Serum expression levels of Mb and NT-proBNP and NF-κB expression in neutrophils in patients with MODS and the clinical significance

CONGMEI WANG[1*], QINGLIANG CHEN[2*], ZHENYU CUI[1], ZHIJING XU[1], YANGANG SHI[1], SHIQIONG SU[1], LU QI[1], YU'AN GENG[1] and RUIFANG LIU[1]

[1]ICU and [2]Department of Radiology, The Third People's Provincial Hospital of Henan Province, Zhengzhou, Henan 466000, P.R. China

Received January 29, 2018; Accepted August 6, 2018

DOI: 10.3892/etm.2018.6659

Abstract. This study was designed to investigate the expression of myoglobin (Mb), N-terminal pro-brain natriuretic peptide (NT-proBNP) in serum and the expression of nuclear factor-κB (NF-κB) in neutrophils in patients with multiple organ dysfunction syndrome (MODS) and the clinical significance. From July 2014 to December 2015, 314 patients with severe disease were selected during their stays at the emergency ward of the Third People's Provincial Hospital of Henan Province (Zhengzhou, China). Seventy patients with MODS were selected as MODS group, 108 patients with single organ or system injury were selected as the key prevention group, and 136 patients without obvious organ or systemic injury were selected as prevention group. Serum levels of Mb and NT-proBNP were measured by ELISA. Neutrophils were isolated by flow cytometry, and the activity of NF-κB in neutrophils of MODS patients was measured by electrophoretic mobility shift assay (EMSA). At 1, 7 and 14 days after treatment, the levels of Mb, NT-proBNP and NF-κB in the three groups were significantly reduced compared with pretreatment levels ($p<0.05$). Logistic regression analysis showed that serum Mb, NT-proBNP and NF-κB activity were positively correlated with the progression of the disease ($r=0.809$, 0.785 and 0.833, $p=0.012$, 0.025 and 0.004), and negatively correlated with the treatment time ($r=-0.773$, -0.734 and -0.815; $p=0.033$, 0.041 and 0.078). Mb, NT-proBNP and NF-κB may be involved in the pathogenesis and development of MODS, and may play an important role in the prevention and treatment of MODS.

Correspondence to: Dr Ruifang Liu, ICU, The Third People's Provincial Hospital of Henan Province, 198 Funiu Road, Zhengzhou, Henan 466000, P.R. China
E-mail: rz26ag@163.com

*Contributed equally

Key words: myoglobin, N-terminal pro-brain natriuretic peptide, neutrophils, nuclear factor-κB, multiple organ dysfunction syndrome

Introduction

Multiple organ dysfunction syndrome (MODS) refers to the simultaneous occurrence of two or more organ dysfunctions or failure. MODS is common in severely infected and severely traumatized patients, and is the most common complication of acute and critically ill patients (1,2). MODS is also a common cause of death in acute and critically ill patients, and mortality rate can be as high as 80%, if dysfunction or failure of three organs exist (3). It's very difficult to control the development of MODS, therefore, prevention, early detection and early treatment are the key for survival (4).

Myoglobin (Mb) is widely present in muscle tissue to transport and store oxygen. Mb level increases in cardiac function damage or failure, and renal failure. Heart and kidneys are two of the most common early-affected organs in MODS (5,6). N-terminal pro-brain natriuretic peptide (NT-proBNP) is a newly discovered biomarker in recent years. NT-proBNP has been widely used in the detection of heart failure due to its high specificity and sensitivity (7). Changes in the level of NT-proBNP have also been found in sepsis, renal failure and other diseases (8,9). Nuclear factor-κB (NF-κB) is a pleiotropic and inducible nuclear transcription factor regulated by inflammatory response and stress response. Organs or system damage can stimulate the release of inflammatory cytokines, activate NF-κB signaling system, and promote the release of NF-κB. NF-κB can also promote the release of proinflammatory cytokines to aggregate the organ or system injury (10,11).

