miR-148 family members are putative biomarkers for sepsis

LEI DONG1*, HONGWEI LI1*, SHUNLI ZHANG2 and GUANZHENG YANG3

1Intensive Care Unit; Departments of 2Pediatrics and 3Respiratory Medicine, Jining No. 1 People's Hospital, Jining, Shandong 272011, P.R. China

Received February 6, 2018; Accepted October 1, 2018

DOI: 10.3892/mmr.2019.10174

Abstract. Sepsis is a type of systemic inflammatory response caused by infection. The present study aimed to identify novel targets for the treatment of sepsis. We conducted bioinformatic analysis of the microarray Gene Expression Omnibus dataset GSE12624, which includes data on 34 patients with sepsis and 36 healthy individuals without sepsis. Differentially expressed genes (DEGs) in sepsis patients were identified using Bayesian methods included in the limma package in R. Correlations among the expression values of DEGs were analyzed using the weighted gene co-expression network analysis (WGCNA) to construct a co-expression network. Subsequently, the generated co-expression network was visualized using Cytoscape 3.3 software. Additionally, a protein-protein interaction (PPI) network was constructed based on all the DEGs using STRING. Finally, the integrated regulatory network was constructed based on DEGs, microRNAs (miRNAs) and transcription factors (TFs). A total of 407 DEGs were identified in the sepsis samples, including 227 upregulated DEGs and 180 downregulated DEGs. WGCNA grouped the DEGs into 13 co-expressed modules. Additionally, MAP3K8 and RPS6KA5 in the MEyellow module were enriched in the MAPK and TNF signaling pathways. In addition, the PPI network comprised 48 nodes and 112 edges, which included the pairs MAP3K8-RPS6KA5, MAP3K8-IL10, RPS6KA5-EXOSC4 and EXOSC4-EXOSC5. Lastly, the TF-miRNA-target DEG regulatory network was constructed based on eight TFs (NF-κB), seven miRNAs (miR152, miR-148A/B), and 52 TF-miRNA-target gene triplets (17 upregulated genes, including MAP3K8, and 10 downregulated genes, including RPS6KA5). Our analysis showed that the members of the miR-148 family (miR-148A/B and miR-152) are candidate biomarkers for sepsis.

Introduction

Sepsis is a type of systemic inflammatory response syndrome (SIRS) and is mediated by an immune response triggered by infection, which can progress from sepsis to severe sepsis and septic shock (1). Sepsis can lead to symptoms, including fever, increased heart rate, breathing rate and confusion (2). In 2015, the incidence rates of sepsis and severe sepsis in high-income countries were 0.44 and 0.27%, respectively (3). Meanwhile, the mortality rates for sepsis were reported to be 3 and 75 cases per 1,000 individuals in two Chinese military hospitals (4). Sepsis remains difficult to predict, diagnose and treat (5). Thus, there is an urgent need to identify target genes and microRNAs (miRNAs) that can serve as biomarkers for sepsis.

Previous studies have demonstrated that proinflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), are key mediators of inflammation during sepsis (6). A previous study showed that IL-6, IL-1β, IL-8 and TNF-α levels were significantly upregulated in culture-proven sepsis (6). In addition, procalcitonin (PCT) is elevated in patients with SIRS, and has been approved by the Food and Drug Administration (U.S. FDA) for the assessment of risk for developing severe sepsis in patients (9). Although PCT is closely associated with inflammation, there are some limitations specific for infection resulting in questionable efficacy as PCT can also be increased in noninfectious disease conditions (10). Generally, the concentration value of PCT <0.5 ng/ml indicates a low risk while values of 0.5-2.0 ng/ml represent an intermediate likelihood of sepsis and/or septic shock. Wacker et al reported (11) that PCT had a modest diagnostic performance with 77% sensitivity and 79% specificity. Therefore, PCT is not specific for diagnosis in patients with values in the intermediate range. Importantly, multiple miRNAs have various biological functions in inflammation, metabolism and tumor progression. These candidate miRNAs show high accuracy and sensitivity, and are expected to be ideal biomarkers for sepsis. Evidence suggests that the

