STUDY ON THE RELATIONSHIP BETWEEN LEVELS OF TNF-α, IL-6, AND IL-10 WITH SEVERITY LEVELS IN SEPSIS PATIENTS CAUSED BY GRAM-NEGATIVE BACTERIA

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SUMMARY

Objectives: *To evaluate the relationship between serum levels of TNF-α, IL-6, IL-10 and the severity in patients with sepsis caused by Gram-negative bacteria.* ***Subjects and methods:*** *110 inpatients treated at Hospital E and Military Hospital 103, were diagnosed with sepsis with Gram-negative bacteria, whose serum levels of TNF-α, IL-6, IL-10 were quantified by ELISA technique.* ***Results:*** *IL-6, IL-10 concentrations and the ratio of IL-6/TNF-α in the group of fatal patients were significantly higher than that of the group of surviving patients. Levels of IL-6, IL-10, IL-6/TNF-α, and IL-10/TNF-α ratios in the group of patients with more than 3 dysfunctional organs were significantly higher than those with less than 3 dysfunctional organs. IL-6, IL-10 serum levels, and IL-6/TNF-α ratio had predictive significance in mortality and septic shock in patients with sepsis caused by Gram-negative bacteria.* ***Conclusion:*** *The concentrations of IL-6, IL-10, and the ratio of IL-6/TNF-α had prognostic value in mortality and septic shock in patients with sepsis infected by Gram-negative bacteria.*

** Keywords: Gram-negative sepsis; Concentrations of TNF-α, IL-6, IL-10; Relationship.*

INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock is a sepsis status with hypotension, despite adequate fluid resuscitation, still requires vasopressor therapy to maintain mean blood arterial pressure (MAP) ≥ 65 mmHg and lactate > 2 mmol/L (> 18 mg/dL) [3].

The emerging pathogens of sepsis recently is Gram-negative bacteria because of its prevalence, severe clinical presentation and accompanied by septic shock [4]. The treatment outcome depends on many factors, including the involvement of immune disorders with the role of cytokines, especially TNF-α, IL-6, and IL-10 [1, 5]. The roles of TNF-α, IL-6, and IL-10 in inflammation have been increasingly confirmed, elucidating the pathogenesis of sepsis and multi organ failure syndrome. These cytokines are mostly produced rapidly within a few hours to 24 hours after the systemic bacterial infection, whether the changes of their concentrations influence the severity of sepsis and treatment outcomes [5].

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Therefore, we conducted this study: *To evaluate the relationship between TNF-α, IL-6, and IL-10 serum levels with severity of illness in sepsis patients caused by Gram-negative bacteria*.

SUBJECTS AND METHODS

1. Subjects

110 inpatients were diagnosed with sepsis caused by Gram-negative bacteria at the Infectious Disease Department, Hospital E and Military Hospital 103, from December 2016 to June 2018.

** Inclusion criteria:* Patients were older than 18 years old, diagnosed sepsis according to the guideline of the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) proposed in 2016 [3] and had blood cultures result positive for Gram-negative bacteria.

- Sepsis patients group: Patients presented suspected infection and had at least two of the following three clinical criteria: Tachypnea, respiratory rate ≥ 22 cycles/min; Change in conscious status: Glasgow score less than 15 points; systolic arterial blood pressure ≤ 100 mmHg. Calculating SOFA score, when patients had SOFA score ≥ 2, they were diagnosed with sepsis.

- Septic shock patients group: Sepsis patients with hypotension, despite adequate fluid resuscitation, they still required vasopressors to maintain mean arterial blood pressure (MAP) ≥ 65 mmHg and blood lactate > 2 mmol/L (> 18 mg/dL).

** Exclusion criteria:*

+ Pregnant women, patients with cancer disease or end-stage chronic kidney failure or Child C cirrhosis or immunocompromised (HIV/AIDS infection...).

+ Patients whose serum levels of cytokines TNF-α, IL-6, and IL-10 were not measured in the period of the study.

+ Patients or family members were not willing to participate in the study.

2. Methods

A retrospective, cross-sectional study.

** Data collection:*

- Blood culture: BacT/Alert 3D automatic bacterial detection system at the Department of Microbiology, Hospital E and Military Hospital 103.

- Quantitative testing of cytokines TNF-α, IL-6, and IL-10:

+ Sample collecting time:

. For the group of sepsis patients without septic shock: At the time when the patients were diagnosed with sepsis. Grouping by disease duration (days of illness): The first 1 day and after the 1 day of illness.