Therefore, this study detected the expression of Mb, NT-proBNP and NF-κB in patients with MODS and explored the clinical significance with the expectation of providing reference for clinical prevention and treatment of MODS.

Patients and methods

Subjects. A total of 314 patients with severe disease were selected during their stays at the emergency ward of the Third People's Provincial Hospital of Henan Province (Zhengzhou, China) from July 2014 to December 2015. Among them, 70 patients with MODS were selected as MODS group, including 42 males and 28 females, with a mean age of

Table I. Comparison of basic information among three groups.

Parameters	MODS group	Key prevention group	Prevention group	Statistical value	P-value
No. of patients (cases)	70	108	136		
Sex (male/female)	42/28	63/45	78/58	3.578	0.161
Age (years)	67.5±20.4	59.4±19.8	42.3±20.3	1.274	0.398
Cause of disease (n, %)					
Serious infection	23 (32.86)	37 (34.26)	45 (33.09)	0.378	0.714
Serious trauma	13 (18.57)	17 (15.74)	15 (11.03)	0.135	0.879
Severe burns	11 (15.71)	15 (13.89)	20 (14.71)	0.166	0.855
Severe blood loss	12 (17.14)	19 (17.59)	21 (15.44)	0.174	0.848
Shock	6 (8.57)	7 (6.48)	9 (6.62)	0.118	0.893
Heartbeat, respiratory resuscitation	3 (4.29)	8 (7.41)	10 (7.35)	0.133	0.880
History of smoking	2 (2.86)	5 (4.63)	16 (11.76)	0.162	0.857

MODS, multiple organ dysfunction syndrome.

67.5±20.4 years. A total of 108 patients with single organ or system injury were selected as the key prevention group, including 63 males and 45 females, with a mean age of 59.4±19.8 years; and the remaining 136 patients without obvious organ or systemic injury were selected as prevention group, including 78 males and 58 females, with a mean age of 42.3±20.3 years. No organ dysfunction and MODS occurred during the study. Patients in MODS group all met the MODS diagnostic criteria proposed by Wang *et al* (12), with a course of disease longer than 24 h. Patients with chronic disease at the end of life, cancer patients, patients with autoimmune diseases, patients with three or more organ dysfunctions or systemic damages, patients who died after 48 h of treatment and patients with mental or learning dysfunction were excluded. This study was approved by the Ethics Committee of the Third People's Provincial Hospital of Henan Province, and all patients signed an informed consent.

Research methods. All patients received normal clinical and nursing care. Fasting peripheral venous blood samples (2 ml x 2) were collected by nurses in the morning. Blood (2 ml) was used to separate serum at 2 h after collection by centrifugation at a speed of 2,100 x g at 4˚C for 5 min, and the other 2 ml were used to isolate neutrophils by flow cytometry (Attune NxT; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Mb, NT-proBNP and NF-κB were detected. Mb was detected by enzyme-linked immunosorbent assay (ELISA) using a detection kit purchased from Keshun Biotechnology Co., Ltd. (Shanghai, China), NT-proBNP was also detected by ELISA using a detection kit purchased from Shanghai Jingkang Biological Engineering Co., Ltd. (Shanghai, China). NF-κB was tested by EMSA using a kit purchased from Shanghai YBio Co., Ltd. (Shanghai, China).

Observation indicators. All patients received basic treatment to actively control the primary disease, improve oxygen metabolism, improve metabolic function, regulate immune function and control blood sugar. The levels of Mb, NT-proBNP and NF-kB, and the disease conditions in all three groups were observed before and at 1, 7 and 14 days after treatment, and the associations between them were analyzed.