*Contributed equally

Key words: sepsis, differentially expressed genes, transcription factors, microRNAs, MAPK signaling pathway, TNF signaling pathway
sensitivity and specificity of miR-223 for predicting the occurrence of sepsis after urinary operation were higher than those of PCT (12,13). In addition, miR-155 has been suggested to directly target key genes that are involved in LPS signaling, such as Fas-associated death domain protein, IkB kinase ε, and the receptor (TNFR superfamily)-interacting serine-threonine kinase 1 to enhance TNF-α production (14). Nevertheless, miR-125b targets the 3’-untranslated region of the TNF-α transcript (14). However, the fundamental mechanisms underlying the pathogenesis of sepsis remain unclear. Multiple mechanisms involving complex systemic inflammation networks, genetic polymorphisms, immune dysfunction, abnormal coagulant function, and host response to pathogenic microorganisms and their toxins are likely to be involved in sepsis. Therefore, the pathogenesis of sepsis warrants further investigation.

In the present study, we performed bioinformatics analysis to identify the differentially expressed genes (DEGs) in sepsis, as well as the TFs and miRNAs of these DEGs. Subsequently, an integrated regulatory network was constructed based on the DEGs, miRNAs and TFs. Finally, we investigated the interactions among the DEGs and TFs/miRNAs and their corresponding functions. Our current findings provided insights into the pathogenesis of sepsis and identified novel targets for the treatment of sepsis.

Materials and methods

Microarray data. The GSE12624 dataset was downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) and contains gene expression data of 34 sepsis patients and 36 healthy individuals without sepsis. The inclusion criteria for the study are described in (15). The microarray platform was GPL4204 GE Healthcare/Amersham Biosciences CodeLink UniSet Human I Bioarray. Raw data were available in TXT format.

Data preprocessing and identification of DEGs. The probes corresponded to gene symbols according to the latest annotation file from the NCBI gene database. When more than one probe corresponded to the same gene symbol, the expression level of the gene was calculated as the median of the two expression values. Subsequently, the data were fitted to a log-normal distribution using the log2 function, normalized using the median function, and compared with septic samples and non-septic samples using Bayesian methods from the limma package in R (Linear Models for Microarray Data, http://www.biocductor.org/packages/release/bioc/html/limma.html). Finally, log fold change (FC) >0.585 and adjusted P-value <0.05 were used as the threshold values for considering the DEGs.

Identification of sepsis-related genes and modules based on WGCNA. WGCNA is a systematic method for identifying putative target genes involved in a disease. It is used to describe the correlation among genes by finding significant modules from high-throughput sequencing data (16). In the present study, WGCNA was performed based on the following analysis workflow. i) The correlations among the expression values of DEGs in the dataset were determined. A higher correlation value indicates higher consistency of gene expression in each dataset, which is a prerequisite for the construction of a WGCNA network. ii) The correlation matrix of gene co-expression values was constructed based on $S_{mn} = lcor(m,n)$, where $S_{mn}$ indicates the correlation coefficient of co-expression patterns between genes m and n. iii) The adjacency is defined as $a_{mn} = power(S_{mn})^\beta$, which measures the pairwise correlation between the expression levels of two genes. iv) Adjacency functions for both weighted and unweighted networks require the user to choose threshold parameters. The threshold of ≥0.9 was considered for the correlation coefficient between log2 k (node count) and log2 p(k) (frequency of node). v) The correlation matrix $S_{mn}$ was transformed to the adjacency matrix $a_{mn}$. Afterwards, the adjacency matrix $a_{mn}$ was transformed to a topological matrix using the following equation:

$$l_{mn} + a_{mn}$$

$$\min\{k_m, k_n\} + 1 - a_{mn}$$

where $l_{mn}$ indicates the sum of adjacency coefficient of the common edge between genes m and n and $k_m$ indicates sum of connection strengths of m with the other network genes.

vi) Gene significance (GS) measures were used to incorporate external information into the co-expression network. Module significance was determined by calculating the average IGSi for all genes in a module.

Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of the key modules. The Database for Annotation, Visualization and Integration Discovery (DAVID, a public high-throughput functional annotation tool (version 6.8, https://david-d.ncifcrf.gov/) is an online bioinformatics tool that can be used for functional annotation and microarray analysis by integrating data mining environments and analyzing gene lists (17). DEGs in the modules were used as input for DAVID, and GO and KEGG enrichment analyses were conducted using MEGene and MEGene Yellow DEGs. P-value <0.05 and the enriched gene count ≥2 were considered significant.

Construction of the PPI network and module analysis. The PPI network was constructed based on all the DEGs using STRING from a well-known online server (version 10.0, http://www.string-db.org/) (18). A combined score of >0.4 was defined as the threshold value for constructing the PPI network. The PPI network was visualized using Cytoscape software (version 3.2.0, http://cytoscape.org/) (19). In addition, MCODE (version 1.4.2, http://apps.cytoscape.org/apps/MCODE) in Cytoscape software was used to analyze the most significant module, with the threshold value of 5 (20).

Construction of the TF-miRNA-target DEGs regulatory network. The miRNA-target DEGs and TF-target DEGs were predicted using Overrepresentation Enrichment Analysis enrichment method in WebGestalt (http://www.webgestalt.org/). Gene pairs with P-value <0.05 were integrated into the TF-miRNA-target DEGs regulatory network, which was visualized using Cytoscape.

Results

Sepsis-related genes and modules. A total of 407 DEGs, including 227 upregulated DEGs and 180 downregulated
DEGs, were identified. According to the standard scale-free network model, the power value was set to 12 when the square of the correlation coefficient was 0.9 (Fig. 1). The network conformed to a scale-free model when the square of the correlation coefficient was set to the highest value. Subsequently, the WGCNA network was constructed under power = 12. Gene cluster dendrogram was obtained according to dissTOM using the hierarchical clustering method. The dynamic tree cut method was employed to estimate the number of clusters in the dataset. Finally, the DEGs were divided into 13 co-expressed modules (Fig. 2), and genes in the grey module contained genes that could not clustered under the other modules. Subsequently, the most highly connected intramodular hub gene in each module was considered as the module representative. The analysis identified a total of 7 modules with correlation coefficients >0.5. The correlation coefficients of the MEblue and MEyellow modules were higher than 0.6. To ensure the reliability of the key network module, the IGSi was used to further identify two key modules (Fig. 3). Finally, the MEblue (Fig. 3A) and MEyellow (Fig. 3B) modules were defined as the key modules. All of DEGs in the two modules are shown in the Table I.

**GO function and KEGG pathway analysis.** A total of 66 DEGs were identified in the MEblue and MEyellow modules, including 46 upregulated DEGs and 20 downregulated DEGs. *F8, PLAU* and *SERPING1* in the MEblue module were enriched with complement and coagulation cascades. *EXOSC4* and *EXOSC5* in the MEblue module were enriched in the RNA degradation pathway. *MAP3K8* and *RPS6KA5* in the MEyellow module were enriched in the MAPK and TNF signaling pathways. The GO functions of the genes in the two modules are shown in Table II.

**PPI network based on the MEblue and MEyellow modules.** The PPI network (Fig. 4) contained 48 nodes (genes) and 112 edges (protein-protein interrelations), such as *MAP3K8-RPS6KA5, MAP3K8-IL10, RPS6KA5-EXOSC4* and *EXOSC4-EXOSC5*. One sub-network (hub module) had an MCODE score ≥5 and comprised 7 nodes (e.g. IL10) and 15 edges (Fig. 4).

