Abstract. The aim of this study was to analyze the dynamic changes and predictive values of nuclear factor-κB (NF-κB) combined with interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in peripheral blood in multiple organ dysfunction syndrome (MODS) in patients with severe multiple trauma. Seventy patients diagnosed with severe multiple trauma in Emergency Department of Sichuan Provincial People’s Hospital (Chengdu, China) from April 2014 to April 2016 were selected and retrospectively analyzed. The patients enrolled were divided into the MODS group (n=25) and the non-MODS group (n=45). The injury severity scores (ISSs), acute physiology and chronic health evaluation II (APACHE II) scores, NF-κB, IL-6 and TNF-α levels in patients were detected at different time points (12, 24 and 48 h after admission), the changes in different indexes and the areas under the receiver operating characteristic (ROC) curve (AUC) were analyzed. The predictive values of different detection methods in MODS patients were discussed and compared. The ISS, APACHE II score, NF-κB, IL-6 and TNF-α levels in the MODS group at admission and 24 and 48 h after admission were higher than those in the non-MODS group (P<0.05). Those indexes in the deceased patients at 12, 24 and 48 h after admission were higher than those in survivors (P<0.05). The ISS, APACHE II score, NF-κB, IL-6 and TNF-α levels were not the risk factors of MODS in patients with severe multiple trauma (P>0.05). AUCs of ISS >22 points and APACHE II score >14 points in predicting MODS were lower than that of combined detection of NF-κB >1.20. In conclusion, the combined detection of NF-κB, IL-6 and TNF-α in peripheral blood of patients with acute multiple trauma is more helpful to predict the occurrence of MODS, which has a certain guiding significance for the prognosis of patients with MODS.

Introduction

Acute multiple trauma is a common disease in clinic, especially in emergency rescue, which will not only develop more rapidly, but also greatly increase the mortality rate if it occurs or is complicated by multiple organ dysfunction syndrome (MODS), causing adverse effects on the prognosis (1). Numerous studies have shown that when patients suffer from acute multiple trauma, the inflammation out of control will lead to the occurrence of MODS, namely the unbalance between inflammatory response system composed of immune inflammatory cells and tissues and anti-inflammatory response system, among which immune inflammatory cells play key roles (2). At present, finding a detection method that can predict MODS quickly and effectively is a common problem (3,4). Many MODSs are developed from systemic inflammatory response syndrome (SIRS); nuclear factor-κB (NF-κB) in neutrophils is a mediator involved in the genetic transcription and regulation of inflammatory factors, while interleukin-6 (IL-6) is one of the common factors of MODS, playing a major position. The injection of high-dose TNF-α into the human body can induce the occurrence of SIRS, eventually leading to MODS (6,7). Therefore, the occurrence of MODS in patients with acute multiple trauma was predicted in this study through detecting the NF-κB, IL-6 and TNF-α levels in peripheral blood, helping the clinical diagnosis and treatment.

Materials and methods

General data. A total of 70 patients diagnosed with severe multiple trauma in Emergency Department of Sichuan Provincial People’s Hospital (Chengdu, China) from April 2014 to April 2016 were selected and retrospectively analyzed. The patients enrolled were divided into the MODS group (n=25), including 18 males and 7 females with an average of
34.6±7.6 years old, and the non-MODS group (n=45), including 28 males and 17 females with an average of 33.8±8.5 years old. The histories of drinking, smoking and chronic diseases of all patients were collected. There were no statistically significant differences in general data and past medical history between the MODS and non-MODS groups (P>0.05), and the data were comparable (Table I). Inclusion criteria: patients diagnosed with severe multiple trauma; patients aged above 14 years old; patients admitted within 6 h after trauma. Exclusion criteria: patients receiving glucocorticoid or immunosuppressive therapy at admission; patients aged below 14 years old; patients with severe liver or kidney disease, heart disease, cardiogenic shock or cancer; pregnant women; patients with incomplete clinical data or who quit halfway. The study was approved by the Ethics Committee of Sichuan Provincial People's Hospital (Chengdu, China) and written informed consents were signed by the patients and/or guardians.

