Original Article

Differential protein expression in patients with urosepsis

Xu-Kai Yang, e, Nan Wang, e, Cheng Yang, e, Yang-Min Wang, a, *, Tuan-Jie Che

a Department of Urology, Lanzhou General Hospital PLA, Lanzhou 730050, China
b Department of Infection, Xi’an Central Hospital, Xi’an 710033, China
c Student teams, Basic Medical College, The Fourth Military Medical University, Xi’an, 710032 China
d Lanzhou Baiyuan Gene Technology Co. Ltd, Lanzhou 730000, China

A R T I C L E   I N F O

Article history:
Received 10 May 2018
Received in revised form 30 July 2018
Accepted 10 August 2018
Available online 2 October 2018

Keywords:
Protein expression
Urosepsis
Proteomics

A B S T R A C T

Purpose: Urosepsis in adults comprises approximately 25% of all sepsis cases, and is due to complicated urinary tract infections in most cases. However, its mechanism is not fully clarified. Urosepsis is a very complicated disease with no effective strategy for early diagnosis and treatment. This study aimed to identify possible target-related proteins involved in urosepsis using proteomics and establish possible networks using bioinformatics.

Methods: Fifty patients admitted to the Urology Unit of Lanzhou General PLA (Lanzhou, China), from October 2012 to October 2015, were enrolled in this study. The patients were further divided into shock and matched-pair non-shock groups. 2-DE technique, mass spectrometry and database search were used to detect differentially expressed proteins in serum from the two groups.

Results: Six proteins were found at higher levels in the shock group compared with non-shock individuals, including serum amyloid A-1 protein (SAA1), apolipoprotein L1 (APOL1), ceruloplasmin (CP), haptoglobin (HP), antithrombin-III (SERPINC1) and prothrombin (F2), while three proteins showed lower levels, including serotransferrin (TF), transthyretin (TTR) and alpha-2-macroglobulin (A2M).

Conclusion: Nine proteins were differentially expressed between uroseptic patients (non-shock groups) and severe uroseptic patients (shock groups), compared with non-shock groups, serum SAA1, APO1, HP, SERPINC1 and F2 at higher levels, while TF, TTR and A2M at lower levels in shock groups. These proteins were mainly involved in platelet activation, signaling and aggregation, acute phase protein pathway, lipid homeostasis, and iron ion transport. They deserve further research as potential candidates for early diagnosis and treatment. (The conclusion seems too simple and vague, please re-write it. You may focus at what proteins have been expressed and introduce more detail about its significance.)

© 2018 Daping Hospital and the Research Institute of Surgery of the Third Military Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Urosepsis is defined as sepsis caused by a urogenital tract infection. Urogenital tract infections are considered to be responsible for about 30% of sepsis cases, with a mortality of 20%–40%. As the population ages, the incidence of urosepsis is likely to rise.1,2 Although blood culture has been considered the gold standard for sepsis diagnosis, it is too slow and limited by false negatives.3 This has prompted studies to identify methods for sepsis diagnosis in the early stages, where treatment outcomes are more favorable. Indeed, the prognosis of urosepsis must be assessed as early as possible. Recently, proteomic technologies have been used to detect new biomarkers. A large number of differentially expressed proteins have been reported as potential biomarkers for the diagnosis and prognosis of several diseases. In this study, two-dimensional liquid chromatography-tandem mass spectrometry (2D-LC-MS/MS) was applied to assess protein profiles in uroseptic patients with different prognoses, to identify potential prognostic biomarkers.

Methods

Patients

Fifty patients with urinary tract infection, admitted to the Urology Unit of Lanzhou General PLA (Lanzhou, China) from...
October 2012 to October 2015 and diagnosed according to the 2001 and 2008 International Sepsis Definition Conference, were enrolled in this study. According to their conditions, these patients were divided into shock and matched-pair non-shock groups. The study was approved by the Lanzhou General PLA Hospital Ethics Committee; informed consent was obtained from all patients or their relatives. Exclusion criteria were: (1) liver dysfunction, (2) abnormal blood coagulation, (3) age < 18 years, (4) patients with a history of cancer, and (5) autoimmune diseases.

