Urinary microRNAs in sepsis function as a novel prognostic marker

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Abstract. Renal dysfunction is a common complication of sepsis. Early diagnosis and prompt treatment of sepsis with renal insufficiency are crucial for improving patient outcomes. Diagnostic markers can help identify patients at risk for sepsis and AKI, allowing for early intervention and potentially preventing the development of severe complications. The aim of the present study was to investigate the expression difference of urinary microRNAs (miRNAs/miRs) in elderly patients with sepsis and secondary renal insufficiency, and to evaluate their diagnostic value in these patients. In the present study, RNA was extracted from urine samples of elderly sepsis-related acute renal damage patients and the expression profiles of several miRNAs were analyzed. In order to evaluate the expression profile of several miRNAs, urine samples from elderly patients with acute renal damage brought on by sepsis were obtained. RNA extraction and sequencing were then performed on the samples. Furthermore, multiple bioinformatics methods were used to analyze miRNA profiles, including differential expression analysis, and Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis of different miRNA target genes, to further explore miRNAs that are suitable for utilization as biomarkers. A total of four miRNAs, including hsa-miR-31-5p, hsa-miR-151a-3p, hsa-miR-142-5p and hsa-miR-16-5p, were identified as potential biological markers and were further confirmed in sepsis using reverse transcription-quantitative PCR. The results of the present study demonstrated that the four urinary miRNAs were differentially expressed and may serve as specific markers for prediction of secondary acute kidney injury in elderly patients with sepsis.

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Introduction

Sepsis can cause poor tissue hypoperfusion and a series of life-threatening organ dysfunctions, which are common causes of death in hospitalized patients. Sepsis remains a significant concern globally, with an estimated 48.9 million cases and 11 million deaths occurring worldwide in 2017 (1). Septic shock refers to sepsis with persisting low blood pressure requiring vasopressors to maintain the mean arterial pressure (2). It has been clinically confirmed that sepsis is caused by bacteria or foci of infection (3-5). Sepsis is the pathological process through which the body responds to infectious factors (6).

Septic shock belongs to a subset of sepsis in which underlying circulatory, cellular, and metabolic abnormalities are associated with a greater risk of death than sepsis alone (7,8). The underlying pathogenesis of sepsis is still not clear, and it involves complex systemic inflammatory network effects, gene polymorphisms, immune dysfunction, abnormal blood coagulation, tissue damage and abnormal host responses to different infectious pathogenic microorganisms and their toxins (9-11). Sepsis is closely related to the pathophysiological changes of multiple systems and organs in the body, and the pathogenesis of sepsis still needs to be further clarified (12-14). Sepsis is a systemic inflammatory response syndrome caused by infection and is a dangerous condition. Elderly individuals are prone to shock and multiple organ failure, especially acute kidney injury (AKI), due to their weakened immunity (15). Early evaluation and timely treatment of sepsis are particularly important; however, since the clinical manifestations of sepsis are more diverse and nonspecific compared with fever and tachycardia, especially in elderly patients with sepsis, the common signs of sepsis in the elderly are changes in mental status (delirium, lethargy or coma), gastrointestinal dysfunction and shortness of breath (16,17). Fever and tachycardia are

relatively easy to be detected by doctors. Sepsis in the elderly is likely to be absent from fever and tachycardia, so sepsis symptoms in the elderly are atypical and more difficult to detect early. There are several biological indicators related to sepsis, such as C-reactive protein, procalcitonin, soluble triggering receptor expressed on myeloid cells 1 (TREM-1) and various inflammatory factors, including IL-6, IL-8, TNF-α and caspase-11; however, their specificity and sensitivity are not ideal (18,19). Therefore, novel biomarkers that can be used to diagnose sepsis and assess prognosis, as well as potential therapeutic targets, are required (20-22).

AKI is a clinical syndrome characterized by a rapid decline in renal function. Sepsis is one of the common causes of AKI in hospitalized and intensive care unit (ICU) patients (23). Sepsis-associated AKI increases the risk of developing chronic comorbidities and is associated with high mortality (24,25). A prospective observational study of 1,753 patients at 54 hospitals in 23 countries found that septic AKI had a higher in-hospital case-fatality rate compared with non-septic AKI (70.2 vs. 51.8%; P<0.001). After adjustment for covariates, septic AKI remained associated with higher risk of mortality (1.48; 95% Confidence Interval (CI) 1.17 to1.89; P=0.001) (26). Therefore, it is of great significance to find novel biomarkers for the early, reliable and noninvasive diagnosis of sepsis-associated AKI.

MicroRNAs (miRNAs/miRs) are a class of noncoding single-stranded RNA molecules with a length of ~22 nucleotides encoded by endogenous genes (27-30). They are involved in post-transcriptional gene expression regulation in animals and plants. miRNAs are involved in a series of important life processes, including early development (31), cell proliferation, apoptosis, cell death (32), fat metabolism (33) and cell differentiation (34). In 2008, it was reported for the first time that circulating miRNAs have the potential to become a novel marker of solid tumors (35). Subsequently, the noninvasive acquisition of circulating miRNAs through plasma or serum attracted the attention of researchers. Studies have reported that miRNAs can exist in a variety of body fluids, such as blood, urine, saliva, sweat, tears, cerebrospinal fluid, semen and milk (36-40). During organ damage, miRNAs are usually released into biological fluids and are stably expressed (41). Therefore, miRNAs in blood or urine can be used as noninvasive biomarkers to detect renal disease and toxicity, and the detection method is simple, economical and efficient (42). Thus, miRNA provides a novel platform and ideas for the diagnosis and treatment of diseases and may become a useful tool in the field of precision medicine. Studies have demonstrated that a variety of miRNAs are involved in the inflammatory process of sepsis, and they serve an important role by targeting the toll-like receptor/NF-κB signaling pathway (43,44). Circulating miR-150 was the first miRNA reported as a biomarker for sepsis (45). miR-146a, miR-143 (46,47), miR-25 (48), miR-15a/16 (49,50), miR-1333a, miR-297 and miR-574-5p have been indicated to be useful as markers for the diagnosis of sepsis (51-54). Currently, sepsis is a significant cause of death in the ICU (55-57), and effective prevention and diagnostic techniques are still lacking.