**miRNA-TF-target gene regulatory network.** Overall, the analysis identified 8 TFs (*NF-kB*) and 7 miRNAs (miR152 and miR-148A/B), which comprised 52 TF-miRNA-target gene pairs (17 upregulated genes, such as *MAP3K8* and 10 downregulated genes, such as *RPS6KA5*) and were used to construct an miRNA-TF-target gene regulatory network (Fig. 5).
Figure 3. The two key modules [(A) MEblue and (B) MEyellow] were identified by calculating |Gene significance (GS)| values.
Discussion

In the present study, we identified a total of 407 DEGs in the sepsis samples, including 227 upregulated DEGs and 180 downregulated DEGs. Subsequently, these DEGs were grouped into 13 co-expressed modules after WGCNA. Additionally, MEblue and MEyellow modules with a correlation coefficient >0.6 were defined as the key modules; these modules included 6 upregulated and 20 downregulated DEGs. EXOSC4 and EXOSC5 in the MEblue module were enriched in the RNA degradation pathway. MAP3K8 and RPS6KA5 in the MEyellow module were enriched in the MAPK and TNF signaling pathways. In addition, the resulting PPI network comprised 48 nodes and 112 edges such as MAP3K8-RPS6KA5, MAP3K8-IL10, RPS6KA5-EXOSC4 and EXOSC4-EXOSC5. Finally, the analysis identified 8 TFs (NF-κB) and 7 miRNAs (miR-152 and miR-148A/B) that corresponded to 52 TF-miRNA-target gene pairs (17 upregulated genes, such as MAP3K8 and 10 downregulated genes, such as RPS6KA5).

Table I. Differentially expressed genes in the MEblue and MEyellow modules.

| Genes   | Module | Description | Genes   | Module | Description |
|---------|--------|-------------|---------|--------|-------------|
| ACN9    | blue   | up          | ADAMTS5 | yellow | down        |
| ANPEP   | blue   | down        | CALCRL  | yellow | up          |
| CA4     | blue   | up          | DGKG    | yellow | down        |
| CDADC1  | blue   | up          | DHRS9   | yellow | up          |
| COQ2    | blue   | up          | ERLIN1  | yellow | up          |
| CPNE5   | blue   | up          | FAM105A | yellow | up          |
| CYP19A1 | blue   | up          | FAR2    | yellow | up          |
| ENN3    | blue   | up          | GADD45A | yellow | up          |
| EMR3    | blue   | down        | HMGB2   | yellow | up          |
| EXOSC4  | blue   | up          | IL18RAP | yellow | up          |
| EXOSC5  | blue   | down        | KLHL2   | yellow | up          |
| F8      | blue   | up          | LEPROT  | yellow | up          |
| FOXN2   | blue   | up          | LRPPRC  | yellow | up          |
| FZD6    | blue   | up          | MAP3K8  | yellow | up          |
| HAS3    | blue   | up          | NAALADL1| yellow | down        |
| HPGD    | blue   | up          | PECR    | yellow | up          |
| ID11    | blue   | up          | PROS1   | yellow | up          |
| IDNK    | blue   | up          | RPS6KA5 | yellow | down        |
| IL10    | blue   | up          | SAMSIN1 | yellow | up          |
| IPO11   | blue   | up          | SDPR    | yellow | up          |
| JUND    | blue   | up          | SIPA1L2 | yellow | up          |
| LOC643641| blue  | up          | SP140   | yellow | down        |
| MREG    | blue   | up          | SPIB    | yellow | down        |
| NOV     | blue   | down        | UBE2H   | yellow | up          |
| OGN     | blue   | up          | URGCP   | yellow | down        |
| PDK2    | blue   | down        | USP46   | yellow | down        |
| PIDD    | blue   | down        | VNN1    | yellow | up          |
| PLA2    | blue   | down        |         |        |             |
| PNMAL1  | blue   | up          |         |        |             |
| PPEF1   | blue   | up          |         |        |             |
| PSTPIP2 | blue   | up          |         |        |             |
| RABGEF1 | blue   | up          |         |        |             |
| RCC1    | blue   | up          |         |        |             |
| SERPINB1| blue   | up          |         |        |             |
| SERPING1| blue   | down        |         |        |             |
| SLC25A1 | blue   | down        |         |        |             |
| SLC51A  | blue   | up          |         |        |             |
| SPON2   | blue   | down        |         |        |             |
| Stard10 | blue   | down        |         |        |             |