**Blood collection and serum preparation**

Blood samples were drawn within 24 h of diagnosis from septic patients, and centrifuged at 15,000 g in the absence of prostacyclin, followed by addition of 20 μL/ml protease inhibitor cocktail to the resulting serum samples. Storage was carried out at −80°C.

**Two-dimensional gel electrophoresis**

All protein samples from non-shock and shock groups were assessed by 2-DE; a minimum of three samples were run per group to ensure reproducibility. In the first dimension of the 2-DE, protein samples (80 μg) were applied to immobilized pH gradient (IPG) strips (17 cm, pH 3–10, NL; Fig. 1), which were rehydrated overnight at room temperature according to the manufacturer’s instructions with DeStreak Rehydration Solution containing 0.4% ampholytes with pH 3–10. The IPG strips were initially run at 50 V for 12 h, and subsequently focused at 250 V for 30 min, 1000 V for 1 h, 10000 V for 5 h, and maintained at 10000 V until a total of 60,000 V/h was achieved. Following is on electric focusing (IEF), the IPG strips were equilibrated with 1.5 M Tris-HCl (pH 8.8), 6 M urea, 87% glycerol, 2% sodium dodecyl sulfate (SDS) and 0.2% bromophenol blue. After initially treatment with 15 ml 1% DTT for 10 min with constant shaking, alkylation with 15 ml 2.5% indole-3-acetic acid was performed for 15 min. Following IEF, the equilibrated strips were subjected to second dimensional separation (SDS-PAGE) using 1.0 mm-thick 10% polyacrylamide gels on a Bio-Rad PROTEAN II xi Cell system. Staining was carried out with silver nitrate (CWBIO, Beijing, China).

**Image acquisition and data analysis**

The 2D gels were scanned on a Densitometer GS-800 (Bio-Rad). Spot detection, quantification and analysis were performed with the PDQuest8.0.1 software (Bio-Rad), followed by manual matching.

**Protein identification by mass spectrometry**

MALDI-TOF MS/MS analysis of protein spots was conducted on an Applied Biosystems 4700 Proteomics Analyzer (Framingham, MA, USA). Differentially expressed protein spots were excised from the 2-DE gels and washed with water before digestion. Digestion and peptide extraction were performed according to previous methods. Mass spectra of peptide mixtures were obtained using an Applied Biosystems 4700 Proteomics Analyzer operated in delayed reflector mode with an accelerated voltage of 20 kV. Spectra were calibrated using trypsin auto-digested ion peaks (m/z = 842.5 and 2211.1) as internal standards. Peptide mass fingerprinting (PMF) data were used to search for candidate proteins using the MASCOT (http://www.matrixscience.com) software. A hit was considered to be positive when MASCOT revealed a global score exceeding 60 (p < 0.05).

**Interaction network of the identified proteins**

We used a web-based bioinformatics tool to analyze the interacting proteins: STRING software version 10.0 (http://string-db.org). The results included a detailed network featuring several core proteins.

**Statistical analysis**

SPSS 16.0 (SPSS, Chicago, IL, USA) was used for data analysis. Measured data were expressed as mean ± standard deviation (SD), and compared by t-test. Qualitative variables were expressed as percentage, and compared by the Chi-square test. p < 0.05 was considered statistically significant.