However, there are few studies on the relationship between miRNAs and sepsis-associated AKI in elderly individuals. In the present study, the differential expression of miRNAs in the urine of elderly patients with sepsis was screened to explore the value of miRNAs in the noninvasive diagnosis of elderly patients with sepsis-associated AKI.

Materials and methods

Study population. Elderly patients (>65 years old) diagnosed with sepsis were enrolled as the research subjects. For enrollment, patients had to meet the international diagnostic criteria for sepsis. The diagnosis of AKI was based on the 2012 Kidney Disease Improving Global Outcomes diagnostic criteria (58). These criteria involve a sharp decline in renal function within 48 h, manifested by an increase in serum creatinine >0.3 mg/dl (26.5 μmol/l) or an increase >50% (According to KDIGO, AKI is defined as an increase in serum creatinine levels by at least 0.3 mg/dl within 48 h with 1.5-fold being the baseline), patient age >65 years, expected survival time >3 days, and pathogen culture or laboratory test results showing gram-negative bacteria. The diagnostic criteria for sepsis were based on the Third International Consensus on the Management of Sepsis and Septic Shock (Sepsis-3) in 2016, which entails a joint diagnosis by >2 attending physicians. The exclusion criteria were as follows: i) Patients with tumor, acute stroke, rheumatic immune system disease and mental illness; ii) patients with viral myocarditis; iii) patients with severe hepatitis and cirrhosis; iv) patients who received anti-infective treatment before enrollment; v) patients with end-stage renal diseases; vi) patients who died or were discharged within 48 h after admission; and vii) patients who do not have complete clinical records or do not cooperate with urine sample collection.

The control group included healthy elderly individuals [elderly people with no previous history of chronic disease, age (75.29±5.46), female (58.82%)] who underwent a physical examination during the same period. All subjects were recruited between August 2020 and December 2021. The current study was approved by the Ethics Committee of Huadong Hospital Affiliated to Fudan University (Shanghai, China). All patients or their family members (some older people lose the ability to write) signed informed consent forms before enrollment.

Collection of clinical samples. Urine samples were collected within 24 h after the onset of sepsis in elderly patients admitted to Huadong Hospital Affiliated to Fudan University (Shanghai, China). Urine samples were collected in the morning of the physical examination day in healthy subjects. All urine samples were centrifuged at 845 x g for 10 min at 4˚C, and the supernatant was aliquoted into 1.8-ml Eppendorf tubes and frozen within 4 h of collection at -80˚C.

miRNA-sequencing. Total RNA was extracted using the mirVana™ miRNA Isolation kit (cat. no. AM1561; Thermo Fisher Scientific, Inc.) and the samples were extracted for total RNA according to the standard procedure provided by the manufacturer, and the extracted total RNA was electrophoresed by an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) for quality control. The samples were then prepared for use by Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). The purified total RNA was subjected to 3' end-joining, 5' end-joining, reverse transcription, amplification, cDNA library size selection and purification according to the experimental instructions to complete the library construction of

the sequenced samples. Total RNA was separated using 17% denaturing polyacrylamide gels and small RNAs between 10 and 60 nucleotides (nt) were collected. Then, 5'- and 3'-RNA adaptors were ligated to the small RNAs, followed by reverse transcription to produce cDNAs. These cDNAs were subsequently amplified by PCR and subjected to Solexa/Illumina sequencing by Shanghai Biotechnology Corporation. The libraries were created using the Qubit 2.0 Fluorometer (; Thermo Fisher Scientific, Inc.) for concentration and the Agilent 2100 for library size. Cluster generation and first-way sequencing primer hybridization were performed on the Illumina HiSeq sequencer's cBot (Illumina, Inc.) according to the appropriate procedure shown in the cBot User Guide. Sequencing reagents are prepared according to the Illumina User Guide and flow cells with clusters are loaded onto the machine. Single-ended sequencing was performed using the single-read program. The sequencing process was controlled by Illumina's data collection software (Illumina, Inc.) and real-time data analysis was performed. FastX software (https://anaconda.org/biobuilds/fastx-toolkit,fastx-toolkit 0.0.14) was used to preprocess the original reads for sequencing, remove linker sequences and low-quality sequences (including ambiguous base N sequences with a base quality <10 nt and length <18 nt), and provide (statistical analysis based on the processed results table and length distribution diagram. The sequences obtained through the Sanger miRBase database (https://www.mirbase.org; such as those of known ribosomal RNA, transfer RNA and repeat regions), RefSeq database (https://www.ncbi.nlm.nih.gov/refseq/) and other noncoding RNA databases, including the non-coding RNA, PIWI-interacting RNA (https://www.smallrnagroup.uni-mainz.de/piRNAclusterDB) and Rfam databases (https://rfam.xfam.org/), were compared, and the known miRNAs were annotated. The sequence obtained by sequencing was compared with the genome database corresponding to the species, the annotated reads were classified and counted, and the known miRNAs and various other types of small RNA molecules were identified and counted. The DEGseq R language package combined with Perl script was used to group samples according to the current requirements (such as the control and experimental groups) for comparative analysis of miRNA expression. In the differential analysis, the transcripts per million (TPM) formula (single miRNA reads x 10^6/total reads) was used to present the data.

