

## ORIGINAL ARTICLES

# Relationship between the expression of TLR4 and TIRAP and sepsis in peripheral blood

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## Abstract

**Objective:** To explore the relationship between the expression of TLR4 and TIRAP and sepsis severity.

**Methods:** The study selected 30 healthy examinees as the control group and 53 patients with sepsis as the observation group. The patients in the observation group were assessed by Acute Physiology and Chronic Health Evaluation II (APACHE II). 40 patients with APACHE II score  $\leq 20$ , were classified into the low-score sepsis group; 13 patients with APACHE II score  $> 20$ , were classified into the high-score sepsis group. The levels of TLR4 and TIRAP in venous serum were detected in all subjects by enzyme-linked immunosorbent assay (ELISA).

**Results:** The levels of TLR4 and TIRAP in serum were  $0.886 \pm 0.058$  ng/ml and  $5.216 \pm 0.410$  ng/ml in the control group;  $2.253 \pm 0.379$  ng/ml and  $9.540 \pm 2.294$  ng/ml in the low-score sepsis group;  $4.494 \pm 0.709$  ng/ml and  $19.206 \pm 1.755$  ng/ml in the high-score sepsis group. The observation group (low-score and high-score sepsis groups) was significantly different from the control group ( $p = .000$ ), and the low-score sepsis group was significantly different from the high-score sepsis group ( $p = .000$ ). With APACHE II score of 20 as the cut-off point, the low-score sepsis group was consisted of low-risk patients and the high-score group was consisted of the high-risk, which can indicate the sepsis severity. According to Pearson's correlation analysis, the level of TLR4 was positively correlated with sepsis severity ( $r = 0.931$ ,  $p < .05$ ), the level of TIRAP was also positively correlated with the sepsis severity ( $r = 0.972$ ,  $p < .05$ ); the level of TLR4 was positively correlated with the level of TIRAP ( $r = 0.936$ ,  $p < .05$ ).

**Conclusions:** The levels of TLR4 and TIRAP in septic patients can be used to predict and determine the severity of sepsis.

**Key Words:** Sepsis, Toll-like receptor-4, Toll/interleukin-1 receptor adapter protein

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Systemic inflammatory response syndrome (SIRS), in which various inflammatory mediators and effector cells that cause out-of-control responses mutually participate, happens after severe infections, burns, shocks, major surgical operations and traumas. It is a subset of self-cascade inflammatory cytokine storm. SIRS is also closely related to sepsis, in which case patients suffer from bacterial infections or have a highly suspected focus of infection. If sepsis progresses, it will lead to septic shock and multiple organ dysfunction syndrome (MODS) and have an ex-

tremely high incidence and mortality in ICU.<sup>[1,2]</sup> Toll-like receptors (TLRs) are bound to extracellular receptors to activate the inflammatory signaling pathway by identifying the pathogen-associated molecular pattern.<sup>[3]</sup> The adapter protein of TLR is toll/interleukin-1 receptor adapter protein (TIRAP), which plays an important role in the downstream of TLR signaling.<sup>[4]</sup> The study is intended to explore the relationship between the levels of TLR4 and TIRAP and the severity of sepsis by detecting their levels in serum in patients with sepsis.

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# 1 Data and methods

## 1.1 Research objects

According to standards defined in “International Sepsis Definitions Conference” held in Washington in December of 2001,<sup>[5]</sup> 53 cases of subjects (28 males and 25 females with the age of  $59.8 \pm 13.2$ ) who were diagnosed as sepsis were selected in MICU in our hospital during October of 2012 to May of 2013. According to Acute Physiology and Chronic Health Evaluation II (APACHE II), the patients with sepsis were divided into two groups: the low-score sepsis group (APACHE II  $\leq 20$ , 23 males and 17 females with the age of  $61.1 \pm 12.3$ ); and the high-score sepsis group (APACHE II  $> 20$ , 7 males and 6 females, with the age of  $62.2 \pm 11.4$ ). Exclusion criteria were as follows: (1) patients with acquired immunodeficiency syndrome; (2) patients who received chemotherapy within the previous 8 weeks; (3) patients who were given the treatment with immunosuppressants after organ transplantation; (4) patients who used glucocorticoids within the previous 4 weeks; (5) patients who were more than 80 years old or with hepatic/renal failure. The control group was consisted of 30 healthy examinees (18 males and 12 females, with the age of  $60.9 \pm 12.5$ ) without a history of special diseases and recent infections. There was no significant difference in age and gender between the observation group (the low-score sepsis group and the high-score sepsis group) and the control group ( $p > .05$ ). The research was approved by Ethics Committee of our hospital and all patients signed informed consent forms.

