STUDY ON THE RELATIONSHIP BETWEEN TNF-α, IL-6, IL-10 CONCENTRATIONS AND PROGNOSIS IN GRAM-NEGATIVE SEPSIS PATIENTS

Vu Manh Cuong[1], Hoang Vu Hung[2], Vu Xuan Nghia[2], Le Van Nam[2]

SUMMARY

Objectives: *To evaluate the association between TNF-α, IL-6, IL-10 concentrations and prognosis (alive or dead) in patients with Gram-negative sepsis.* ***Subjects and methods:*** *124 patients who were diagnosed with Gram-negative sepsis based on blood culture results at Military Hospital 103 and E Hospital. TNF-α, IL-6, and IL-10 concentration were quantified by ELISA technique and compare the cytokin concentrations at the time-points of T0, T24 between the death group and the survival group.* ***Results:*** *Concentrations of TNF-α, IL-6, and IL-10 at the time-points of T0, T24 were higher in the death group than in the survival group, but statistically significant difference was found in IL-6 concentration alone ($p < 0.05$). Serum IL-6 concentration at the time of $T_{24} > 51.48$ pg/mL was capable of predicting mortality in patients with Gram-negative sepsis. The ratios of IL-6/IL-10 and IL-10/TNF-α at the time of T24 were higher in the death group, but the difference was not statistically significant with $p > 0.05$.* ***Conclusion:*** *Concentrations of TNF-α, IL-6, and IL-10 at the time-points of T_0, T_{24}, and ratios IL-6/IL-10, IL-10/TNF-α at the time-points of T24 in the death group were higher than the survival group. Serum IL-6 concentration at the time of T24 is a good predictor of mortality in patients with Gram-negative sepsis.*

** Keywords: Gram-negative sepsis; TNF-α, IL-6, IL-10 concentrations; Prognosis.*

INTRODUCTION

Sepsis is the body's (host) response to an infection that gets out of control, causing organ dysfunction and life-threatening. Septic shock is the sepsis with hypotension, life-threatening, and still requires vasopressors to maintain mean arterial pressure (MAP) ≥ 65 mmHg and lactate > 2 mmol/L (> 18 mg/dL) despite achieving complete resuscitation [1].

The common causative agent of sepsis is Gram-negative bacteria due to its prevalence, severe clinical manifestations, and associated septic shock. The mortality rate in patients with septic shock can be up to 80%. The treatment of Gram-negative sepsis is very difficult due to their high resistance to antibiotics [2]. Moreover, the outcome of treatment depends on many factors, including the involvement of immune disorders with the role of cytokines,

[1]*E Hospital*
[2]*Military Hospital 103,* ***Vietnam Military Medical University***
Corresponding author: Vu Manh Cuong (vumanhcuongbve@gmail.com)
Date received: 25/01/2021
Date accepted: 28/3/2021

especially TNF-α, IL-6, and IL-10 [3, 4]. The inflammatory roles of TNF-α and IL-6, as well as the anti-inflammatory role of IL-10, have been identified and helped to clarify sepsis pathophysiology and multi-organ failure syndrome. These cytokines are mostly produced quickly within a few hours to 24 hours after the entry of a pathogen into the body. Whether the change in these cytokine concentrations affects disease severity and treatment outcomes or not is still under debate [3].

Therefore, we conducted this study: *To evaluate the relationship between TNF-α, IL-6, IL-10 concentrations and prognosis in patients with Gram-negative sepsis.*

SUBJECTS AND METHODS

1. Subjects

124 patients treated in the Clinical Departments at E Hospital and Military Hospital 103, who were diagnosed as Gram-negative sepsis based on the blood culture results from December 2016 to June 2018. These patients were divided into 2 groups:

- Group 1: The survival group (including 103 patients who were cured and discharged from the hospital)

- Group 2: The death group (including 21 patients who were dead or critically ill for home requirement.

** Inclusion criteria:*

+ Age of 18 and above, regardless of gender and occupation.

+ Two positive blood cultures with the same Gram-negative bacteria.

+ Measuring TNF-α, IL-6, and IL-10 concentrations at two times of study: T_0 (time of blood culture) and T_{24} (after 24h).

** Exclusion criteria:*

+ Pregnancy.

+ End-stage cancer, end-stage chronic kidney failure, Child-C cirrhosis, immunodeficiency such as HIV/AIDS, etc.

