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The predictive value of microRNA-21 for sepsis risk and its correlation with disease severity, systemic inflammation, and 28-day mortality in sepsis patients

Lei Na[1] | Huajie Ding[2] | Enhong Xing[3] | Yan Zhang[4] | Jun Gao[1] | Bin Liu[5] | Jian Yu[1] 🆔 | Yanjun Zhao[1] 🆔

[1]Emergency Department, Affiliated Hospital of Chengde Medical College, Chengde, China

[2]Ultrasonography Department, Affiliated Hospital of Chengde Medical College, Chengde, China

[3]Clinical Laboratory, Southern District of Affiliated Hospital of Chengde Medical College, Chengde, China

[4]Science and Education Department, Chengde Maternal and Child Health-Care Hospital, Chengde, China

[5]Radiology Department, Affiliated Hospital of Chengde Medical College, Chengde, China

Correspondence
Jian Yu and Yanjun Zhao, Emergency Department, Affiliated Hospital of Chengde Medical College, No. 36 Nanyingzi Street, Chengde 067000, China.
Emails: 2417685225@qq.com and nobody50@163.com

Abstract

Background: This study aimed to investigate the value of microRNA (miR)-21 for predicting sepsis risk and its correlation with inflammation, disease severity as well as 28-day mortality in sepsis patients.

Methods: Totally, 219 sepsis patients and 219 healthy controls (HCs) were recruited. Plasma samples were obtained from sepsis patients within 24 hours after admission and from HCs at the enrollment to detect miR-21 expressions by real-time quantitative polymerase chain reaction. Besides, the clinical characteristics of sepsis patients were recorded and the 28-day mortality of sepsis patients was evaluated.

Results: MiR-21 expression was decreased in sepsis patients compared with HCs, and further receiver operating characteristic (ROC) curve analysis revealed that miR-21 was of a good value in predicting sepsis risk (area under the curve [AUC]: 0.801, 95% CI: 0.758-0.844). Besides, miR-21 expression was negatively associated with acute pathologic and chronic health evaluation II (APACHE II) and sequential organ failure assessment (SOFA) score in sepsis patients. Furthermore, miR-21 expression was negatively correlated with serum creatinine, C-reactive protein, tumor necrosis factor-α, interleukin (IL)-1β, IL-6, and IL-17, while positively correlated with albumin in sepsis patients. However, there was no correlation of miR-21 expression with white blood cell, smoke, or comorbidities in sepsis patients. Additionally, ROC curve analysis displayed that miR-21 exhibited a poor predictive value for 28-day mortality risk in sepsis patients (AUC: 0.588, 95% CI: 0.505-0.672).

Conclusion: MiR-21 might serve as a potential biomarker for the development and progression of sepsis, while not for prognosis prediction in sepsis patients.

KEYWORDS

disease severity, inflammation, MiR-21, prognosis, sepsis

1 | INTRODUCTION

Sepsis, a systemic inflammatory response syndrome, is induced by bacterial, viral, or fungal infections, which results in dysregulated systemic inflammatory response, multiple organ dysfunction or failure, and even death.[1,2] It is estimated that 19.4 million individuals develop sepsis annually worldwide, and the mortality rate remains as high as 30% for sepsis patients.[3,4] Blood culturing is a routine assessment for sepsis in clinical practice. However, this technique is time-consuming and lacks adequate sensitivity, which leads to delay in results, unnecessary uses of antimicrobial agents, and the emergency of resistant pathogens in some situations.[5] Therefore, it is essential to explore novel, sensitive, and rapidly measurable biomarkers for aiding in sepsis risk prediction and disease monitoring in sepsis patients.

MicroRNAs (MiRNAs) are a class of small non-coding endogenous molecules RNA (approximately 19-25 nucleotides in size), which regulate gene expression at post-transcriptional level by influencing the stability and translation of mRNA.[6] MicroRNA (miR)-21, a commonly studied miRNA, is shown to regulate inflammation and several organ injuries in the pathogenesis of sepsis.[7-9] For instance, miR-21 suppresses lipopolysaccharide (LPS)-induced inflammation through the toll-like receptor 4 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway in sepsis.[8] Another study illuminates that upregulation of miR-21 decreases apoptosis and proinflammatory cytokines production in kidneys, hearts, livers, and lungs, implying that miR-21 exerts protective effect on multiple organ failure induced by sepsis.[9] Given the effect of miR-21 on inflammation inhibition and organ protection in sepsis, we hypothesized that miR-21 might serve as a potential biomarker for assessing sepsis risk and prognosis of sepsis. However, limited related study has been reported. Therefore, this study was to investigate the value of miR-21 for predicting sepsis risk and its correlation with inflammation, disease severity as well as 28-day mortality in sepsis patients.