Statistical analysis. Statistical analysis was performed by using SPSS 22.0 software [AsiaAnalytics (formerly SPSS China), Shanghai, China]. Count data were expressed as rate and measurement data were expressed as mean ± SD. χ^2 test was used to compare the count data. Variance analysis was used to compare measurement data among multiple groups, and paired t-test was used for comparisons between two groups. Repeated measures ANOVA was used for intragroup comparisons and Dunnett's test was used as a post hoc test. Logistics regression analysis was used to analyze the correlations between Mb, NT-proBNP, NF-κB and patient's disease development and treatment time. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of basic information. There were no significant differences in age, sex, cause of disease, history of smoking among the three groups (p>0.05) (Table I).

Comparison of Mb levels in peripheral venous. Significant differences in the blood levels of Mb were found among the three groups before and at 1, 7 and 14 days after treatment (p<0.05). Comparisons between two groups at the same time-points were significantly different (p<0.05) except the comparisons between key prevention and prevention groups at 7 and 14 days after treatment. After treatment, the levels of Mb decreased gradually in all three groups, and significant differences were found from the comparison of one time-point with the former time-point (p<0.05) (Table II).

Comparison of NT-proBNP levels in peripheral venous. Significant differences in the blood levels of NT-proBNP were

Table II. Comparison of Mb levels in peripheral venous (μg/l).

Treatment	MODS group	Key prevention group	Prevention group	Statistical value	P-value
Before treatment	913.19±210.78	357.67±184.32[d]	288.87±115.49[d,e]	40.49	<0.001
1 day after treatment[a]	301.33±161.17	274.18±177.49[d]	149.33±154.26[d,e]	26.87	<0.001
7 days after treatment[b]	90.66±54.84	69.36±32.25[d]	72.21±38.69[d]	6.455	0.002
14 days after treatment[c]	42.75±11.16	37.59±14.88[d]	38.14±15.24[d]	3.174	0.043

[a]Compared with pretreatment, p<0.05; [b]compared with 1 day after treatment, p<0.05; [c]compared with 7 days after treatment, p<0.05; [d]compared to MODS group at the same time-points, p<0.05; [e]compared to key prevention group at the same time-points, p<0.01. Mb, myoglobin; MODS, multiple organ dysfunction syndrome.

Table III. Comparison of NT-proBNP levels in peripheral venous (fmol/ml).

Treatment	MODS group	Key prevention group	Prevention group	Statistical value	P-value
Before treatment	1,161.5±342.6	1,014.3±321.5[d]	984.8±294.7[d,e]	7.598	<0.001
1 day after treatment[a]	964.2±272.4	879.5±263.8[d]	755.3±132.1[d,e]	23.11	<0.001
7 days after treatment[b]	648.5±191.5	431.6±112.4[d]	358.2±84.6[d,e]	68.25	<0.001
14 days after treatment[c]	216.8±59.3	246.9±53.2[d]	215.7±10.6[d,e]	18.69	<0.001

[a]Compared with pretreatment, p<0.05; [b]compared with 1 day after treatment, p<0.05; [c]compared with 7 days after treatment, p<0.05; [d]compared to MODS group at the same time-points, p<0.05; [e]compared to key prevention group at the same time-points, p<0.01. NT-proBNP, N-terminal pro-brain natriuretic peptide; MODS, multiple organ dysfunction syndrome.

Table IV. Comparison of NF-κB levels in peripheral venous (%).

Treatment	MODS group	Key prevention group	Prevention group	Statistical value	P-value
Before treatment	284.3±18.6	212.2±16.9[d]	182.7±12.6[d,e]	16.92	<0.001
1 day after treatment[a]	231.7±15.3	195.5±14.3[d]	156.2±11.8[d,e]	7.535	0.023
7 days after treatment[b]	174.6±11.2	102.7±9.8[d]	92.5±8.4[d,e]	8.103	0.017
14 days after treatment[c]	103.4±7.8	88.4±7.1[d]	80.9±6.7[d,e]	2.164	0.039

[a]Compared with pretreatment, p<0.05; [b]compared with 1 day after treatment, p<0.05; [c]compared with 7 days after treatment, p<0.05; [d]compared to MODS group at the same time-points, p<0.05; [e]compared to key prevention group at the same time-points, p<0.01. NF-κB, nuclear factor-κB.