. For the group of septic shock patients: When the patients occurred shock and left shock. Grouping by the time of onset of shock: First 2 days and after 2 days of illness.

+ Sample preparation: Collecting 2 mL of peripheral venous blood in a test tube with EDTA, citrate or heparin anticoagulant. After that, they were centrifuged at 1,000 rpm for 30 minutes at the Department of Hepatology, Hospital E and Department

of Microbiology, Military Hospital 103 to separate plasma before refrigerating at -80°C and transferring to the laboratory of the Institute of Biomedicine and Pharmacy, Vietnam Military Medical University. The plasma samples were defrosted before testing. Reagents and samples were kept at room temperature (18 - 25°C) before use. Then, the technical process was performed immediately or the samples were stored at ≤ 20°C in case the process was not performed.

+ The TNF-α, IL-6, and IL-10 kits were made by MULTISCIENCES (LIANKE) BIOTECH to quantify the concentrations of TNF-α, IL-6, and IL-10 in plasma samples with ELISA method. The limit and mean values of manufacturer's kit are 2.9 - 11.7 (7.4 pg/mL); 0 - 18.9 (5.0 pg/mL); 1.8 - 14.7 (6.8 pg/mL), respectively.

+ Analyzing the results with the US ELX800DA machine, connecting to the computer system at Institute of Biomedicine and Pharmacy, Vietnam Military Medical University.

* *Statistical analysis:* Both descriptive and analytical statistics were executed by SPSS (IBM SPSS Statistics for Windows, v. 20.0. Armonk, NY: IBM Corp). Demographic and descriptive continuous variables with normal distribution are shown as mean (standard deviation, SD), while non-normally distributed data are illustrated as median values (interquartile range, IQR). Categorical variables are presented as proportion. Chi-square or Fisher's exact test was used to compare dichotomous, whereas Mann-Whitney or T-test was calculated for continuous non-standard and standard variables, respectively. A statistically significant level of $p < 0.05$ was used.

RESULTS AND DISCUSSION

Table 1: Patient characteristics by time of disease detection.

Disease detection time (days)	Number of patients (n)	Percentage (%)
< 1	15	13.6
1 - 2	18	16.4
3 - 7	42	38.2
> 7	35	31.8
Total	110	100.0
Mean (Min - max)	5 (2 - 11)	

The mean time for sepsis detection and cytokine production was 5 days. Most patients were diagnosed on the 3rd - 7th day of illness. About 30% of patients were diagnosed within the first 2 days (of which about 13.6% were diagnosed within the first 1 day).

Pham Thi Ngoc Thao et al. witnessed that the average onset time of sepsis patients before admission was 6.1 days [1]. The American Society of Emergency Resuscitation's sepsis control campaign updated in 2018 introduced a strategy of "1-hour packages" instead of "3-hour and 6-hour packages". This illustrated that the duration time for the diagnosis and treatment of sepsis is an essential issue. The early detection and diagnosis of sepsis contribute to improving prognosis and reducing mortality rate [6].

Table 2: Comparison of TNF-α, IL-6, IL-10 levels between two groups of fatal and surviving sepsis patients.

Cytokine	Mortal patients (n = 21)	Surviving patients (n = 89)	p-value
IL-6 (pg/mL)	60.91 (18.16 - 169.93)	17.07 (6.58 - 35.4)	< 0.005
IL-10 (pg/mL)	24.67 (6.57 - 84.24)	6.92 (3.25 - 21.72)	< 0.01
TNF-α (pg/mL)	163.13 (7.58 - 394.2)	168.13 (21.04 - 271.23)	> 0.05
IL-6/IL-10 ratio	4.11 (1.17 - 7.1)	1.89 (0.61 - 7.47)	> 0.05
IL-6/TNF-α ratio	0.49 (0.1 - 6.94)	0.09 (0.02 - 1.11)	< 0.05
IL-10/TNF-α ratio	0.28 (0.03 - 1.41)	0.06 (0.02 - 0.42)	> 0.05

The mean concentrations of IL-6, IL-10, the IL-6/TNF-α ratio in the group of fatal patients were significantly higher than that of survivors ($p < 0.005$ to $p < 0.05$).