RNA isolation and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted using TransZol Up reagent (Beijing Transgen Biotech Co., Ltd.). Briefly, the process was as follows: A total of 1 ml TransZol UP reagent was added into 500 μl of urine sample, followed by mixing with 200 μl chloroform and centrifugation at 4˚C for 15 min at 10,000 x g. The aqueous phase containing the RNA was transferred to a new Eppendorf tube and the same volume (~500 μl) isopropyl alcohol was added. A total of 1 ml pre-cooled (4˚C) 75% ethanol was added, and centrifugation at 4˚C for 5 min at 7,500 x g. The RNA precipitate was air-dried, followed by dissolution in RNA solution buffer. cDNA synthesis was conducted with TransScript miRNA First-Strand cDNA Synthesis SuperMix (TransGen Biotech Co., Ltd.). The RT kit was used according to the manufacturer's protocol. The reactions were performed in a PCR instrument and the reaction program was set to 37˚C for 1 h and 85˚C for 5 sec. The Hieff qPCR SYBR Green Master Mix kit (Shanghai Yeasen Biotechnology Co., Ltd.) was used to perform RT-qPCR assays. The qPCR cycling conditions were 95˚C for 10 sec, 55˚C for 30 sec and 72˚C for 30 sec for 40 cycles. Relative quantification of hsa-miR-31-5p, hsa-miR-151a-3p, hsa-miR-142-5p and hsa-miR-16-5p was performed by normalization to U6 small nuclear (sn)RNA expression levels. The $2^{-\Delta\Delta Cq}$ method was used to analyze miRNA levels (59). The primer sequences used are presented in Table SI.

Bioinformatics analysis. Bioinformatics analysis was performed to preprocess sequencing data and analyze the results. Bioinformatics analyses included miRNA expression quantitative analysis, expression correlation analysis, miRNA differential expression analysis, differential miRNA target gene prediction, and Gene Ontology (GO; http://www.geneontology.org) and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.kegg.jp/kegg/pathway.html) enrichment analysis of different miRNA target genes. The results of the GO enrichment analysis were displayed in plots, where Rich Factor=(the number of miRNA target genes in a GO term/the number of all target genes that can correspond to the GO database)/(the number of genes contained in a GO term/the total number of genes that can correspond to the GO database). The greater the Rich Factor is, the greater the degree of enrichment, while the smaller the Q-value is, the more significant the enrichment (60).

Statistical analysis. All the experimental results in this study were verified by three biological repetitions to ensure the accuracy of the experimental results. Data analysis was performed using SPSS 23 software (IBM Corp.). Data are presented as the mean ± SEM. Each experiment, controlling a single variable and setting up two experimental groups (the AKI and non-AKI group) and a control group, had at least three biological repetitions. EdgeR (http://www.R-project.org/) was used to analyze the difference in miRNAs between samples. After obtaining the P-value, multiple hypothesis test correction was performed, and the P-value threshold was determined by controlling the false discovery rate, thereby providing the Q-value. Fold-change was calculated as the differential expression based on the TPM value. The screening conditions for differential genes were as follows: Q-value ≤0.05; fold-change ≥2. One-way ANOVA followed by Dunnett's multiple comparisons test was used to compare the groups. Receiver operating characteristic (ROC) curves were plotted to analyze the predictive value of miR-31-5p, miR-151a-3p, miR-142-5p and miR-16-5p for the prognosis and 28-day mortality of elderly patients with sepsis. The ROC curve analysis and the derived area under the curve (AUC) statistic provide a global and standardized appreciation of the accuracy of a marker or a composite score for predicting an event (61). ROC curves were generated by plotting sensitivity against 1-specificity. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient demographic and baseline characteristics. Patients were diagnosed with sepsis, and the main infection sites were the lung, urinary system and gastrointestinal tract.

According to the general clinical data, the 74 study subjects included 17 healthy elderly patients, 29 septic patients with AKI and 28 septic patients without AKI. The mean age was 81 years, with a range of 65-97 years. After 28 days of follow-up in the observation group, 18 patients of the 57 patients with sepsis succumbed, accounting for 31.58% (Table SII).

Differential expression of miRNAs in the sepsis AKI vs. sepsis non-AKI group. Details of the patients are provided in Table I. Heatmap and cluster analysis demonstrated that there were differentially expressed miRNAs between the sepsis AKI and non-AKI groups. The sepsis AKI and non-AKI groups were compared with the normal group. Among the differentially expressed miRNAs in the sepsis AKI group, six miRNAs were upregulated compared with the normal group (Fig. 1A). Among the differentially expressed miRNAs in the sepsis non-AKI group, 28 miRNAs were upregulated compared with the normal group (Fig. 1C). The volcano plots show the differentially expressed miRNAs under the two different conditions (AKI vs. non-AKI groups) (Fig. 1B and D).