**Results**

**Identification of differentially expressed 2-DE spots by mass spectrometry**

Table 1 summarizes the clinical data of all the patients involved in this study. Protein samples of the non-shock and shock groups were run on 2-DE gels, and each sample was run at least three times to ensure reproducibility. For the non-shock group, an average of 645 ± 45 spots was obtained (Fig. 1A). For those of the shock group, 704 ± 84 spots were obtained (Fig. 1B), indicating no difference in spot count between the two groups (p > 0.05). Up- and down-regulation in the shock group was defined with a fold change cutoff value of 2.0 in comparison with the non-shock group. Eight spots were found to be differentially expressed (Fig. 2A). The up-regulated spots were further analyzed using mass spectrometry and were identified as gelsolin (spots 1 and 2). Spot 3 was identified as α-1-antitrypsin, a protein involved in the acute phase response, while spot 4 was identified as a novel protein, spot 5 was identified as maldehydrogenase, a protein involved in the metabolic process. Spot 6 was identified as gelsolin, and spot 7 was identified as a novel protein. Spot 8 was identified as α-1-antitrypsin, a protein involved in the acute phase response. The results of mass spectrometry analysis are shown in Table 2.
protein spots with differential levels were statistically significant at $p < 0.01$, including spots 10, 19, 2, 24, 31, 26, 9 and 30 (Fig. 2).

Identification of differentially expressed proteins by MALDI-TOFMS

Spots representing the differentially expressed proteins were excised from stained gels, in situ digested with trypsin, and analyzed by MALDI-TOFMS. The identified proteins using MASCOT scores, MS/MS matched sequences, apparent and theoretical MWs, pl values, sequence coverage and regulation are listed in Table 2. There were 9 differentially expressed proteins. Among them, 6 proteins (SAA1, APOL1, CP, HP, SERPINC1 and F2) were upregulated, while another 3 proteins (IF, TTR, and A2M) were down regulated in the shock group compared with the non-shock group.

MALDI-TOF mass spectrum and database query results of the representative spot 19 are shown in Figs. 3, 4, 5. A total of 22 monoisotopic peaks were input into the Mascot search engine to search the Swiss-Prot database, and query results showed that protein spot 19 was APOL1.

Protein–protein interaction analysis

Protein–protein interaction (PPI) analysis was performed through STRING. A total of 9 differentially expressed proteins were imputed into STRING, and a complex network was obtained. The obtained protein–protein interactions are shown in Fig. 6. In biological process analysis, these proteins were mainly involved in platelet activation, signaling and aggregation, acute phase proteins highly expressed in response to inflammation and tissue injury, lipid homeostasis, cartilage development, iron ion transport, and select metabolic processes.

Discussion

Urosepsis mainly results from obstructed uropathy of the upper urinary tract, with ureterolithiasis being the most common cause. The complex pathogenesis of sepsis is initiated when pathogen or injury-associated molecular patterns recognized by pattern recognition receptors of the host innate immune system generate pro-inflammatory cytokines.6,7 In this study, the 2-DE-based proteomics approach was undertaken to identify altered proteins in shock group compared with the non-shock group. Nine differentially expressed proteins were successfully identified by MALDI-TOF-MS, with six upregulated (SAA1, APOL1, CP, HP, SERPINC1 and F2) and three proteins downregulated (TF, TTR and A2M).

Table 1
Clinical characteristics of patients involved in this study.

| Characteristics | Non-shock | Shock | $p$ value |
|-----------------|-----------|-------|-----------|
| Age (years)     | 49.64 ± 18.02 | 49.16 ± 15.62 | 0.903 |
| Gender (Male/Female) | 11/14 | 12/13 | 0.500 |
| WBC counts ($ \times 10^9$/L) | 13.12 ± 2.72 | 14.32 ± 3.29 | 0.176 |
| CRP (mg/dl)     | 14.32 ± 4.44 | 14.74 ± 4.34 | 0.778 |
| Serum PCT (ng/ml) | 5.76 ± 2.31 | 7.88 ± 2.19 | 0.001 |

Fig. 2. Results of 2-DE analysis. A, C: Representative 2-DE images of non-shock group samples; B, D: Representative 2-DE images of shock group samples.
During sepsis. Chiarla C hypothesized that change in transferring expression of TF is associated with sepsis, and TF is consistently low expressed. Sialylation could re-modulatory properties in inflammation. A previous study indicated that prothrombin gene-variant associated with sepsis, respiratory distress syndrome, and perinatal asphyxia, as well as other thrombophilic disorders, could be a risk factor for the development of neonatal thrombosis. There are many complex pathophysiological changes of the coagulation system in sepsis. Survivors and non-survivors show significant differences in anti-thrombin as revealed by coagulation tests.