The research by Truong Ngoc Hai et al. (2009) presented that concentrations of TNF-α, IL-6, IL-8, IL-10, and ratio of IL-10/TNF-α in the group of multi-organ dysfunction patients were 1.75 times higher than that of surviving patients [2]. In 2021, Georgescu AM et al. observed that TNF-α level was significantly higher in infection-induced fatal patients treated in the ICU (53.71 ± 10.21 pg/mL) compared to survivors (39.68 ± 10.21 pg/mL), $p = 0.02$ [7].

Table 3: Comparison of concentrations of TNF-α, IL-6, IL-10 between groups of patients with more or less than 3 dysfunctional organs.

Cytokine	≥ 3 dysfunctional organs (n = 35)	< 3 dysfunctional organs (n = 75)	p
IL-6 (pg/mL)	34.35 (16.55 - 174.15)	13.61 (5.23 - 36.45)	< 0.001
IL-10 (pg/mL)	16.3 (5.62 - 70.57)	6.36 (3.44 - 19.37)	< 0.01
TNF-α (pg/mL)	124.45 (6.74 - 374.48)	171.57 (25.17 - 268.88)	> 0.05
IL-6/IL-10 ratio	4,98 (0.81 - 7.61)	1.84 (0.63 - 7.06)	> 0.05
IL-6/TNF-α ratio	0.49 (0.06 - 7.1)	0.09 (0.02 - 0.86)	< 0.05
IL-10/TNF-α ratio	0.17 (0.03 - 1.28)	0.04 (0.02 - 0.32)	< 0.05

The group of patients with more than 3 dysfunctional organs had significantly higher levels of IL-6 and IL-10, ratios of IL-6/TNF-α and IL-10/TNF-α compared with the group of patients with less than 3 dysfunctional organs ($p < 0.001$ to $p < 0.05$).

The study by Pham Thi Ngoc Thao et al. on severe sepsis patients displayed that the IL-10 concentrations at T24 in the group of patients with more than 3 dysfunctional organs and group with less than or equal 3 dysfunctional organs were 19.99 and 7.15, respectively. This difference was statistically significant with p < 0.001 [1].

Table 4: Predictive value of cytokines level for septic shock in sepsis patients with Gram-negative bacteria by ROC curve.

Variable	AUC	p-value	Cut-off point	Sensitivity (%)	Specificity (%)
IL-6 level	0.775	< 0.001	37.47	66.7	82.3
IL-10 level	0.715	< 0.005	36.56	46.7	88.8
TNF-α level	0.553	> 0.05	-	-	-
IL-6/IL-10 ratio	0.578	> 0.05	-	-	-
IL-6/TNF-α ratio	0.628	< 0.05	0.17	70.0	60.0
IL-10/TNF-α ratio	0.608	> 0.05	-	-	-

The results demonstrated that levels of IL-6, IL-10, and IL-6/TNF-α ratio had a prognostic value of septic shock status in sepsis patients caused by Gram-negative bacteria. In which, IL-6 level had the best predictive value with AUC = 0.775; followed by IL-10 level with AUC = 0.715; the last was the ratio of IL-6/TNF-α with an AUC = 0.628 (p-values ranged from p < 0.001 to p < 0.05).

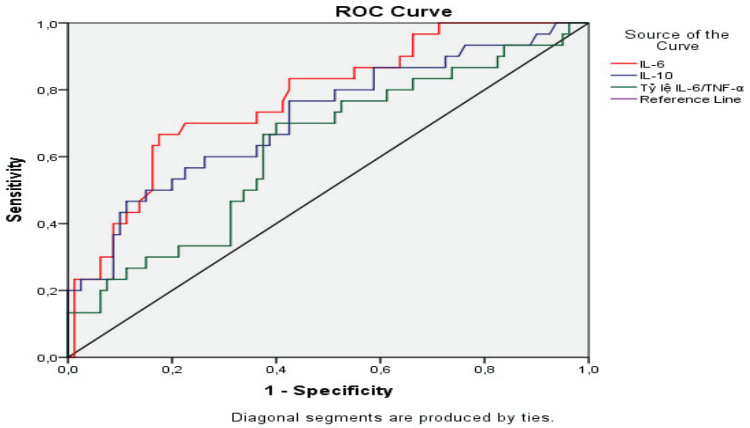

Figure 1: Predictive value of cytokines level for septic shock in sepsis patients with Gram-negative bacteria by ROC curve.