GO function and KEGG signaling pathway analysis of differentially expressed miRNA target genes. Firstly, the number of target genes corresponding to the three GO elements, biological process, cellular component and molecular function, was counted. Only the top 30 GO entries are shown in Fig. 2A. Using the same principle as for GO enrichment analysis, KEGG pathway enrichment analysis was also performed for target genes of differentially expressed miRNAs, and the results are shown in Fig. 2B.

miRNAs with differentially upregulated expression in patients with sepsis in the AKI and non-AKI groups. There were six upregulated miRNAs in the AKI group (Table II) and 27 in the non-AKI group (Table SIII) compared with the control. Analysis of the data revealed that several miRNAs in the sepsis AKI and non-AKI groups were differentially expressed and upregulated compared with the control. A high expression trend was found in the sepsis AKI and non-AKI groups.

Differentially expressed miRNAs verified by RT-qPCR. To verify the expression levels of these four miRNAs in sepsis, 17 samples from healthy controls, 29 samples from patients with sepsis and AKI and 28 samples from patients with sepsis without AKI were collected. Details of the patients are provided in Table SII. Compared with those in the control group, the expression levels of miR-31-5p, miR-151a-3p, miR-142-5p and miR-16-5p were significantly increased in the sepsis AKI and sepsis non-AKI groups (Fig. 3A-D).

Clinical predictive value of miRNAs for AKI occurrence in elderly patients with sepsis. ROC curve analysis of miR-31-5p, miR-151a-3p, miR-142-5p and miR-16-5p was performed to assess their predictive value in the diagnosis of AKI in elderly patients with sepsis. Fig. 4 shows that the AUC for miR-142-5p and miR-16-5p expression was 0.746 and 0.820, respectively, indicating a good predictive value of miR-142-5p and miR-16-5p for patients with sepsis-induced AKI. The AUC for miR-31-5p and miR-151-3p expression was 0.416

Table I. Clinical data of the control, sepsis AKI and sepsis non-AKI groups.

	Control (n=3)			Non-AKI (n=5)					AKI (n=6)					
Characteristic	1	2	3	1	2	3	4	5	1	2	3	4	5	6
Sex	Female	Male	Female	Female	Male	Male	Female	Male	Female	Male	Female	Male	Male	Female
Age	82	81	76	80	77	85	86	89	97	87	77	67	88	83
Lung	no	no	no	yes	no	no	yes	no	yes	yes	yes	no	no	yes
Urine	no	no	no	yes	yes	yes	no	yes	no	no	yes	yes	no	yes

AKI, acute kidney injury; CKD, chronic kidney disease.