Methods. The injury severity scores (ISS), acute physiology and chronic health evaluation II (APACHE II) scores, NF-κB, IL-6 and TNF-α levels in patients enrolled were detected at different time-points (12, 24 and 48 h after admission). Peripheral venous blood (8 ml) after anticoagulation was collected at different time points, and the serum was separated and stored at -80˚C for standby application. 1) The level of NF-κB was detected via electrophoretic mobility shift assay (EMSA). After specimen collection, the nucleoprotein was extracted using the nucleoprotein extraction kit provided by Active Motif (Carlsbad, CA, USA), and the activity of nucleo-protein was detected via enzyme-linked immunosorbent assay (ELISA). The optical density (OD) at the wavelength of 500 nm was measured using the kit provided by Active Motif. 2) Detection of IL-6 and TNF-α levels: The peripheral IL-6 and TNF-α specimens were centrifuged at 1,800 x g for 20 min, and the upper-layer serum was collected. The nucleoprotein was collected via ELISA and placed into the solubilizing buffer; after incubation and washing for many times, the goat anti-rabbit horseradish peroxidase (HRP)-labeled polyclonal antibody (dilution, 1:1,000; cat no. 7074; Cell Signaling Technology, Danvers, MA, USA) was added, and the mixture was incubated and washed again. Finally, the IL-6 and TNF-α levels were detected using the Bio-Rad 680 microplate reader provided by Active Motif, and OD at the wavelength of 450 nm was measured. All of the above indexes were measured by professional detection physicians in strict accordance with the manufacturer's instructions.

Statistical analysis. Statistical Product and Service Solutions (SPSS) 19.0 software (SPSS, Inc., Chicago, IL, USA) was used for data processing. Data were collected and presented as mean ± SD, ANOVA was used for the comparison between multiple groups and the post hoc test was the Least Significant Difference test. Multivariate logistic regression analyses were performed for the factors with statistically significant difference, and the area under the receiver operating characteristic (ROC) curve (AUC) was calculated. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparisons of ISS, APACHE II score, NF-κB, IL-6 and TNF-α levels at admission between the two groups. The peripheral serum was collected from patients at 12 h after admission to detect the serum NF-κB, IL-6 and TNF-α levels. The ISSs, APACHE II scores, NF-κB, IL-6 and TNF-α levels in the MODS group at 12, 24 and 48 h after admission were higher than those in the non-MODS group, and the differences were statistically significant (P<0.001) (Table II).

Comparisons of ISS, APACHE II score, NF-κB, IL-6 and TNF-α levels at different time points (12, 24 and 48 h after admission) between the two groups. The ISSs, APACHE II scores, NF-κB, IL-6 and TNF-α levels in the MODS group at 12, 24 and 48 h after admission were higher than those in

| Groups | n  | ISS  | APACHE II score | NF-κB (µg/l) | IL-6 (µg/l) | TNF-α (ng/ml) |
|--------|----|------|-----------------|-------------|-------------|---------------|
| Non-MODS | 45 | 18.3±2.8 | 11.9±2.6 | 1.0±0.3 | 23.2±4.6 | 1.96±0.23 |
| MODS    | 25 | 22.6±3.9 | 18.2±3.2 | 1.4±0.2 | 35.3±3.8 | 3.62±0.21 |
| P-value |    | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

P>0.05, the difference is not statistically significant.
Comparisons of ISS, APACHE II score, NF-κB, IL-6 and TNF-α levels at different time-points after admission between the two groups.

| Groups    | n  | ISS (µg/l) | APACHE II score | NF-κB (µg/l) | IL-6 (µg/l) | TNF-α (ng/ml) |
|-----------|----|------------|-----------------|--------------|-------------|---------------|
| Non-MODS  | 45 |            |                 |              |             |               |
| 12 h after admission |    | 18.3±2.8   | 11.9±2.6        | 1.0±0.3      | 23.2±4.6    | 1.96±0.23     |
| 24 h after admission |    | 21.6±3.2   | 14.2±3.1        | 1.2±0.2      | 26.5±4.9    | 2.22±0.32     |
| 48 h after admission |    | 23.2±2.7   | 15.9±2.9        | 1.3±0.3      | 28.2±3.4    | 2.91±0.41     |
| MODS      | 25 |            |                 |              |             |               |
| 12 h after admission |    | 22.6±3.9   | 18.2±3.2        | 1.4±0.2      | 35.3±3.8    | 3.62±0.21     |
| 24 h after admission |    | 29.3±4.2   | 25.3±2.7        | 1.9±0.2      | 41.6±4.1    | 4.93±0.24     |
| 48 h after admission |    | 36.5±3.6   | 29.9±2.3        | 2.3±0.3      | 49.3±3.7    | 5.71±0.31     |

*Compared with the non-MODS group; †compared with 12 after admission; ‡compared with 24 h after admission. P<0.05, the difference is statistically significant.