### Platelet activation, signaling and aggregation

TF (serotransferrin) related pathways are platelet activation and signaling and aggregation, as well as transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds. The inflammatory process is associated with alterations in iron metabolism. Transferrin, an acute-phase N-glycosylated glycoprotein, plays an important role in iron transport. Decreased expression of TF is associated with sepsis, and TF is consistently low expressed during sepsis. Chiarla C hypothesized that change in transferring sialylation could reflect the intensity of the inflammatory response, and is insufficient if under-expressed and detrimental when over-expressed. In this study, TF showed lower levels in the shock group; the most likely explanation is transferring degradation by neuraminidase. Further studies, including measurement of blood neuraminidase concentrations and activity, are needed to understand the exact role of sialic acid decrease in septic patients.

SERPINC1 (antithrombin-III) inhibits thrombin and other serine proteases of the coagulation system, and regulates the blood coagulation cascade. The related pathways encompass platelet activation, signaling and aggregation, as well as the clotting cascade. Postoperative hemorrhagic disorders manifest as both hypo- and hyper-coagulation, with thrombinemia in most cases. Patients with urosepsis show latent hyper-coagulation phase of DIC. There is insufficient evidence to support SERPINC1 substitution in any category of critically ill participants, including those with sepsis and DIC. SERPINC1 does not impact mortality, but increases the risk of bleeding. SERPINA1 is a highly effective inhibitor of neutrophil elastase, plasmin, thrombin, trypsin, chymotrypsin, and plasminogen activator. In addition, it is an acute-phase protein and positively associated with necrosis and inflammation. As shown above, compared with the non-shock group, SERPINA1 showed higher levels in the shock group.

A2M (alpha-2-macroglobulin) is a protease inhibitor and cyto-kine transporter. It inhibits many proteases, including trypsin, thrombin and collagenase. Kelly BJ reported that A2M concentrations were significantly lower in surgical intensive care unit (SICU) patients; meanwhile, PCT levels were significantly higher in subjects with bacterial sepsis. Interestingly, combination of A2M and PCT can discriminate bacterial sepsis from other SIRS among SICU patients with suspected sepsis. A2M is also a protein with modulatory properties in inflammation. A previous study indicated that A2M has been microencapsulated could be an effective strategy to harness the complex biology of A2M, enhancing outcomes of fundamental processes of the innate immune response; this paves the way to potential future strategies in controlling sepsis. The current findings corroborate previous reports demonstrating that A2M plays an important role in urosepsis regulation.

F2 (prothrombin) coagulation factor II is proteolytically cleaved to form thrombin in the first step of the coagulation cascade, which ultimately results in blood loss. F2 also plays a role in maintaining vascular integrity during development and postnatal life. It was suggested that prothrombin gene-variant associated with sepsis, respiratory distress syndrome, and perinatal asphyxia, as well as other thrombophilic disorders, could be a risk factor for the development of neonatal thrombosis. There are many complex pathophysiological changes of the coagulation system in sepsis. Survivors and non-survivors show significant differences in anti-thrombin as revealed by coagulation tests.

### Acute phase proteins

SSA1 (serum amyloid A-1 protein) is a major acute phase protein that is highly expressed in response to inflammation and tissue injury. This protein also plays an important role in HDL metabolism and cholesterol homeostasis. Apolipoprotein A is a major apoprotein (45%) constituting HDL in the early phase of sepsis, and slowly replaced by apolipoprotein A-I during recovery. In severe sepsis, HDL is shifted to acute phase HDL, which is enriched in serum amyloid A and depleted of cholesterol and apolipoprotein A-1. In addition, high apolipoprotein A-1 levels are found in patients with sepsis.