## 1.2 Research methods

4 ml of peripheral venous blood was taken from each subject in these three groups, and 2 ml of blood was injected into the anticoagulant tube containing 2% EDTA anticoagulant, placed and frozen at  $-80^{\circ}\text{C}$ . Gradient freezing was applied to the freezing process. The rest 2 ml of blood was

placed and centrifuged in the refrigerated centrifuge ( $4^{\circ}\text{C}$ ) at 3,000 r/min for 5-7 minutes, and the serum was isolated, and then drawn by the pipette in the freezing tube for freezing at  $-80^{\circ}\text{C}$  with the application of gradient freezing as well. ELISA kits made by R&D Corporation (USA) were used to detect the levels of TLR4 and TIRAP in serum according to the instruction. The automatic microplate reader (Thermo Fisher Scientific, USA, 450 nm) was used to measure the value of optical density (OD) in each well in order. Standard curves were plotted according to standard concentrations, which can be calculated by use of OD values.

(1) Standard curve plotting: the X-axis was represented by the concentration of standard substances known in practice, and the Y-axis was represented by the measured corresponding OD value ( $\text{OD}_{450}$ ). The curve was plotted on this coordinate system.

(2) According to the OD value of each sample to be measured (serum), the corresponding concentration of proteins (i.e. the protein concentration of sample to be measured) can be found on the plotted standard curve mentioned above.

Patients with sepsis were assessed by APACHE II, a type of clinical medical scoring software (2005 Version).

## 1.3 Statistical treatment

The experimental data were analyzed by SPSS 17.0 statistical software and represented by  $\bar{x} \pm s$ . The analysis of variance was applied to the data analysis in all groups. When the variance was homogeneous, one way ANOVA was applied to the comparison of correlation, and least significant difference (LSD) was used in the test; when the variance was not homogeneous, rank sum test was used in the comparison, and the difference  $p < .05$  was of statistical significance. Pearson’s correlation test was applied to bivariate analysis correlation.

**Table 1:** Expressions of TLR4 and TIRAP in serum in each group

	Control Group	Low-score Sepsis Group	High-score Sepsis
n	30	40	13
TLR4 (ng/ml)	$0.886 \pm 0.058$	$2.253 \pm 0.379^*$	$4.494 \pm 0.709^{*\Delta}$
TIRAP (ng/ml)	$5.216 \pm 0.410$	$9.540 \pm 2.294^*$	$19.206 \pm 1.755^{*\Delta}$

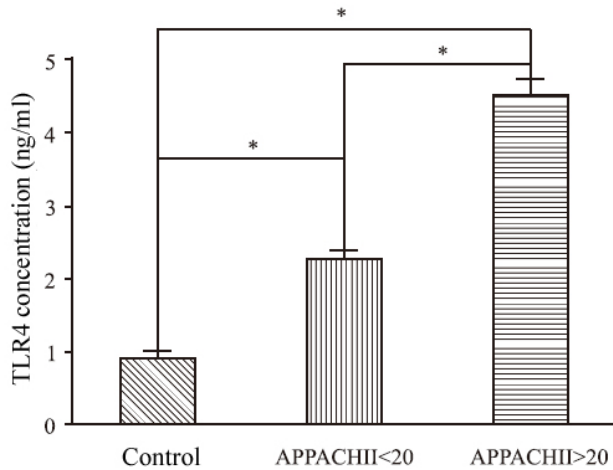
Note. In comparison with the control group, \*  $p = .000$ ; In comparison with the low-score sepsis group,  $\Delta p = .000$