Comparisons of ISS, APACHE II score, NF-κB, IL-6 and TNF-α levels at different time-points after admission between the deceased and survivor.

| Groups    | n  | ISS (µg/l) | APACHE II score | NF-κB (µg/l) | IL-6 (µg/l) | TNF-α (ng/ml) |
|-----------|----|------------|-----------------|--------------|-------------|---------------|
| Deceased  | 19 |            |                 |              |             |               |
| 12 h after admission |    | 25.3±2.9   | 29.5±3.5        | 1.5±0.2      | 29.3±4.6    | 4.66±0.25     |
| 24 h after admission |    | 26.5±2.6   | 32.3±4.1        | 1.8±0.2      | 35.3±3.9    | 4.93±0.31     |
| 48 h after admission |    | 28.3±1.9   | 33.6±3.3        | 2.1±0.3      | 45.9±4.2    | 5.63±0.22     |
| Survivor  | 51 |            |                 |              |             |               |
| 12 h after admission |    | 19.5±3.2   | 18.3±4.4        | 1.0±0.1      | 24.4±5.1    | 1.72±0.36     |
| 24 h after admission |    | 20.8±2.9   | 19.6±3.9        | 1.2±0.2      | 26.3±4.4    | 2.74±0.29     |
| 48 h after admission |    | 26.2±2.3   | 22.3±4.2        | 1.4±0.1      | 28.1±3.2    | 3.95±0.41     |

*Compared with the Survivor group; †compared with 12 after admission; ‡compared with 24 h after admission. P<0.05, the difference is statistically significant.

The analyses with ISS, APACHE II score, NF-κB, IL-6 and TNF-α levels as the independent variables, and whether MODS occurred as the dependent variable showed that the

Table V. Multivariate logistic regression analyses of influencing factors of MODS in patients with severe multiple trauma.

| Indexes | Odds ratio (OR) | 95% confidence interval (CI) |
|---------|----------------|-----------------------------|
| ISS     | 0.823          | 0.919                       | 0.659, 1.087 |
| APACHE II score | 0.610  | 0.957 | 0.823, 1.316 |
| NF-κB   | 0.463          | 3.562                       | 0.314, 3.989 |
| IL-6    | 0.185          | 2.159                       | 0.992, 4.024 |
| TNF-α   | 0.366          | 1.944                       | 0.765, 3.566 |

ISS, APACHE II score, NF-κB, IL-6 and TNF-α levels were not the risk factors of MODS in patients with severe multiple trauma, and the differences were not statistically significant (P>0.05) (Table V).
theories argue that MODS, instead of MOF, is more reason
multiple organ failure (MOF). At present, more and more
in the body consecutively or simultaneously are defined as
In the disease or severe trauma, failures of two or more organs
Discussion
the differences were statistically significant (P<0.001) (Fig. 2).
 ROC curves of combined detection of NF-κB, IL-6 and TNF-α
levels and ISS and APACHE II score of patients with severe
multiple trauma in predicting MODS. AUCs of ISS ≥22 points
and APACHE II score >14 points in predicting MODS were
0.598 (95% CI=0.483, 0.812) and 0.664 (95% CI=0.614, 0.903),
which were lower than that of combined detection of NF-κB
>1.20; IL-6 >25.1 µg/l and TNF-α >2.20 ng/ml in peripheral
blood in predicting MODS (0.853, 95% CI=0.659, 0.977),
and the differences were statistically significant (P<0.001) (Fig. 2).

