triggering Receptor Expressed on Myeloid Cells-1 (sTERM-1) into a biological scoring system, higher diagnostic efficiency on sepsis could occur, and the AUC was as high as 0.95 [16]. Besides, the combination of IL-6, PCT and sTERM-1 could effectively predict the prognosis of infected patients [5]. With larger sample size and more accurate monitoring of cytokines, we may be able to draw more convincing conclusions into this area.

As a newly developed technology of pathogen detection, the diagnostic value of mNGS was proven by numerous studies [17-21]. In current study, the results demonstrated that reads of pathogens were positively correlated with plasma concentrations of various inflammatory factors including M-CSF. Reads of pathogens reflects the pathogen load in blood of human body, while higher the pathogen load in the blood, the more severe the disease and poorer prognosis maybe. Correlation between reads of pathogens and M-CSF may indirectly affirmed the indicative effect of M-CSF on prognosis.

There were shortcomings in current study. First of all, blood samples were only collected on the day of enrollment but failed to monitor the dynamic changes of cytokines during the development of the disease; Secondly, a verification queue to verify the effect of M-CSF on the prognosis of patients was not set up; Thirdly, the sample size was small, unable to compare and distinguish patients with different types of infection, and the results of data analysis may be biased.

In conclusion, plasma levels of cytokines were higher in patients with sepsis and 30-day death group, and M-CSF > 8.21pg/ml was an independent risk factor for 30-day death. A variety of cytokines were positively correlated with the reads of pathogens, which indirectly proved the indicative effect of cytokines on the severity and prognosis of infected patients. The complex process of immune status in patients with sepsis requires further studies. We also believe that with the deepening of research, the mechanism of cytokines functioning in patients with sepsis will be explained more clearly, so as to monitor the process of infection more efficiently.

Trail Registration and Ethical Approval

The study has been registered in the Chinese Clinical Trials Registry. Registration number: ChiCTR1800019187, October

30, 2018, retrospectively registered. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Zhongshan Hospital affiliated to Fudan University (No: B2018-182R).

Patient Consent for Publication

Before enrollment, all patients were informed fully and written informed consent was obtained from the patients for the publication of clinical information.

Availability of Data and Material

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

Funding

This work was supported by the National Natural Science Foundation of China (grant number: 81970035); and Shanghai Top-Priority Clinical Key Disciplines Construction Project (grant number: 2017ZZ02013).

Conflicting Interest

No conflicts of interest exist.

References

1. Ahn JY, Song JE, Ann HW, *et al.* Effects of Early Exercise Rehabilitation on Functional Recovery in Patients with Severe Sepsis. Yonsei Med J. 2018;59(7):843-851.
2. Singer M, Deutschman CS, Seymour CW, *et al.* The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-810.
3. Kidson KM, Henderson WR, Hutcheon JA. Case Fatality and Adverse Outcomes Are Reduced in Pregnant Women With Severe Sepsis or Septic Shock Compared With Age-Matched Comorbid-Matched Nonpregnant Women. Crit Care Med. 2018;46(11):1775-1782.
4. Han Y, Li Y, Jiang Y. The Prognostic Value of Plasma MicroRNA-155 and MicroRNA-146a Level in Severe Sepsis and Sepsis-Induced Acute Lung Injury Patients. Clin Lab. 2016;62(12):2355-2360.
5. Dolin HH, Papadimos TJ, Stepkowski S, *et al.* A Novel Combination of Biomarkers to Herald the Onset of Sepsis Prior to the Manifestation of Symptoms. Shock (Augusta, Ga.). 2018;49(4):364-370.
6. Takahashi H, Tashiro T, Miyazaki M, *et al.* An essential role of macrophage inflammatory protein 1alpha/CCL3 on the expression of host's innate immunities against infectious complications. J Leukocyte Biol. 2002;72(6):1190-1197.
7. Kalechman Y, Gafter U, Gal R, *et al.* Anti-IL-10 therapeutic strategy using the immunomodulator AS101 in protecting mice from sepsis-induced death: dependence on timing of immunomodulating intervention. Journal of immunology (Baltimore, Md.: 1950). 2002;169(1):384-392.
8. Emonts M, Sweep FC, Grebenchtchikov N, *et al.* Association between high levels of blood macrophage migration inhibitory factor, inappropriate adrenal response, and early death in patients with severe sepsis. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2007;44(10):1321-1328.
9. Ramnath RD, Ng SW, Guglielmotti A, *et al.* Role of MCP-1 in endotoxemia and sepsis. Int Immunopharmacol. 2008;8(6):810-818.
10. Hidaka T, Fujimura M, Nakashima A, *et al.* Macrophage colony-stimulating factor (M-CSF) prevents infectious death induced by chemotherapy in mice, while granulocyte-CSF does not. Japanese journal of cancer research: Gann. 2002;93(4):426-435.
11. Karnezis T, Farnsworth RH, Harris NC, *et al.* CCL27/CCL28-CCR10 Chemokine Signaling Mediates Migration of Lymphatic Endothelial Cells. Cancer Res. 2019;79(7):1558-1572.
12. Homey B, Alenius H, Müller A, *et al.* CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nat Med. 2002;8(2):157-165.
13. Karakawa M, Komine M, Hanakawa Y, *et al.* CCL27 is downregulated by interferon gamma via epidermal growth factor receptor in normal human epidermal keratinocytes. J Cell Physiol. 2014;229(12):1935-1945.
14. Sandquist M, Wong HR. Biomarkers of sepsis and their potential value in diagnosis, prognosis and treatment. Expert Rev Clin Immu. 2014;10(10):1349-1356.
15. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nature reviews. Immunology. 2013;13(12):862-874.
16. Li X, Yuan X, Wang C. The clinical value of IL-3, IL-4, IL-12p70, IL17A, IFN-γ, MIP-1β, NLR, P-selectin, and TNF-α in differentiating bloodstream infections caused by gram-negative, gram-positive bacteria and fungi in hospitalized patients: An Observational Study. Medicine. 2019;98(38):e17315.
17. Watson RS. Sick or Not SSIC? Time to Develop a Screening Tool for Early Identification of Sepsis in Children*. Pediatr Crit Care Me. 2018;19(8):790-792.
18. Yan HP, Li M, Lu XL, *et al.* Use of plasma mitochondrial DNA levels for determining disease severity and prognosis in pediatric sepsis: a case control study. Bmc Pediatr. 2018;18(1):267.
19. Miao Q, Ma Y, Wang Q, *et al.* Microbiological Diagnostic Performance of Metagenomic Next-generation Sequencing When Applied to Clinical Practice. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2018;67:S231-S240.
20. Li N, Cai Q, Miao Q, *et al.* High-Throughput Metagenomics for Identification of Pathogens in the Clinical Settings. Small methods. 2021;5(1):2000792.
21. Deurenberg RH, Bathoorn E, Chlebowicz MA, *et al.* Application of next generation sequencing in clinical microbiology and infection prevention. J Biotechnol. 2017;243:16-24.