Up, upregulated; down, downregulated.
Table II. Gene Ontology functions for genes in the two modules.

| Module | GO-ID-Name | Count | P-value | Genes |
|--------|------------|-------|---------|-------|
| **MEblue** | **GO_BP** | | | |
| GO:0007596~blood coagulation | 3 | 1.75E-02 | F8, SERPING1, PLAU |
| GO:0050817~coagulation | 3 | 1.75E-02 | F8, SERPING1, PLAU |
| GO:0007599~hemostasis | 3 | 1.95E-02 | F8, SERPING1, PLAU |
| GO:0050878~regulation of body fluid levels | 3 | 3.19E-02 | F8, SERPING1, PLAU |
| GO:0008299~isopenoid biosynthetic process | 2 | 3.92E-02 | COQ2, IDI1 |
| GO:0032101~regulation of response to external stimulus | 3 | 3.98E-02 | SERPING1, IL10, PLAU |
| GO:0045861~negative regulation of proteolysis | 2 | 4.30E-02 | SERPING1, IL10 |
| **GO_CC** | GO:0044421~extracellular region part | 7 | 1.88E-02 | NOV, OGN, F8, SERPING1, EMR3, SPON2, IL10 |
| GO:000178~exosome (RNase complex) | 2 | 2.69E-02 | EXOSC4, EXOSC5 |
| **GO_MF** | GO:000175~3'-5' exoribonuclease activity | 2 | 2.74E-02 | EXOSC4, EXOSC5 |
| GO:0004532~exoribonuclease activity | 2 | 2.96E-02 | EXOSC4, EXOSC5 |
| GO:0016896~exonuclease activity, producing 5'-phosphomonoesters | 2 | 2.96E-02 | EXOSC4, EXOSC5 |
| GO:0016796~exonuclease activity, active with either ribo- or deoxyribonucleic acids and producing 5'-phosphomonoesters | 2 | 4.52E-02 | EXOSC4, EXOSC5 |
| **MEyellow** | **GO_BP** | | | |
| GO:0006508~proteolysis | 6 | 1.64E-02 | RPS6KA5, USP46, ERLIN1, UBE2H, NAAALDL1, ADAMTS5 |
| GO:0006511~ubiquitin-dependent protein catabolic process | 3 | 4.90E-02 | USP46, ERLIN1, UBE2H |
| **GO_MF** | GO:0008237~metallopeptidase activity | 3 | 2.89E-02 | RPS6KA5, NAAALDL1, ADAMTS5 |
| GO:0070011~peptidase activity, acting on L-amino acid peptides | 4 | 4.40E-02 | RPS6KA5, USP46, NAAALDL1, ADAMTS5 |
| GO:0008233~peptidase activity | 4 | 4.92E-02 | RPS6KA5, USP46, NAAALDL1, ADAMTS5 |

GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function.
Figure 4. The protein-protein interaction (PPI) network based on the differentially expressed genes (DEGs) in the MEblue and MEyellow modules. Triangles indicate upregulated DEGs, and arrows indicate downregulated DEGs. Yellow and blue colors indicate genes in the MEyellow and MEblue modules, respectively.