### Lipid homeostasis, iron ion transport, and select metabolic processes

APOL1 (apolipoprotein L1) plays a role in lipid exchange and transport throughout the body, and it is involved in reverse cholesterol transport from peripheral cells to the liver. Several transcript variants encoding different isoforms have been described for this gene. A negative correlation between Apol 1 expression in neutrophils and C-reactive protein (CRP) levels was reported as well as a positive association of apoptotic neutrophil count with Apol1 and 2 mRNA levels. The degree of neutrophil apoptosis in critically ill patients is therefore correlated with modified expression levels of ApoLs.

Table 2

| Spot No. | Target protein          |Aliases| Nominal mass (Mr) KD | Calculated pl | Protein score | Sequence coverage (%) | Expression |
|----------|------------------------|-------|----------------------|--------------|---------------|-----------------------|------------|
| 10       | Serotransferrin TF     |       | 79.294               | 6.81         | 88            | 7                     | Decreased  |
| 19       | Serum amyloid A-1 protein SSA1 | | 13.581               | 6.28         | 341           | 63                    | Increased  |
| 2        | Transthyretin TTR      |       | 15.877               | 5.52         | 68            | 8                     | Decreased  |
| 19       | Apolipoprotein L1 APOL1 |       | 44.004               | 5.60         | 119           | 15                    | Increased  |
| 24       | Ceruloplasmin CP       |       | 122.983              | 5.44         | 53            | 4                     | Increased  |
| 31       | Haptoglobin HP         |       | 45.861               | 6.13         | 69            | 9                     | Increased  |
| 26       | Antithrombin-III SERPINC1 |     | 53.025               | 6.32         | 280           | 15                    | Increased  |
| 9        | Prothrombin F2         |       | 71.475               | 5.64         | 70            | 7                     | Increased  |
| 30       | Alpha-2-macroglobulin A2M |      | 163.188              | 6.03         | 26            | 0                     | Decreased  |
Fig. 3. MALDI-TOF MS mass spectrum of protein spot 19 identified as APOL1 according to the matched peaks.

Fig. 4. Protein sequence of APOL1, and matched peptides are bold red.

Fig. 5. Database query results and score of protein spot 19.
patients. An inef
canter in neonatal septicemia, but is not useful as a tool for early
case compared with the urosepsis group, and mainly involved in
case. Therefore, a TTR (transthyretin) is a carrier protein that transports thyroid
tein to Fe (III) transferrin. Serum CP level estimate may help

Conclusion
This study firstly provided an overview of serum proteomics
changes between patients with urosepsis (non-shock group) and
severe urosepsis (shock group). Interestingly, 9 differentially
expressed proteins were found in the serum of severe urosepsis
cases compared with the urosepsis group, and mainly involved in
platelet activation, signaling and aggregation, acute phase protein
pathway, lipid homeostasis, and iron ion transport. This study
revealed molecular markers of early diagnosis and prognosis of
urosepsis, and the differentially expressed proteins might be new
therapeutic targets for urosepsis treatment.

Fund
This study was supported by a grant from Gansu science and
technology support project, China (15Q4FKCA101).

Appendix A. Supplementary data
Supplementary data to this article can be found online at
https://doi.org/10.1016/j.cjtee.2018.07.003.

Conflict of interests
All authors declare that they have not any conflict of interests.