# 2 Results

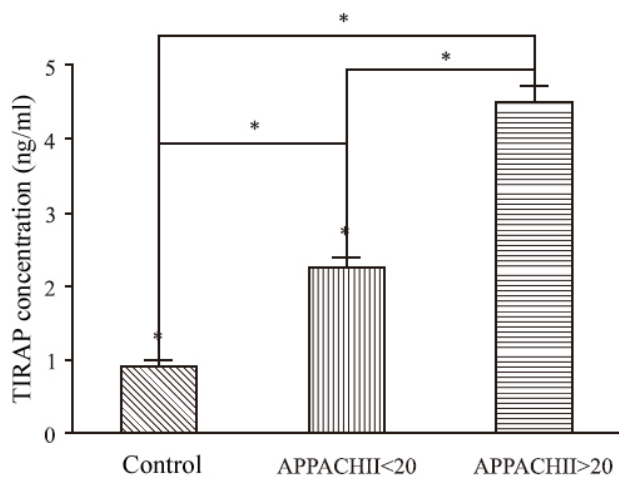
The experimental results were as follows: the levels of TLR4 and TIRAP: the control group:  $0.886 \pm 0.058$  ng/ml and  $5.216 \pm 0.410$  ng/ml; the low-score sepsis group:  $2.253 \pm 0.379$  ng/ml and  $9.540 \pm 2.294$  ng/ml; the high-score sepsis group:  $4.494 \pm 0.709$  ng/ml and  $19.206 \pm 1.755$  ng/ml.

There were significant differences in the levels of TLR4 and TIRAP among these three groups ( $p = .000$ , see Table 1 and Figures 1-2). With APACHE II score of 20 as the cut-off point, the low-score sepsis group was consisted of low-risk patients and the high-score group was consisted of the high-risk, which can indicate the sepsis severity. According to Pearson’s correlation analysis, the level of TLR4 was pos-

itively correlated with sepsis severity ( $r = 0.931, p < .05$ , see Figure 3); the level of TIRAP was also positively correlated with sepsis severity ( $r = 0.972, p < .05$ , see Figure 4); the level of TLR4 was positively correlated with the level of TIRAP ( $r = 0.936, p < .05$ , see Figure 5).



**Figure 1:** Expression of TLR4 in each group (\* $p < .001$ )

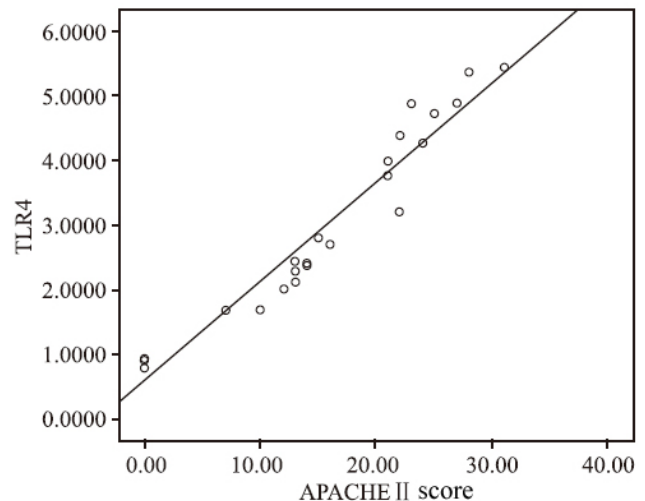


**Figure 2:** Expression of TIRAP in each group (\* $p < .001$ )

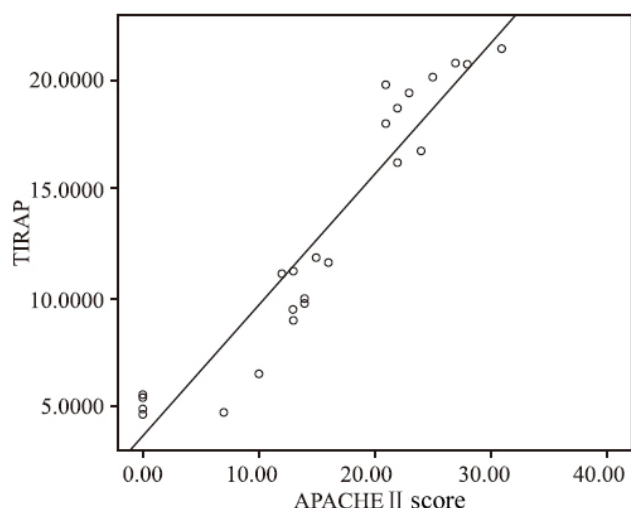
### 3 Discussion

Studies have shown that 45%-60% of sepsis was caused by gram-negative bacteria infections; and LPS, the toxic ingredient of gram-negative bacteria cell walls, is the main cause of sepsis. LPS, which enters the host body, is bound to the LPS binding protein and transferred to the membrane or