Figure 5. Constructed TF-miRNA-target DEG regulatory network. Triangles indicate upregulated DEGs; arrows indicate downregulated DEGs; hexagons indicate TFs; and the circles indicate miRNAs. Yellow and blue colors indicate genes in the MEyellow and MEblue modules, respectively. Arrows indicate the direction of regulation. TF, transcription factor; DEG, differentially expressed gene.
MAP3K8 is a serine-threonine kinase that plays a critical role in innate immunity and is known to induce tumor necrosis factor (TNF) production by activating ERK (21). In addition, TNF-α has been implicated as a key mediator in inflammation, morbidity and mortality associated with sepsis. TNF-α has been demonstrated to be responsible for the initial hypothermia and lethality in septic mice (22). In addition, host reactions during sepsis, septic shock, and multiple organ failure are associated with increased TNF production in humans (23). TNF-α is a strong pro-inflammatory cytokine associated with septic patients and has been considered as a target for the treatment of sepsis (24). In the present study, MAP3K8 expression levels were found to be upregulated in sepsis samples relative to those of the control samples. As indicated above, MAP3K8 induces TNF production, consistent with increased TNF levels in the sepsis samples in the present study. Importantly, MAP3K8 and RPS6KA5 in the MElowyer module were enriched in the MAPK and TNF signaling pathways. In addition, MAP3K8 interacts with IL10 based on the constructed PPI network. IL10 is an anti-inflammatory agent that can improve disease outcome in the model of sepsis syndrome (25). Therefore, these findings indicate that MAP3K8 is involved in sepsis through the MAPK and TNF signaling pathways. The nuclear transcription factor NF-κB is known to be activated following hemorrhagic shock and sepsis (26). In the present study, NF-κB acts as the upstream TF of the MAP3K8 gene. Proinflammatory cytokines, such as TNF-α and IL-1, activate important signaling pathways. In particular, cytokines activate members of the NF-κB group of TFs, which play central roles in inflammation and innate immunity (27). Activation of NF-κB and other TFs involved in the innate immune/inflammatory response can upregulate the expression of various genes, such as MMP-9, VEGF and TNF (28). Therefore, MAP3K8 is potentially involved in sepsis through the activation of NF-κB and is likely to be involved in the MAPK and TNF signaling pathways.

In the PPI network, RPS6KA5 interacted with MAP3K8, and these two genes were enriched in the MAPK and TNF signaling pathways. RPS6KA5, also known as mitogen- and stress-activated protein kinase 1 (MSK1), is a downstream target of both p38 and ERK1/2 (29). RPS6KA5 stimulates the transcription of various pro-inflammatory genes, such as IL-6, IL-8 and TNF-α, by activating TFs (30). Therefore, RPS6KA5 was associated with sepsis through the MAPK and TNF signaling pathways. In the miRNA-TF-target gene regulatory network, RPS6KA5 was the target gene of miR-152, miR-148A and miR-148B. A previous study indicated that members of the miR-148 family (miR-148A, miR-148B and miR-152) negatively regulated antigen presentation and Toll-like receptor (TLR)-triggered cytokine secretion in dendritic cells (31). TLRs are a class of proteins that play key roles in the innate immune system and secrete proinflammatory cytokines, such as TNF-α, IL-6 and IL-12 (32,33). In addition, soluble TLR2 is a biomarker for sepsis in critically ill patients with multi-organ failure within 12 h of ICU admission (34). Although there was no direct evidence to identify that miR-148 is better than PCT or TLR2, miRNAs with high accuracy and sensitivity, are expected to be ideal biomarkers for sepsis (13). Thus, the receiver operating characteristic (ROC) curve of the miR-148 family (including sensitivity and specificity) should be compared with those of PCT or TLR2 in diagnostic performance of sepsis patients. It is one of the limitation of the present study. Therefore, members of the miR-148 family (miR-148A, miR-148B and miR-152) may be candidate biomarkers for sepsis.

The present study has certain limitations. First, limited samples were collected from the sepsis patients, and experimental validation of the results was not performed. PCR or western blotting will be performed in subsequent studies to verify the findings. In addition, experiments should be conducted to verify whether RPS6KA5 is a target of miR-148A/B and miR-152 in sepsis. In addition, the micro-array dataset GSE12624 from the Gene Expression Omnibus only included 34 patients with sepsis and 36 healthy individuals without sepsis. Therefore, correlation among the miR-148 family (miR-148A/B and miR-152), and the type of infection was not performed. In addition, an ROC curve of the miR-148 family should be assayed in the diagnostic performance of sepsis patients. However, the present results will not be affected by these limitations.

Therefore, MAP3K8 is potentially induced during sepsis through NF-κB activation and is potentially involved in the MAPK and TNF signaling pathways. Meanwhile, RPS6KA5 interacted with MAP3K8 in the PPI network and was also found to be enriched in the MAPK and TNF signaling pathways. Members of the miR-148 family (miR-148A/B and miR-152) are candidate biomarkers for sepsis.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

All data generated or analyzed during this study are included in the published article.