+ Not agree to participate in the study.

2. Methods

** Study design: descriptive, prospective follow-up.*

The concentrations TNF-α, IL-6, and IL-10 between the two groups were compared at the timepoints of T0, T24.

** Study material:*

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

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

- Using TNF-α, IL-6, and IL-10 kits of AviBion - Orgenium (Finland) to quantify TNF-α, IL-6, and IL-10 concentrations in plasma samples of patients.

- Reading the ELISA results using BECKMAN-COULTER-DTX 880 system of Beckman-Coulter (USA) at D3, Institute of Biomedicine and Pharmacy - Vietnam Military Medical University.

- The normal ranges: TNF-α (< 11 pg/mL); IL-6 (< 1.23 pg/mL); IL-10 (< 1.9 pg/mL).

** Data processing:* Using SPSS 20.0 software.

RESULTS AND DISCUSSIONS

Table 1: Some clinical characteristics in two groups of patients

Characteristics	Group 1 (n = 103)	Group 2 (n = 21)	p
Age (year) ($\overline{X}$ ± SD)	64 (53 - 75)	79 (57 - 83)	< 0.05
Males (n, %)	57 (55.3)	15 (71.4)	> 0.05
History of comorbidities (n, %)	85 (82.5)	17 (81)	> 0.05
Duration of hospitalization (days) ($\overline{X}$ ± SD)	17 (13 - 27)	15 (5 - 32)	> 0.05
APACHE II score ($\overline{X}$ ± SD)	10 (7 - 13)	14 (12.5 - 17.5)	< 0.001
SOFA score ($\overline{X}$ ± SD)	3 (1 - 6)	8 (5.5 - 11.5)	< 0.001
More than 3 organs with dysfunction (n, %)	21 (20.4)	18 (85.7)	< 0.001

It can be seen from table 1 the median age of patients, APACHE II score, SOFA score, and the number of organs with dysfunction was significantly different between the two groups Meanwhile, there was no significant difference in gender, the history of comorbidities, and the duration of hospitalization between the two groups.

Pham Thi Ngoc Thao et al., when studying on 123 severe sepsis patients, found that the APACHE II score, SOFA score, and the number of organs with dysfunction also had a statistically significant difference between the death group and survival group. In the meantime, age, gender, and duration of hospitalization were not significantly different between the two groups [1].

Table 2: Some laboratory characteristics in two groups

Characteristics	Group 1 (n = 103)	Group 2 (n = 21)	p
Mean hemoglobin concentration (g/L)	111.47 ± 23.87	103.9 ± 15.91	> 0.05
White blood cell count (G/L) $\overline{X}$ ± SD	11.9 (7.8 - 16.5)	12.18 (7.7 - 18.63)	> 0.05
Platelet count (G/L) $\overline{X}$ ± SD	151 (96 - 247)	116 (77 - 170.45)	< 0.05
Urea (mmol/L) $\overline{X}$ ± SD	6.78 (4.7 - 9.5)	10.26 (6.63 - 16.4)	< 0.05
Prothrombin time (s) $\overline{X}$ ± SD	16.1 (14.2 - 18.4)	18.2 (15.1 - 21.6)	< 0.05
CRP (mg/L) $\overline{X}$ ± SD	100 (32.1 - 171.9)	197.6 (67.89 - 251.7)	< 0.05
Procalcitonin (ng/mL) $\overline{X}$ ± SD	7.9 (1.63 - 26.97)	11.77 (0.85 - 25.06)	> 0.05
Lactate (mmol/L) $\overline{X}$ ± SD	1.9 (1.3 - 3)	3.2 (1.75 - 4.5)	> 0.05

There was a statistically significant difference in the platelet count, urea concentration, prothrombin time, and CRP between the two groups with $p < 0.05$. Lactate concentration and other indices changed but not statistically significant with $p > 0.05$.