ROC curves of combined detection of NF-κB, IL-6 and TNF-α
levels and ISS and APACHE II score of patients with severe
multiple trauma in predicting MODS. AUCs of ISS ≥22 points
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blood in predicting MODS (0.853, 95% CI=0.659, 0.977),
and the differences were statistically significant (P<0.001) (Fig. 2).

Discussion
In the disease or severe trauma, failures of two or more organs
in the body consecutively or simultaneously are defined as
multiple organ failure (MOF). At present, more and more
theories argue that MODS, instead of MOF, is more reason-
able and scientific (8). Clinically, acute multiple trauma is a
common disease in emergency, often seriously threatening the
life of patients. There are also increasingly more studies on
severe multiple trauma, but there is a lack of more accurate
predictive indexes for severe multiple trauma or MODS (9).
After severe trauma, infection or major surgery, the body will
exhibit a significant acute inflammatory reaction, thus causing
abnormal inflammatory mediator expression levels, among
which inflammatory as well as anti-inflammatory mediators
are the most common (10). In particular, inflammatory and
anti-inflammatory mediators are in a relatively unstable state
in patients with severe multiple trauma (11), and the expres-
son levels of anti-inflammatory mediators are enhanced with
the increase in inflammatory mediators, showing balance and
fluctuation between inflammatory and anti-inflammatory
mediators (12). In addition to the expression of inflammatory
mediators, more indexes are involved in the regulation
process of inflammatory mediators, which can indirectly
reflect the degree of inflammatory response (13). Many studies
have shown that NF-κB is an important regulatory factor in
the inflammatory response process, which in turn affects the
expression disorder of inflammatory mediators. Moreover,
NF-κB activates the immune system in the body via early acti-
vation, exhibiting SIRS (14); continuous attack and multiple
organ dysfunctions under stress state result in MODS (15).
After activation, NF-κB enters neutrophils and monocytes
quickly, and specifically binds to gene promoter and enhancer,
which is expressed by cell regulatory factors and promotes
the synthesis and release of a large number of inflammatory
factors after binding and regulation, such as IL-6, IL-8 and
TNF-α. The most important factors stimulating the synthesis
of acute reactive protein in SIRS are IL-6 and TNF-α (16,17).

In this study, the ISS, APACHE II score, NF-κB, IL-6 and
TNF-α levels in the MODS group were higher than those in
the non-MODS group. With the prolongation of time, these
indexes were increased. The ISS, APACHE II score, NF-κB,
IL-6 and TNF-α levels in the deceased patients were obviously
higher than those in survivors. With the prolongation of time,
these indexes were increased, and the differences were statisti-
cally significant. It can be seen that ISS and APACHE II score,
or NF-κB, IL-6 and TNF-α have great reference significance
in the diagnosis of MODS. However, the multivariate logistic
regression analyses of influencing factors of MODS in patients
with severe multiple trauma showed that the ISS, APACHE II
score, NF-κB, IL-6 and TNF-α levels were not the risk factors
of MODS in patients with severe multiple trauma. Besides, the
ROC curves of combined detection of NF-κB, IL-6 and TNF-α
levels and ISS and APACHE II score of patients with severe
multiple trauma in predicting MODS revealed that AUCs of
ISS ≥22 points and APACHE II score >14 points in predicting
MODS were significantly lower than that of combined detec-
tion of NF-κB >1.20, IL-6 >25.1 µg/l and TNF-α >2.20 ng/ml
in peripheral blood, suggesting that the combined detection
of NF-κB, IL-6 and TNF-α in peripheral blood of patients
with severe multiple trauma has important significance in
predicting MODS (18).

The pathogenesis and pathophysiological process of
MODS are extremely complex, and the states of corre-
sponding inflammatory and anti-inflammatory mediators at
different stages of disease are different (19). Maintaining the
relative balance between inflammation and anti-inflammation in human body is the most critical step in the treatment of MODS. The detection of inflammation-related factors, such as IL-6 and TNF-α, can reflect the autoreaction. Moreover, the intervention in NF-κB activity can ultimately interfere in the pathogenesis of MODS and improve the prognosis (20).

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Availability of data and materials

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

Authors' contributions

JZ wrote the manuscript and performed EMSA and ELISA.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Sichuan Provincial People’s Hospital (Chengdu, China) and written informed consents were signed by the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The author declares that he has no competing interests.

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