References
1. Wagenlehner FM, Alidjanov J, Pilatz A. Urosepsis. Update on diagnosis and
treatment. Urol A. 2016;55:454–459. https://doi.org/10.1016/j.uroil.016-
0066-9.
2. Dreger NM, Degener S, Ahmad-Nejad P, et al. Urosepsis-etiology, diagnosis, and
treatment. Dtsch Arztebl Int. 2015;112:837–847. https://doi.org/10.3238/arz-
tebl.2015.0837, quiz 848.
3. Delanghe JR, Speeckaert MM. Translational research and biomarkers in
neonatal sepsis. Clin Chim Acta. 2015;451(Pt A):46–64. https://doi.org/10.1016/
j.cca.2015.01.031, Epub 2015 Feb 4.
4. Levy MM, Fink MP, Marshall JC, et al. 2001 SCoM/ESICM/ACCP/ATS/SIS
international sepsis definitions conference. Crit Care Med. 2003;31:1250–1256.
https://doi.org/10.1016/j.ccm.2003.12.005.
5. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international
guidelines for management of severe sepsis and septic shock: 2012. Intensive Care
Med. 2013;39:165–228. https://doi.org/10.1007/s00134-012-2769-8.
6. Hildebrandt S, Steinhart H, Paschke A. Comparison of different extraction so-
lutions for the analysis of allergens in hen’s egg. Food Chem. 2008;108(3):
1085–1093.
7. Degoricia V, Sharma M, Legac A, et al. Survival analysis of 314 episodes of
sepsis in medical intensive care unit in university hospital: impact of intensive
care unit performance and antimicrobial therapy. Croat Med J. 2006;47:
385–397.
8. Szatanik M, Hong E, Ruckly C, et al. Experimental meningococcal sepsis in
congenic transgenic mice expressing human transferrin. PloS One. 2011;6(7),
e22210. https://doi.org/10.1371/journal.pone.0022210.
9. Piagnerelli M, Boudjeltia KZ, Nuyens V, et al. Rapid alterations in transferrin
sialylation during sepsis. Shock. 2005;24:48–52.
10. Chiara C, Giovannini I, Siegel JH. Hypo transferrinemia and changes in plasma
lipid and metabolic patterns in sepsis. Amino Acids. 2009;36:327–331. https://
doi.org/10.1007/s00726-008-0072-3.
11. Chiara C, Giovannini I, Siegel JH. The relationship between plasma cholesterol,
amino acids and acute phase proteins in sepsis. Amino Acids. 2004;27:97–100.
https://doi.org/10.1007/s00726-004-0064-9.
12. Gornik O, Gornik I, Kolejdjak IZ, et al. Change of transferrin sialylation differs
between mild sepsis and severe sepsis and septic shock. Intem Med. 2011;50:
861–869.
13. Wiklund CG, Erdahcan E, Tunali Y, et al. The effects of intravenous, enteral and
combined administration of glutamine on malnutrition in sepsis: a randomized
clinical trial. Asia Pac J Clin Nutr. 2014;23:34–40. https://doi.org/10.6133/
apjn.2014.23.1.11.
14. Allingstrup M, Weterslev J, Raven FB, et al. Antithrombin III for critically ill
patients: a systematic review with meta-analysis and trial sequential analysis.
Intensive Care Med. 2016;42:505–520. https://doi.org/10.1007/s00134-016-
4225-7.
15. Allingstrup M, Weterslev J, Raven FB, et al. Antithrombin III for critically ill
patients. Cochrane Database Syst Rev. 2016;2:CDB005370. https://doi.org/
10.1002/14651858.CD005370.pub3.
16. Law RH, Zhang Q, Mckowan S, et al. An overview of the serpin superfamily.
Genome Biol. 2006;7:2-11. https://doi.org/10.1186/gb-2006-7-5-216.
17. Farshchian M, Kivisaari A, Ala-Alho R, et al. Serpin peptidase inhibitor clade A
member 1 (SerpinA1) is a novel biomarker for progression of cutaneous
squamous cell carcinoma. Am J Pathol. 2011;179:1110–1119. https://doi.org/
10.1016/j.ajpath.2011.05.012.
18. Subramaniyam D, Zhou H, Liang M, et al. Cholesterol rich lipid raft micro-
domains are gateway for acute phase protein, SERPINA1. Int J Biochem Cell
Biol. 2010;42:1562–1578. https://doi.org/10.1016/j.biocel.2010.06.005.7.
19. Normandin K, Piant B, Le Page C, et al. Protease inhibitor SERPINA1 expression
in epithelial ovarian cancer. Clin Exp Metastasis. 2010;27:55–69. https://
doi.org/10.1007/s10433-009-9303-6.
20. Kelly BJ, Lautenbach E, Nachamin I, et al. Combined biomarkers discriminate a
low likelihood of bacterial infection among surgical intensive care unit patients
with suspected sepsis. Diagn Microbiol Infect Dis. 2016;85:109–115. https://
doi.org/10.1016/j.diagmicrobio.2016.01.003.
21. Federici Canova D, Pavlov AM, Norling LV, et al. Alpha-2-macroglobulin loaded
microcapsules enhance human leukocyte functions and innate immune
response. J Control Release. 2015;217:284–292. https://doi.org/10.1016/j.
jjconrel.2015.09.021.
22. Birkenmeier G, Nicklich S, Pockelt C, et al. Polymyxin B-conjugated alpha 2-
macroglobulin as an adjunctive therapy to sepsis: modes of action and impact
on lethality. J Pharmocol Exp Ther. 2006;318:762–771. https://doi.org/10.1124/
jpnet.105.104265.
23. Wiedermann CJ. Clinical review: molecular mechanisms underlying the role of
antithrombin in sepsis. Crit Care. 2006;10:209. https://doi.org/10.1186/cc4822.
24. Mosunjac MB, Sundstrom JB, Mosunjac MI. Unusual presentation of anaplastic
large cell lymphoma with clinical course mimicking fever of unknown origin and
sepsis: autopsy study of five cases. Croat Med J. 2008;49:660–668.