Table V. Logistics regression analysis results.

Items	Mb	NT-proBNP	NF-κB
Disease progression	r=0.809	r=0.785	r=0.833
	p=0.012	p=0.025	p=0.004
Treatment time	r=-0.773	r=-0.734	r=-0.815
	p=0.033	p=0.041	p=0.078

Mb, myoglobin; NT-proBNP, N-terminal pro-brain natriuretic peptide; NF-κB, nuclear factor-κB.

found among the three groups before and at 1, 7 and 14 days after treatment (p<0.05). Comparisons between two groups at the same time-points all yielded significant differences (p<0.05). After treatment, the levels of NT-proBNP decreased gradually in all three groups, and significant differences were found from the comparisons of one time-point with the former time-point (p<0.05) (Table III).

Comparison of NF-κB levels in peripheral venous. Significant differences in the blood levels of NF-κB were found among the three groups before and at 1, 7 and 14 days after treatment; highest levels were found in MODS group, followed by key prevention group and the lowest levels were found in prevention group (p<0.05). Comparisons between two groups at the same time-points all yielded significant differences (p<0.05). After treatment, the levels of NF-κB decreased gradually in all three groups, and significant differences were found from the comparisons of one time-point with the former time-point (p<0.05) (Table IV).

Logistics regression analysis. Logistic regression analysis showed that Mb, NT-proBNP and NF-κB were positively correlated with patients' disease progression (r=0.809, 0.785 and 0.833; p=0.012, 0.025 and 0.004), and negatively correlated with the treatment time of MODS (r=-0.773, -0.734 and -0.815; p=0.033, 0.041 and 0.078) (Table V).

Discussion

At present, the pathogenesis of MODS still hasn't been fully elucidated. Hypothesis on uncontrolled inflammation which causes MODS is described as the internal environment and the microcirculation disorder caused by the release of inflammatory mediators into blood (2,13). Ischemia-reperfusion theory has also been well accepted, because insufficient oxygen delivery can cause direct hypoxic-ischemic damage of tissues and the release of excessive inflammatory cytokines, which is also an important mechanism of MODS (14,15). Although Mb, NT-proBNP and NF-κB are closely related to ischemia-reperfusion and inflammatory reaction (5-11), their involvement in MODS remains unclear. Therefore, our study aimed to detect the expression of Mb, NT-proBNP and NF-κB in patients with MODS with the expectation of providing reference for clinical prevention and treatment of MODS.

This study enrolled 314 critically ill patients, including patients without organ dysfunction (prevention group), patients with single organ dysfunction (key prevention group) as well as MODS patients. Real-time monitoring of serum Mb, NT-proBNP, NF-κB before and after treatment was performed to explore their associations with the occurrence and development of MODS.

Results of this study showed that there were significant differences in serum levels of Mb, NT-proBNP and NF-κB among the three groups before treatment, and serum levels of Mb, NT-proBNP and NF-κB decreased with the progression of treatment. Logistic regression analysis also showed that Mb, NT-proBNP and NF-κB were positively correlated with the progression of the disease, indicating that elevated Mb and NT-proBNP and NF-κB may promote the development of patients with MODS and even the occurrence of MODS. The possible explanation is that the aggregation of disease may increase the degree of anoxia, and the body's defense and protection function may improve the production of Mb to improve the oxygen supply capacity. Correlation between Mb and oxidative damage is also described in 'Oxidative Damage and Repair: Chemical, Biological and Medical Aspects' (16). With the occurrence and development of systemic inflammatory response, production of NF-κB will be promoted. Fan *et al* (17) also found that NF-κB pathway and ischemia-reperfusion injury-induced inflammation and oxidative stress are closely related. However, we cannot determine the cause of increased NT-proBNP. The shortcoming of this study is that the correlation between MODS etiology and organ dysfunction was not investigated. Therefore, we can not confirm the role of MODS and organ dysfunction in the increased level of NT-proBNP, which is also a common shortcoming shared by previous studies (18,19). However, we found that the NT-proBNP level increased with the increased degree of organ dysfunction. After treatment, patients' condition was effectively controlled, and the levels of Mb, NT-proBNP and NF-κB in serum of patients were also effectively improved. NT-proBNP in all patients recovered almost to normal level at 14 days after treatment. Logistic regression analysis also showed that Mb, NT-proBNP and NF-κB levels were negatively correlated with treatment time. Therefore, we believe that reduced levels of Mb, NT-proBNP and NF-κB play an important role in the occurrence and development of MODS.