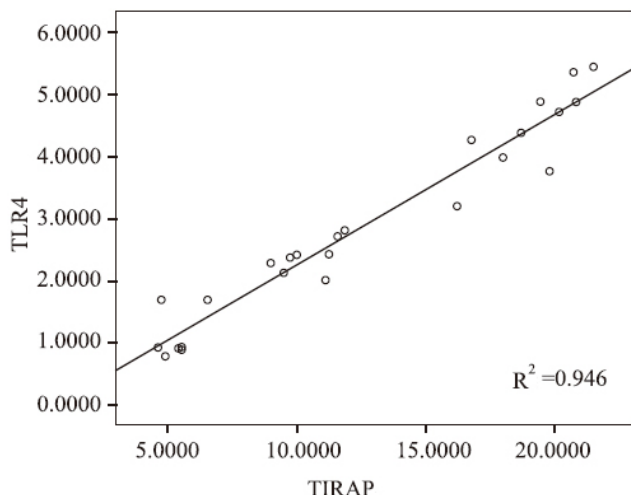
soluble CD-14 (cluster of differentiation 14) to form LPS-LBP-CD14 compound, which activates the complex receptor consisted of TLR4/myeloid differentiation-2 to recruit signaling molecules in the downstream: myeloid differentiation factor 88 (MyD88) and TIRAP activate nuclei factor  $\kappa B$  (NF- $\kappa B$ ) and promote the transcription and the release of cytokines and inflammatory mediators in large amount.<sup>[6]</sup> TLR4 is considered to play an important role in the mediation of sepsis caused by gram-negative bacteria.<sup>[7]</sup> After patients are infected with pathogens, the expression of TLR4 in serum is increased. With the inflammation progresses to sepsis, the expression of TLR4 is obviously increased.<sup>[8]</sup> The research has confirmed that, in acute lung injury (ALI), the expression of TIRAP is increased under the stimulation of TLR2 and TLR4, and TIRAP activates the intracellular signaling, triggers the signaling cascade and activates immuno-inflammatory responses.<sup>[4]</sup> The research has found that sepsis can cause extensive injury of hepatic tissue cells in hepatitis. When the organism is infected with hepatitis viruses, TLR4 will activate the transcription of target genes and release a series of cytokines. The high expression of TLR4 has a critically promotive effect on the process of virus elimination, chronic virus infection and severe liver injury.<sup>[9]</sup> Foreign scholar Branger<sup>[10]</sup> injected 2 groups of mice (TLR4- group and TLR4+ group) with *Streptococcus Pneumoniae*, and the results showed that mice in TLR4-group were more susceptible to *Streptococcus Pneumoniae* than those in TLR4+ group. The number of bacteria was multiplied after 1-2 days, and the mortality was high as well. Therefore, the expression of TLR4 became increased when bacterial infections occurred. In severe abdominal infections, it also can be found that inflammatory cytokines were released in large amount and multiple organ injuries were resulted by increasing the expression of TLR4 to regulate the level of NF- $\kappa B$ .<sup>[11]</sup>



**Figure 3:** Correlation between TLR4 and APACHE II score



**Figure 4:** Correlation between TIRAP and APACHE II score



**Figure 5:** Correlation between TLR4 and TIRAP

In this experiment, we can receive the results from the expression of peripheral blood in patients with sepsis, there were significant differences in the levels of TLR4 and TIRAP among these three groups (the control group, the low-score group and the high-score group) ( $p = .000$ ). With APACHE II score of 20 as the cut-off point to differentiate the severity of sepsis, according to Pearson's correlation analysis, the level of TLR4 was positively correlated with sepsis severity ( $r = 0.931, p < .05$ ); the level of TIRAP was also positively correlated with sepsis severity ( $r = 0.972, p < .05$ ); the level of TLR4 was positively correlated with the level of TIRAP ( $r = 0.936, p < .05$ ).

#### 4 Conclusions

In conclusion, TLR4 is the primary link of immune signal transduction after the body is infected. When sepsis caused by bacterial infections occurs, the expression of TLR4 and TIRAP is obviously increased. It is indicated that TLR4 and TIRAP have an identified effect on the process of anti-infection and play an important role in activating innate immunity. The degree of inflammatory responses, the severity of sepsis and the immune responses are associated with the levels of TLR4 and TIRAP, and they are interacted to play a mutual effect. The increase in the levels of serum TLR4 and TIRAP in patients with sepsis is positively correlated with the severity of sepsis. Conclusions from this research provide an important biological significance for diagnosis, assessment and treatment of sepsis, and promote the further study on the pathogenesis of sepsis.

#### Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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