Fig. 6. Protein–protein interactions.
25. Cinar A, Dilber E, Karagoz T, et al. Association of prothrombin gene mutation with sepsis in a preterm with multiple intracardiac thrombi. *Echocardiography.* 2005;22:340–344. https://doi.org/10.1111/j.1540-8175.2005.03107.x.

26. Bay A, Oner AF, Kose D, et al. Global fibrinolytic capacity in pediatric patients with sepsis and disseminated intravascular coagulation. *Blood Coagul Fibrinolysis.* 2006;17:569–573. https://doi.org/10.1097/01.bcc.0000245304.95138.cf.

27. van Leeuwen HJ, Heezius EC, Dallinga GM, et al. Lipoprotein metabolism in patients with severe sepsis. *Crit Care Med.* 2003;31:1359–1366. https://doi.org/10.1097/01.CCM.0000059724.08290.51.

28. Paiva RA, David CM, Domont GB. Proteomics in sepsis: a pilot study. *Rev Bras Ter Intensiv.* 2010;22:403–412.

29. Liu MJ, Bao S, Napolitano JR, et al. Zinc regulates the acute phase response and serum amyloid A production in response to sepsis through JAK-STAT3 signaling. *Plos One.* 2014;9, e94934. https://doi.org/10.1371/journal.pone.0094934.

30. Raju MS, V J, Kamaraju RS, et al. Continuous evaluation of changes in the serum proteome from early to late stages of sepsis caused by Klebsiella pneumoniae. *Mol Med Rep.* 2016;13:4835–4844. https://doi.org/10.3892/mmr.2016.5112.

31. Akl I, Lelubre C, Uzureau P, et al. Apolipoprotein L expression correlates with neutrophil cell death in critically ill patients. *Shock.* 2017;47:111–118. https://doi.org/10.1097/SHK.0000000000000728.

32. Suri M, Sharma VK, Thirupuram S. Evaluation of ceruloplasmin in neonatal sepsis. *Indian Pediatr.* 1991;28(5):489–493.

33. Su L, Zhou R, Liu C, et al. Urinary proteomics analysis for sepsis biomarkers with iTRAQ labeling and two-dimensional liquid chromatography-tandem mass spectrometry. *J Trauma Acute Care Surg.* 2013;74:940–945. https://doi.org/10.1097/TA.0b013e31828272c5.

34. Devakonda A, George L, Raoof S, et al. Transthyretin as a marker to predict outcome in critically ill patients. *Clin Biochem.* 2008;41:1126–1130. https://doi.org/10.1016/j.clinbiochem.2008.06.018.