This study is a prospective study, and all results and conclusions remain to be further verified by future studies with larger sample size.

In conclusion, reduced Mb, NT-proBNP and NF-κB levels may be involved in the occurrence and development of MODS, and Mb, NT-proBNP and NF-κB may play an important role in the prevention and treatment of MODS.

Acknowledgements

Not applicable.

Funding

This study was funded by the 2015 Medical Science Research Plan of Henan Province: Expression and significance of serum TNF-α, IL-10 and TGF-β in patients with MODS (no. 201503219).

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

CW and QC were major contributors in writing the manuscript. ZC participated in the analysis and interpretation of the data. ZX was responsible for the acquisition of the data and the follow-up management of the patients. YS was a major contributor in designing the methods. CW, QC and SS performed ELISA. LQ participated in the design of the study. YG analyzed and interpreted the patient data. RL was a major contributor in the conception and design of this study and was also responsible for reviewing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Third People's Provincial Hospital of Henan Province (Zhengzhou, China). Signed written informed consents were obtained from the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Tamburro RF and Jenkins TL: Multiple organ dysfunction syndrome: A challenge for the pediatric critical care community. Pediatr Crit Care Med 18 (Suppl 1): S1-S3, 2017.
2. Cheng J, Wei Z, Liu X, Li X, Yuan Z, Zheng J, Chen X, Xiao G and Li X: The role of intestinal mucosa injury induced by intra-abdominal hypertension in the development of abdominal compartment syndrome and multiple organ dysfunction syndrome. Crit Care 17: R283-R283, 2013.
3. Roberts BW, Kilgannon JH, Chansky ME, Mittal N, Wooden J, Parrillo JE and Trzeciak S: Multiple organ dysfunction after return of spontaneous circulation in postcardiac arrest syndrome. Crit Care Med 41: 1492-1501, 2013.
4. Rajasekaran S, Kruse K, Kovey K, Davis AT, Hassan NE, Ndika AN, Zuiderveen S and Birmingham J: Therapeutic role of anakinra, an interleukin-1 receptor antagonist, in the management of secondary hemophagocytic lymphohistiocytosis/sepsis/multiple organ dysfunction/macrophage activating syndrome in critically ill children*. Pediatr Crit Care Med 15: 401-408, 2014.
5. Bolli R, Tang XL, Sanganalmath SK, Rimoldi O, Mosna F, Abdel-Latif A, Jneid H, Rota M, Leri A and Kajstura J: Intracoronary delivery of autologous cardiac stem cells improves cardiac function in a porcine model of chronic ischemic cardiomyopathy. Circulation 128: 122-131, 2013.
6. Petejova N and Martinek A: Acute kidney injury due to rhabdomyolysis and renal replacement therapy: A critical review. Crit Care 18: 224, 2014.
7. Mirjafari H, Welsh P, Verstappen SM, Wilson P, Marshall T, Edlin H, Bunn D, Chipping J, Lunt M, Symmons DP, *et al*: N-terminal pro-brain-type natriuretic peptide (NT-pro-BNP) and mortality risk in early inflammatory polyarthritis: Results from the Norfolk Arthritis Registry (NOAR). Ann Rheum Dis 73: 684-690, 2014.