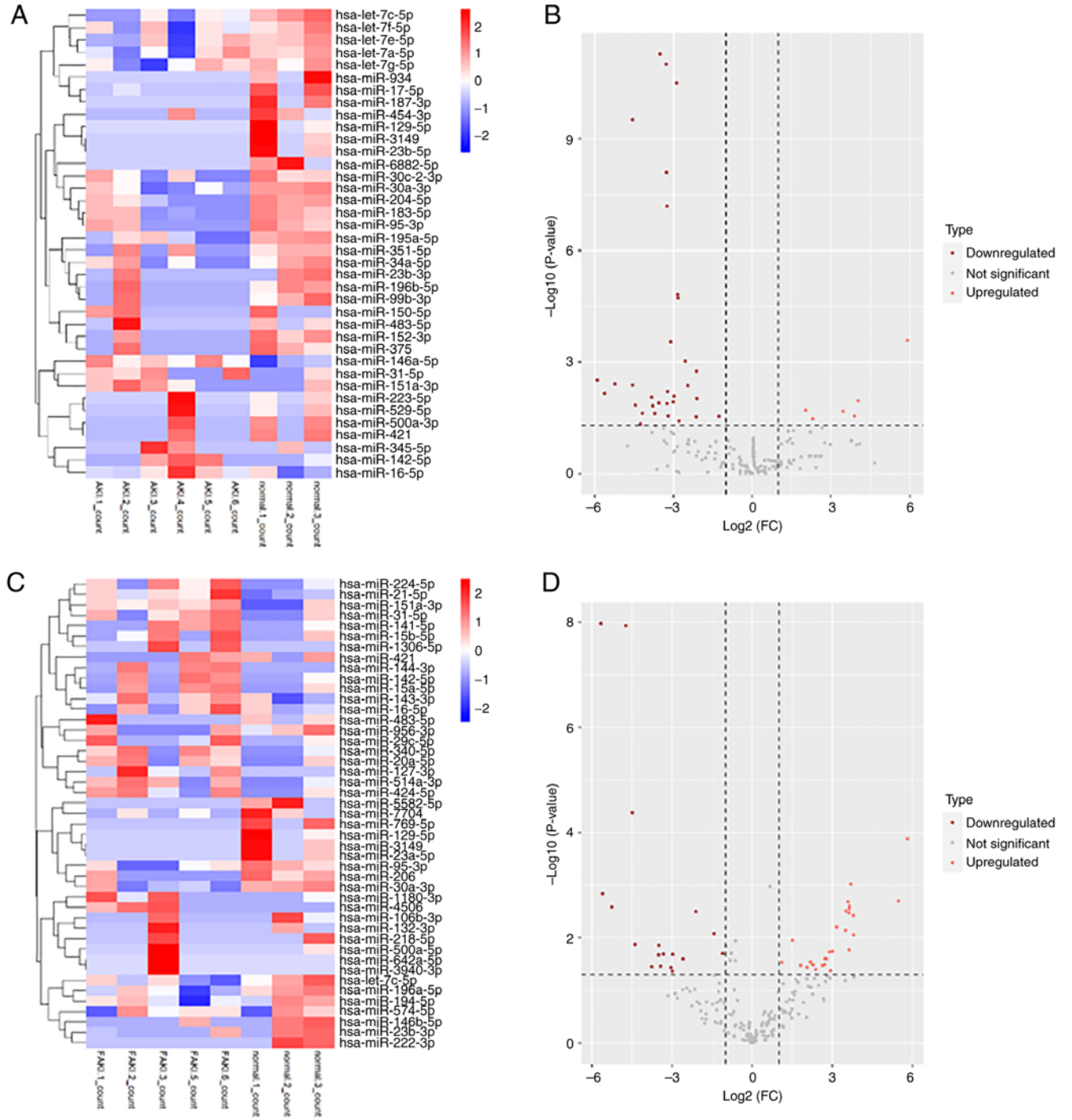

Figure 1. Heat map and volcano plot of differentially expressed miRNAs. Differentially expressed miRNAs in (A) the sepsis AKI group and (B) the sepsis non-AKI group. Rows represent miRNAs, and columns represent specimens. Red represents a relative increase in expression, and blue represents a relative decrease in expression. Volcano plots of (C) the AKI group and (D) the non-AKI group. AKI, acute kidney injury; miRNA/miR, microRNA; FC, fold change; FAKI,non-acute kidney injury.

(P=0.274) and 0.450 (P=0.513) respectively, with no statistical significance.

Prognostic value of miRNAs for the 28-day survival of 57 septic patients. ROC curves were generated to evaluate the predictive value of miR-31-5p, miR-151a-3p, miR-142-5p and miR-16-5p for the 28-day mortality in patients with sepsis (Fig. 5). The AUC for each miRNA was 0.668, 0.747,0.714 and 0.838, respectively. These results indicated a good predictive value of miR-31-5p, miR-151a-3p, miR-142-5p and miR-16-5p in the prognosis of 57 patients with sepsis.

Discussion

Sepsis is an important clinical area in the emergency and critical care medicine field. Clinical management of sepsis remains a major challenge (62). Sepsis can lead to

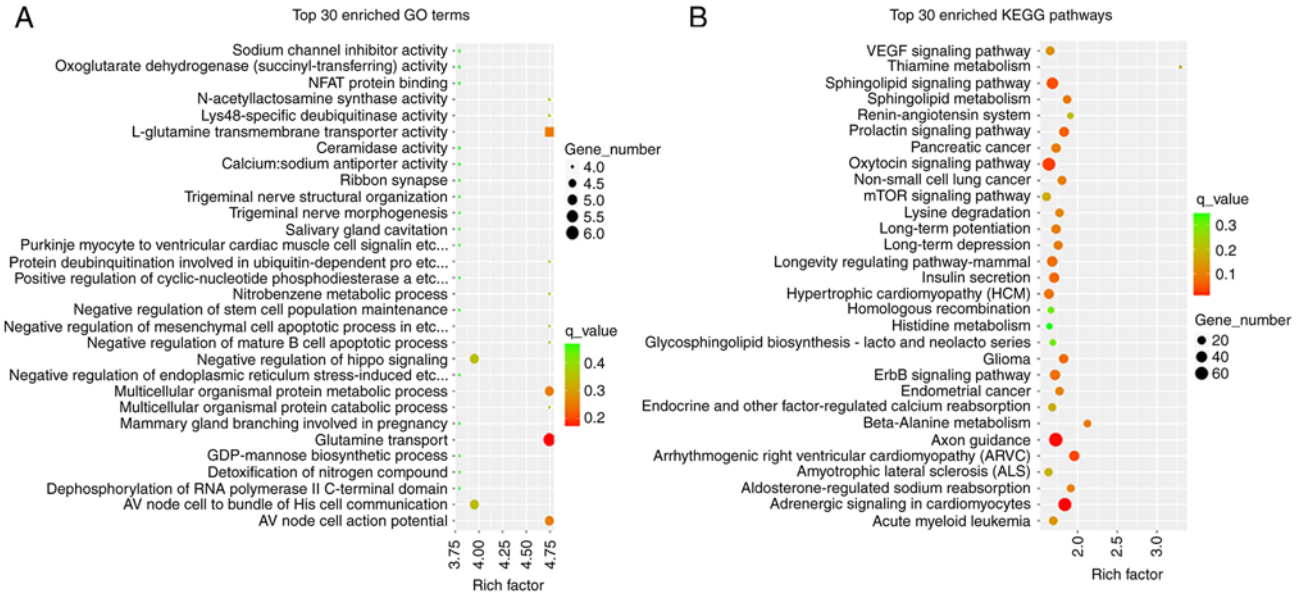

Figure 2. Plots of GO and KEGG enrichment analysis. (A) GO enrichment analysis and (B) KEGG pathway enrichment analysis of differentially expressed microRNA target genes. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