Table 3: Comparison of TNF-α, IL-6, and IL-10 concentrations between two groups at the time of follow-up

Cytokine ($\overline{X}$ ± SD)	**Time**	**Group 1 (n = 103)**	**Group 2 (n = 21)**	**p**
TNF-α *(< 11 pg/mL)*	T_0	137.39 (17.04 - 259.5)	142.26 (27.29 - 298.34)	> 0.05
	T_{24}	146.4 (22.4 - 268.88)	159.27 (6.78 - 384.82)	> 0.05
IL-6 *(< 1.23 pg/mL)*	T_0	26.47 (8.45 - 92.2)	106.19 (16.76 - 819.12)	< 0.01
	T_{24}	15.51 (5.86 - 32.12)	54.14 (10.01 - 194.72)	< 0.01
IL-10 *(< 1.9 pg/mL)*	T_0	9.68 (4.65 - 46.4)	38.38 (9.16 - 183.27)	> 0.05
	T_{24}	5.62 (3.25 - 17.77)	14.44 (3.97 - 61.43)	> 0.05
IL-6 *(pg/mL)*	T24/T0	0.58 (0.26 - 1.14)	0.55 (0.16 - 1.57)	> 0.05
IL-10 *(pg/mL)*		0.73 (0.26 - 1.03)	0.72 (0.12 - 1.88)	> 0.05
IL-6/IL-10 ratio	T_{24}	1.87 (0.6 - 6.62)	2.22 (0.74 - 8.34)	> 0.05
IL-10/TNF-α ratio	T_{24}	0.05 (0.02 - 0.5)	0.18 (0.04 - 0.96)	> 0.05

Concentrations of TNF-α, IL-6, and IL-10 at the time of T_0, T_{24} were higher in the death group than in the survival group, but only IL-6 concentration was statistically significantly different with $p < 0.05$. The changes in concentrations of IL-6 and IL-10 at the time of T_{24} compared with T_0 did not differ between the groups of death and survival. The ratios of IL-6/IL-10 and IL-10/TNF-α at the time of T_{24} were higher in the death group than in the survival group. However, the difference was not statistically significant with $p > 0.05$.

According to Pham Thi Ngoc Thao et al., there was a statistically significant difference in the variation of IL-6 concentration at the time of T_{24} compared with T_0 and ratio of IL10/TNF-α at the time of T_{24} between the two groups of severe sepsis patients (the death and survival) [1].

Maja Surbatovic et al., when studying on 165 patients with severe abdominal infection, found that the concentrations of TNF-α and IL-10 in the death group were 4.7 and 3.3 times higher than those in the survival group with $p < 0.01$. Patients with TNF-α concentration > 0.48 pg/mL and IL-10 > 9.5 pg/mL had a higher risk of death than patients with lower TNF-α and IL-10 concentrations with the sensitivity, the specificity, and the area under the curve of 67%, 76.6%, 0.772 and 72.7%, 80.5%, 0.770, respectively [5].

Meda Georgescu AM et al., when studying on 163 sepsis patients showed that serum TNF-α concentration was significantly higher in sepsis shock patients (57.3 ± 10.21 pg/mL) than in sepsis patients (42.9 ± 10.22 pg/mL) with $p = 0.0007$ [6].

Table 4: Mortality prognostic value of IL-6, IL-10 concentration, IL-10/TNF-α ratio at the time of T_{24} and the differential ratio of IL-6 concentration at time T_{24} compared with T_0 in Gram-negative sepsis patients (n = 124).

Variables	AUC	p-value	Cut-off value	The sensitivity	The specificity
IL-6_T24	0.694	< 0.01	51.48	52.4%	86.4%
IL-10_T24	0.623	> 0.05	10.87	61.9%	69.9%
IL-10/TNF-α_T24	0.629	> 0.05	0.13	61.9%	68%
IL-6_T24/T0	0.502	> 0.05	0.06	19%	92.2%

Serum IL-6 concentration at the time of T24 > 51.48 pg/mL had a prognostic value of mortality in Gram-negative sepsis patients with a sensitivity of 52.4%, a specificity of 86.4%, and the area under the curve of 0.694 (p < 0.01).

Serum IL-10 concentration at the time of T24 > 10.87 pg/mL did not have the prognostic value of mortality in Gram-negative sepsis patients with a sensitivity of 61.9%, a specificity of 69.9%, and the area under the curve of 0.623 (p > 0.05).

The median value of IL-10/TNF-α ratio at the time of T24 > 0.13 pg/mL did not have the prognostic value of mortality in Gram-negative sepsis patients with a sensitivity of 61.9%, a specificity of 68%, and the area under the curve of 0.629 (p > 0.05).

Serum IL-6 concentration at the time of T24 increased 0.06 times compared with the time of T0 and did not have the prognostic value of mortality in Gram-negative sepsis patients with the area under the curve of 0.502, a sensitivity of 19%, and a specificity of 92.2% (p > 0.05).