The ROC curve graph presented the prognostic value of IL-6, IL-10 levels, and IL-6/TNF-α ratio for septic shock in sepsis patients with Gram-negative bacteria. The cut-off point of IL-6 was 37.47 pg/mL with a sensitivity level of 66.7% and a specificity level of 82.3%, while the cut-off point of IL-10 was 36.56 pg/mL with a sensitivity level of 46.7%, and a specificity level of 88.8%. The cut-off point of the IL-6/TNF-α ratio was 0.17, with a sensitivity value of 70% and a specificity value of 60%.

Xu XJ, et al (2019) indicated that PCT, IL-6 and IL-10 levels were advantage biomarkers to predict septic shock with area under the AUC curve (95%CI) being 0.776 (0.726 - 0.826); 0.888 (0.861 - 0.914); 0.866 (0.828 - 0.903), respectively. These biomarkers forecast progression of septic shock with specificity value about 80%, and sensitivity values of PCT, IL-6 and IL-10 were 63.3%; 84.4% and 77.1%, respectively [8].

Table 5: Predictive value of cytokines level for mortality in sepsis patients with Gram-negative bacteria by ROC curve.

Variable	AUC	p-value	Cut-off point	Sensitivity (%)	Specificity (%)
IL-6 level	0.723	< 0.005	35.4	66.7	75.3
IL-10 level	0.685	< 0.01	14.37	66.7	70.8
TNF-α level	0.504	> 0.05	-	-	-
IL-6/IL-10 ratio	0.564	> 0.05	-	-	-
IL-6/TNF-α ratio	0.647	< 0.05	0.095	81.0	51.7
IL-10/TNF-α ratio	0.628	> 0.05	-	-	-

Based on ROC curve model, IL-6, IL-10 levels and IL-6/TNF-α ratio had prognostic significance for mortality in sepsis patients with Gram-negative bacteria. Of which, IL-6 level had the best predictive value with the AUC = 0.723; followed by IL-10 level with the AUC = 0.685; the last was the ratio of IL-6/TNF-α with the AUC = 0.647 (p-values ranged from p < 0.005 to p < 0.05).

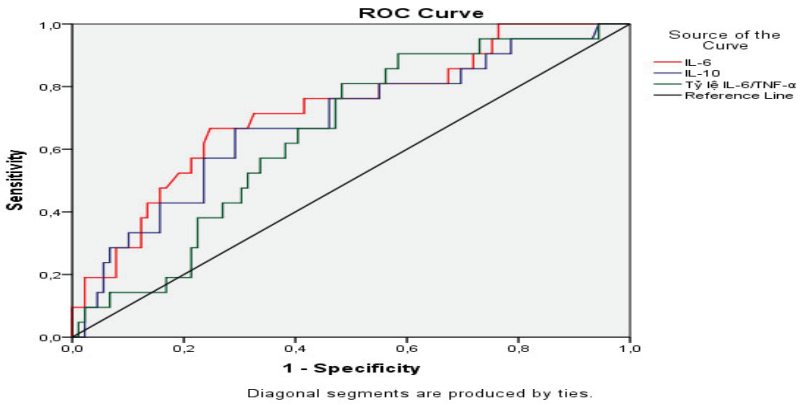

Figure 2: Predictive value of cytokines level for mortality in sepsis patients with Gram-negative bacteria by ROC curve.

The ROC curve graph showed the prognostic value of IL-6, IL-10 levels, and IL-6/TNF-α ratio for fatal rate in sepsis patients with Gram-negative bacteria. The cut-off point of IL-6 level was 35.4 pg/mL with a sensitivity level of 66.7% and a specificity level of 75.3%, while the cut-off point of IL-10 level was 14.37 pg/mL with a sensitivity value of 66.7% and a specificity value of 70.8%. The cut-off point of the IL-6/TNF-α ratio was 0.095, with a sensitivity value of 81% and a specificity value of 51.7%.

Oberholzer A et al. (2005) manifested that IL-6 level was an excellent predictor of mortality in sepsis patients, even when using alone or combined with the APACHE-II or MODS score [9]. Hamishekar H et al. (2010) studied 51 patients with severe sepsis and noted that IL-6 level was significantly higher in the mortality group compared to the surviving group, at the testing time of the 1st day, the 3rd day, and the 7th day after admission in the patients with severe sepsis and septic shock [10].

CONCLUSION

From all the results above, a conclusion can be drawn that the concentrations of IL-6, IL-10 and IL-6/TNF-α ratio in the group of fatal patients were significantly higher than that of the survivors and had prognostic value for septic shock and mortality in sepsis patients with Gram-negative bacteria.

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DISCLOSURE

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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