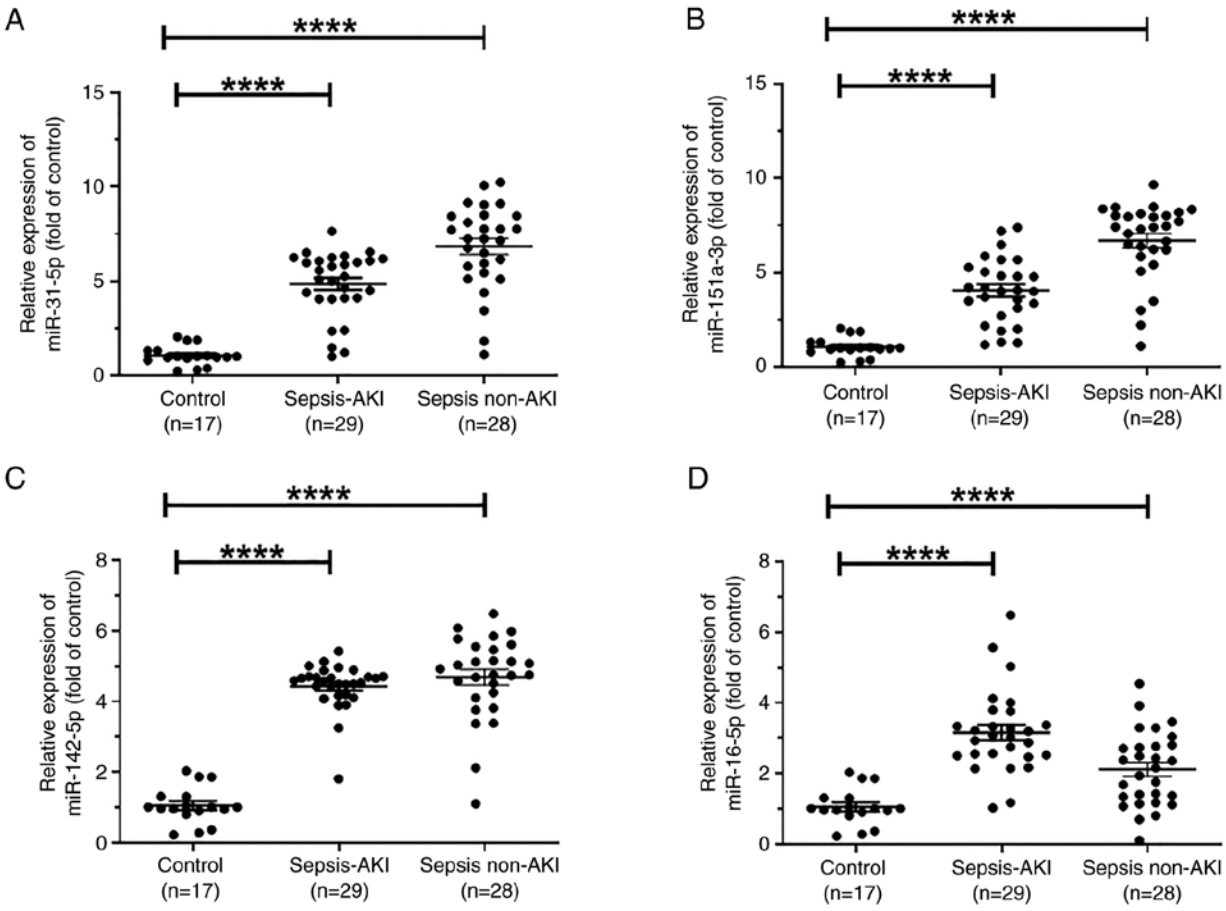

Figure 3. Validation of miRNA expression levels by reverse transcription-quantitative PCR. Expression levels of (A) miR-31-5p, (B) miR-151a-3p, (C) miR-142-5p and (D) miR-16-5p in the control, sepsis-AKI and sepsis non-AKI groups. The one-way ANOVA was used to compare the two groups with ****P<0.0001. AKI, acute kidney injury; miRNA/miR, microRNA.

life-threatening multiple organ dysfunction and has a high fatality rate; therefore, it is important to reduce the incidence and fatality rate of sepsis (63). With the in-depth study of its pathogenesis, biomarkers for the prediction of the prognosis of sepsis have emerged (64). Circulating miRNA can be used as a novel candidate biomarker for the clinical diagnosis and

Table II. Upregulated miRNAs in the sepsis acute kidney injury group compared with the control group (partial results).

Name	LogFC	Average expression	t-value	P-value	Adjusted P-value	B
hsa-miR-345-5p	5.91748	5.52460616	6.261657	0.000262745	0.007849	-0.70176
hsa-miR-31-5p	4.029956	7.20973673	3.300482	0.011146469	0.139838	-2.57026
hsa-miR-151a-3p	3.458328	7.02248642	2.87484	0.021092684	0.200744	-3.19779
hsa-miR-142-5p	3.898493	7.01479061	2.682209	0.028311824	0.235373	-3.45029
hsa-miR-146a-5p	2.308655	10.8501514	2.562092	0.034059476	0.261123	-3.47134
hsa-miR-16-5p	2.04111	16.2923015	2.914069	0.019872937	0.19589	-3.71548

miRNA/miR, microRNA; FC, fold change.