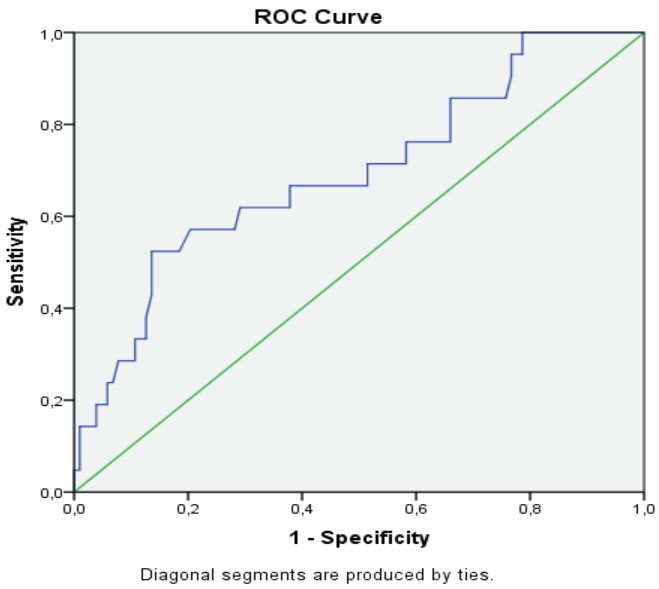

Figure 1: ROC curves predict the mortality of IL-6 at T_{24}

Diepold et al [7] found that IL-6 concentration was the best predictor of sepsis and severe sepsis with high sensitivity and specificity (90% and 85%, respectively).

Sahbudak Bal Z et al found that IL-8 and IL-10 concentrations had a better sensitivity and specificity than IL-6 concentration on predicting sepsis and severe sepsis [8].

Pham Thi Ngoc Thao et al. showed that the median value of IL-10/TNF-α ratio at the time of T_{24} > 0.73 pg/mL had a prognostic value of mortality in severe sepsis patients with a sensitivity of 82.6%, a specificity of 53%, and the area under the curve of 0.752 [1].

CONCLUSIONS

Studying on the relationship between TNF-α, IL-6, and IL-10 concentrations and prognosis in 124 Gram-negative sepsis patients, we found that:

- Concentrations of TNF-α, IL-6, and IL-10 at the time of T_0, T_{24} were higher in the death group than in the survival group, but only IL-6 concentration was statistically significantly different ($p < 0.05$). Serum IL-6 concentration at the time of T_{24} > 51.48 pg/mL had a prognostic value of mortality in Gram-negative sepsis patients.

- The ratio of IL-6/IL-10 and IL-10/TNF-α at the time of T_{24} were higher in the death group than in the survival group. However, the difference was not statistically significant ($p > 0.05$).

REFERENCES

1. Pham Thi Ngoc Thao. Clinical, subclinical research and prognostic value of some cytokin TNF-α, IL-6, IL-10 in patients with severe sepsis. Doctor of Medicine PhD thesis, Vietnam Military Medical University. 2011.

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. Wisplinghoff H, Bischoff T, Tallent SM et al. Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004; 39(3):309-317.

4. Kibe S, Adams K, Barlow G. Diagnostic and prognostic biomarkers of sepsis in critical care. J Antimicrob Chemother 2011; 66 Suppl 2:ii33-40.

5. Surbatovic M, Popovic N, Vojvodic D et al. Cytokine profile in severe Gram-positive and Gram-negative abdominal sepsis. Sci Rep 2015; 5: p11355.

6. Georgescu AM, Banescu C, Azamfirei R et al. Evaluation of TNF-α genetic polymorphisms as predictors for sepsis susceptibility and progression. BMC Infect Dis, 2020; 20(1):221.

7. Diepold M, Noellke P, Duffner U et al. Performance of Interleukin-6 and Interleukin-8 serum levels in pediatric oncology patients with neutropenia and fever for the assessment of low-risk. BMC Infect Dis 2008; 8:28.

8. Sahbudak Bal Z, Karadas Ozdemir N, Sen S et al. Diagnostic accuracy of Interleukin-6, Interleukin-8, and Interleukin-10 for predicting bacteremia in children with febrile neutropenia. Turk J Haematol 2017; 34 (3):254-257.