Authors’ contributions

Conception and design of the research were carried out by LD, HL and GY. Data collection, analysis and interpretation were conducted by SZ. Drafting of the manuscript was performed by LD and HL. Revision of the manuscript for important intellectual content was conducted by GY. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.
Competing interests
The authors declare that they have no competing interests.

References

1. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, et al.: Surviving sepsis campaign: International guidelines for management of sepsis and septic shock: 2016. Crit Care Med 45: 486‑552, 2017.

2. Tsai D, Stewart P, Goud R, Gourley S, Hiewagama S, Krishnasawmy S, Wallis SC, Lipman J and Roberts JA: Total and unbound cerftriaxone pharmacokinetics in critically ill Australian Indigenous patients with severe sepsis. Int J Antimicrob Agents 48: 748‑752, 2016.

3. Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, Angus DC and Reinhart K: International Forum of Acute Care Trialsists: Assessment of global incidence and mortality of hospital-treated sepsis: current estimates and limitations. Am J Respir Crit Care Med 193: 259‑272, 2016.

4. Cheng W, Wang S, Shen C, Zhao D, Li D and Shang Y: Epidemiology of hospitalized burns patients in china: A systematic review and Burn Open 2: 8‑16, 2017.

5. Mitra P, Guha D, Nag SS, Mondal BC and Dasgupta S: Role of plasma fibrinogen in diagnosis and prediction of short term outcome in neonatal sepsis. Indian J Hematol Blood Transfus 33: 195‑199, 2017.

6. Kang S, Jiazhi Z, Masuda K and Kishimoto T: Implications of IL‑6 Targeting Therapy for Sepsis. Immunotherapy (Los Angeles) 3: 138, 2017.

7. Kurt AN, Aygun AD, Godekmerdan A, Kurt A, Dogan Y and Yilmaz E: Serum IL‑Ibeta, IL‑6, IL‑8, and TNF‑alpha levels in early diagnosis and management of neonatal sepsis. Mediators Inflamm 2007: 3197, 2007.

8. Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Thierer F, Hackstein H, Franjkovic I, Colaris T, Martens F, Menges T, König IR, Hossain H, Little S, Tchatalbachev S, Wacker C, Prkno A, Brunkhorst FM and Schlattmann P: Procalcitonin and the role of biomarkers in the diagnosis and management of sepsis. Diagn Microbiol Infect Dis 73: 221‑227, 2012.

9. Wacker C, Prkno A, Brunkhorst FM and Schlattmann P: Procalcitonin as a diagnostic marker for sepsis: A systematic review and meta-analysis. Lancet Infect Dis 13: 426‑435, 2013.

10. Wu X, Yang J, Yu L and Long D: Plasma miRNA‑223 correlates with risk, inflammatory markers as well as prognosis in sepsis patients. Medicine (Baltimore) 97: e11352, 2018.

11. Bao YYX and Chen Z: The early diagnostic value of microRNA‑223 for patients with complication of sepsis after ureteroscopic lithotripsy. Chin J Integr Tradit West Med Intensive Crit Care 24: 465‑468, 2017 (In Chinese).

12. Tili E, Michaille JJ, Cimino A, Costinean S, Dumitrud CD, Adair B, Fabbri M, Alder H, Liu CG, Calin GA and Croce CM: Modulation of miR‑155 and miR‑125b levels following lipopolysaccharide/TNF‑alpha stimulation and their possible roles in regulating the response to endotoxin shock. J Immunol 179: 5082‑5089, 2007.

13. Menges T, König IR, Hossain H, Little S, Tkhatlabachev S, Thierer F, Hackstein H, Franjkovic I, Colaris T, Martens F, et al.: Sepsis syndrome and death in trauma patients are associated with variation in the gene encoding tumor necrosis factor. Crit Care Med 36: 1456‑1462, e1‑e6, 2008.

14. Langfelder P and Horvath S: WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics 9: 724, 2008.

15. Huang W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4: 44‑57, 2009.