8. Li N, Zhang Y, Fan S, Xing J and Liu H: BNP and NT-proBNP levels in patients with sepsis. Front Biosci (Landmark Ed) 18: 1237-1243, 2013.
9. Metra M, Cotter G, Davison BA, Felker GM, Filippatos G, Greenberg BH, Ponikowski P, Unemori E, Voors AA, Adams KF Jr, *et al*; RELAX-AHF Investigators: Effect of serelaxin on cardiac, renal, and hepatic biomarkers in the Relaxin in Acute Heart Failure (RELAX-AHF) development program: Correlation with outcomes. J Am Coll Cardiol 61: 196-206, 2013.
10. De Simone V, Franzè E, Ronchetti G, Colantoni A, Fantini MC, Di Fusco D, Sica GS, Sileri P, MacDonald TT, Pallone F, *et al*: Th17-type cytokines, IL-6 and TNF-α synergistically activate STAT3 and NF-kB to promote colorectal cancer cell growth. Oncogene 34: 3493-3503, 2015.
11. Garding A, Bhattacharya N, Claus R, Ruppel M, Tschuch C, Filarsky K, Idler I, Zucknick M, Caudron-Herger M, Oakes C, *et al*: Epigenetic upregulation of lncRNAs at 13q14.3 in leukemia is linked to the *in cis* downregulation of a gene cluster that targets NF-kB. PLoS Genet 9: e1003373, 2013.
12. Wang C, Su Q, Zhang SW, Yin CH, Wang H and Wang BE: A clinical study on the diagnostic criteria of multiple organ dysfunction syndrome. Zhonghua Wai Ke Za Zhi 47: 40-43, 2009 (In Chinese).
13. El-Hattab AW, Adesina AM, Jones J and Scaglia F: MELAS syndrome: Clinical manifestations, pathogenesis, and treatment options. Mol Genet Metab 116: 4-12, 2015.
14. Szijártó A, Turóczi Z, Szabó J, Kaliszky P, Gyurkovics E, Arányi P, Regáli L, Harsányi L and Lotz G: Rapidly progressing fatal reperfusion syndrome caused by acute critical ischemia of the lower limb. Cardiovasc Pathol 22: 493-500, 2013.
15. Hebbel RP: Ischemia-reperfusion injury in sickle cell anemia: Relationship to acute chest syndrome, endothelial dysfunction, arterial vasculopathy, and inflammatory pain. Hematol Oncol Clin North Am 28: 181-198, 2014.
16. Davies KJA (ed): Oxidative Damage and Repair: Chemical, Biological and Medical Aspects. 1st edition. Pergamon Press, Inc., New York, 2013.
17. Fan Z, Yao J, Li Y, Hu X, Shao H and Tian X: Anti-inflammatory and antioxidant effects of curcumin on acute lung injury in a rodent model of intestinal ischemia reperfusion by inhibiting the pathway of NF-Kb. Int J Clin Exp Pathol 8: 3451-3459, 2015.
18. Licata A, Corrao S, Petta S, Genco C, Cardillo M, Calvaruso V, Cabibbo G, Massenti F, Cammà C, Licata G, *et al*: NT pro BNP plasma level and atrial volume are linked to the severity of liver cirrhosis. PLoS One 8: e68364, 2013.
19. Mishra RK, Li Y, Ricardo AC, Yang W, Keane M, Cuevas M, Christenson R, deFilippi C, Chen J, He J, *et al*; Chronic Renal Insufficiency Cohort Investigators: Association of N-terminal pro-B-type natriuretic peptide with left ventricular structure and function in chronic kidney disease [from the Chronic Renal Insufficiency Cohort (CRIC)]. Am J Cardiol 111: 432-438, 2013.