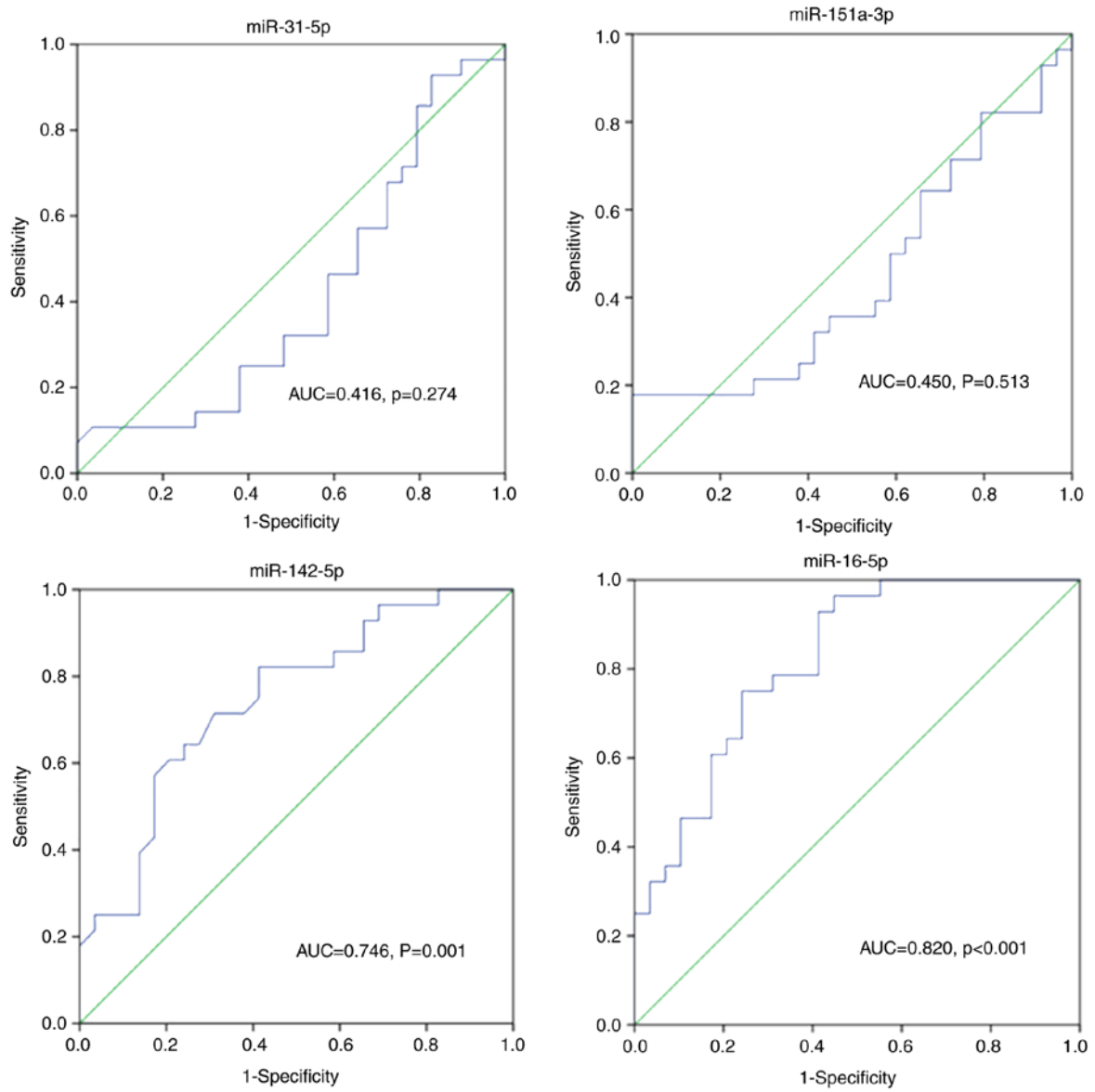

Figure 4. Receiver operating characteristic curve analysis of miR-31-5p, miR-151a-3p, miR-142-5p and miR-16-5p for the prognosis of patients with sepsis. AUC, area under the curve; miR, microRNA.

treatment of sepsis (61). miRNAs have previously been considered as biomarkers in different diseases, such as lung cancer and sepsis. However, several issues should be investigated before their use in clinical practice (65-67). miRNA detection is convenient and fast and its clinical diagnostic and prognostic value for patients with sepsis and related complications has

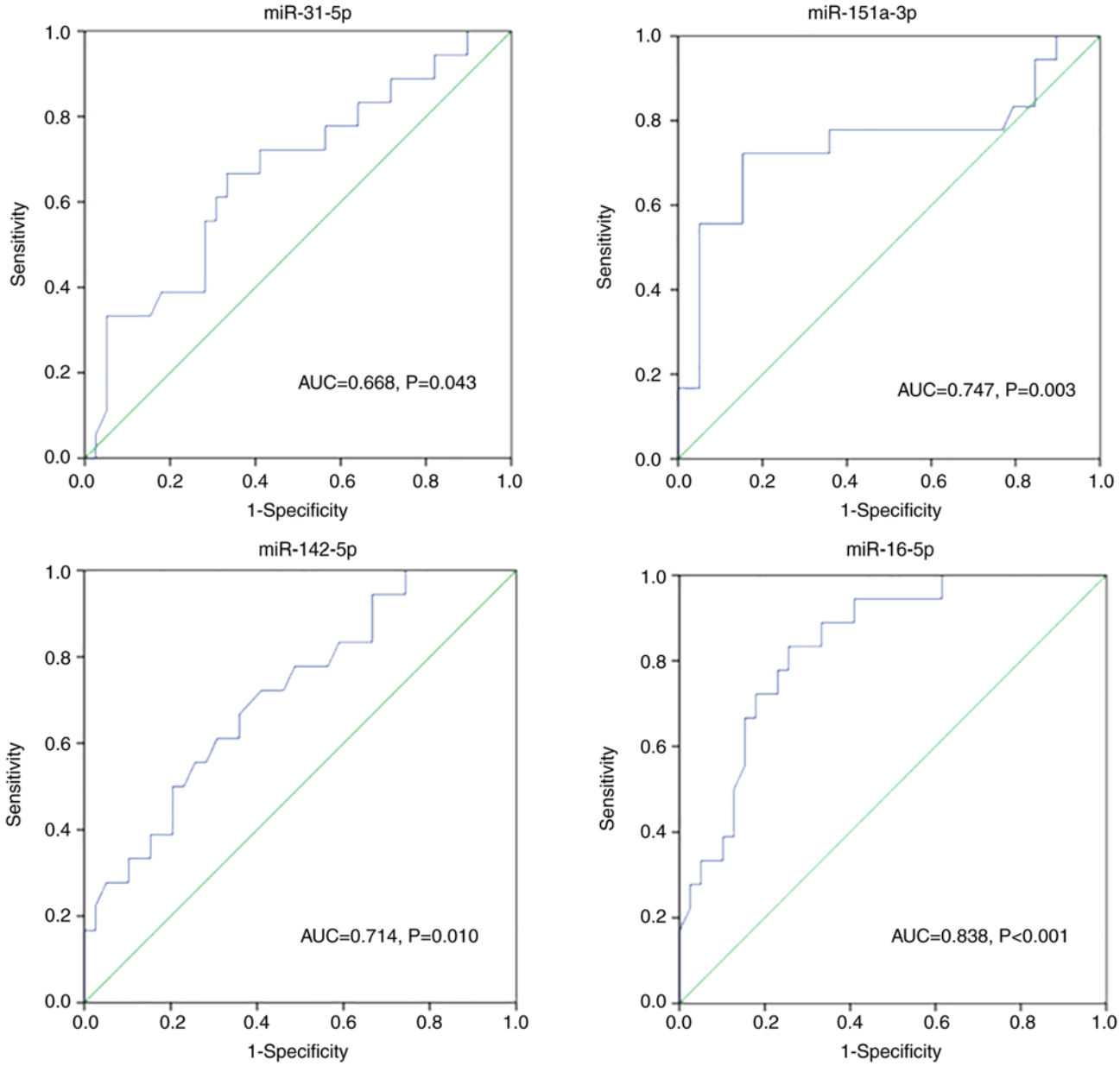

Figure 5. Receiver operating characteristic curve analysis of miR-31-5p, miR-151a-3p, miR-142-5p and miR-16-5p in predicting 28-day mortality in elderly patients with sepsis. The sensitivity of miR-31-5p, miR-151a-3p, miR-142-5p and miR-16-5p was 66.7, 72.2, 72.2 and 83.3%, and the specificity was 66.7, 84.6, 59.0 and 74.4%, respectively. AUC, area under the curve; miR, microRNA.

gained increasing attention (68,69). The complex pathogenesis and diagnostic value of miRNAs in sepsis need to be further explored, as the identification of specific miRNAs will help to further clarify the pathogenesis of the disease and provide a way to screen novel clinical diagnostic indicators or explore molecular targeted therapies (70).

Previous studies show that there are distinct miRNA regulation models in the different cohorts of patients with sepsis. This is due to a lack of standardization of sample collection, data normalization and analysis methods. There is still no optimal normalization strategy for miRNA analysis from serum, urine or other samples. Usually, miR-16 or U6 snRNA is used as an internal gene for normalization. However, U6 snRNA is differentially regulated between healthy subjects and septic patients (71). Aomatsu *et al* (72) demonstrated that the upregulation of miRNA-5100 may inhibit the development of AKI at least partially by regulating multiple apoptotic pathways, and miRNA-5100 can be used as a diagnostic biomarker of AKI. Zhao *et al* (73) found that inhibition of miR-16-5p could reduce the symptoms of AKI in mice with ischemia-reperfusion-induced AKI. In addition, it has been reported that serum miR-16-5p (74) and serum miR-142-5p (75) are downregulated in septic patients with AKI.

The present study screened four miRNAs in urine, namelymiR-31-5p, miR-151a-3p, miR-142-5p and miR-16-5p, as potential biological markers in patients with sepsis-induced AKI. These four miRNAs were confirmed by RT-qPCR to be specific markers for predicting secondary AKI in elderly patients with sepsis. Therefore, the present study provides potential diagnostic biomarkers for the early diagnosis, disease staging and prognosis of elderly patients with sepsis.

Future studies are required to further examine and verify the accuracy and specificity of the four miRNAs as diagnostic markers in sepsis, laying a foundation for clinical application. Originally diagnosed and treated according to conventional methods, our study offers the possibility of early diagnosis and prognostic judgement. Based on the present study results and ROC curve analysis, miR-16-5p showed the best diagnostic results among the four genes examined. Further studies will verify the regulatory mechanism of miR-16-5p, its relationship with the major gene of pyroptosis and gasdermin D and its regulatory mechanism in AKI.As circulating miRNAs have several advantages, such as the easy and noninvasive sample collection from patients, it is possible for them to have a wide use in the clinic (76). The four miRNAs examined in the present study can facilitate the rapid initiation of directed treatment in sepsis and infection.

In summary, the present study indicated that specific miRNAs, and especially miR-16-5p, represent novel candidates for the clinical management of patients with sepsis.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Sequence Read Archive repository (accession no. PRJNA906749; https://www.ncbi.nlm.nih.gov/sra/PRJNA906749).

Authors' contributions

ZM and ZB conceived the experiments. RH and WL developed the methodology. RH, WL, HT, YZ, HZ and WP performed the experiments. WP, XW and LX organized and analyzed the data. RH and ZM wrote the manuscript. WP and ZM confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The current study was approved by the Ethics Committee of Huadong Hospital Affiliated to Fudan University (approval no. 2020K039; Shanghai, China).

Patient consent for publication

All patients or their family members signed informed consent forms before enrollment.

Competing interests

The authors declare that they have no competing interests.

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