2 | METHODS

2.1 | Participants

This study consecutively recruited 219 sepsis patients between July 2016 and June 2019. All patients included in this study must met the following criteria: (a) diagnosed as sepsis based on the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[10]; (b) age above 18 years old; (c) not complicated with hematological malignancies or solid tumors; (d) seronegative for human immunodeficiency virus (HIV); (e) willing to participate in the study. The exclusion criteria were: (a) received immunosuppressant within 3 months before enrollment; (b) died within 24 hours after admission; (c) history of stem cell transplant; (d) complicated with autoimmune disorders (eg, systemic lupus erythematosus, rheumatoid arthritis); (e) pregnant or lactating woman. In addition, 219 healthy subjects who had no history of malignancies or severe infection, or no obvious abnormalities in biochemical indexes were enrolled as healthy controls (HCs), during the same period. The study was approved by the Ethics Committee of our hospital. All participants or their guardians signed the informed consents.

2.2 | Data and sample collection

After enrollment, all clinical features of sepsis patients were recorded. Demographic characteristics were included age, gender, body mass index (BMI), and smoke status. Chronic comorbidities were included chronic obstructive pulmonary disease (COPD), cardiomyopathy, chronic kidney failure, and cirrhosis. Biochemical indexes included serum creatinine (Scr), albumin, white blood cell (WBC), C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-17. Besides, peripheral blood samples of sepsis patients were collected within 24 hours after admission, and peripheral blood samples of HCs were collected on enrollment. Then, the peripheral blood samples were immediately separated for plasma at the condition of 3000 g for 25 min (4°C). The levels of TNF-α, IL-1β, IL-6, and IL-17 in plasma were measured by enzyme-linked immunosorbent assay (ELISA), and the level of miR-21 in plasma was detected by real-time quantitative polymerase chain reaction (qPCR).

2.3 | Severity assessment

The severity of sepsis was assessed using acute pathologic and chronic health evaluation II (APACHE II) score, which included acute physiology score, age score, and chronic health score. The total score of APACHE II ranges from 0 to 71, and the higher score represented more severe disease condition.[11] The severity of organ dysfunction of sepsis patients was evaluated using sequential organ failure assessment (SOFA) score, which included respiration score, coagulation score, liver score, cardiovascular score, central nervous system score, and renal score. The total SOFA score was calculated by summing up each item scores (range 0-24), and higher score represented more severe organ dysfunction.[12]

2.4 | ELISA

Commercial human ELISA Kits (Thermo Fisher Scientific) were used to measure the level of TNF-α, IL-1β, IL-6, and IL-17 in plasma, and all procedures were performed according to the manufacturer's instruction. In brief, plasma samples were added to the wells of target-specific antibody pre-coated microplate. After incubation, the wells were washed to remove unbound material. The sandwich was formed by the addition of the second antibody, and a tetramethylbenzidine substrate was added to produce measurable signal. After stop solution was added, the optical density was measured at 450 nm wavelengths on microplate reader (BioTek).

2.5 | qPCR

The miR-21 relative expression of sepsis patients and HCs was detected by real-time quantitative polymerase chain reaction (qPCR). Initially, total RNA was extracted from plasma using QIAamp RNA Blood Mini Kit (Qiagen). Subsequently, cDNA was synthesized using iScript™ Reverse Transcription Supermix (Bio-Rad). After that, qPCR was conducted using QuantiNova SYBR Green PCR Kit (Qiagen). And the miR-21 relative expression was calculated by $2^{-\Delta\Delta Ct}$ method using U6 as internal reference.[2,9] During the process of qPCR, triplicate samples of miR-21 were used to ensure the reliability of miR-21 assessment in sepsis. The internal consistency of miR-21 among three duplicates was good, which ensured miR-21 the stability among triplicate samples. Primers applied were shown below: miR-21, forward: 5′ ACACTCCAGCTGGGTAGCTTATCAGACTGA 3′, reverse: 5′ TGTCGTGGAGTCGGCAATTC 3′; U6 forward: 5′ CTCGCTTCGGCAGCACATATACTA 3′, reverse: 5′ ACGAATTTGCGTGTCATCCTTGC 3′.

2.6 | Treatment and follow-up

Appropriate treatment regimens were given to the sepsis patients according to guideline of sepsis.[13] All patients were followed up to 28 days after enrollment, during follow-up, the number of deaths was recorded to calculate 28-day mortality. And based on the survival status in 28-day follow-up, patients were categorized into survivors and deaths.

2.7 | Statistical analysis

Statistical analysis was performed by the use of SPSS 24.0 software (IBM), and figure was made using GraphPad Prism 7.01 software (GraphPad Software). Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). Categorical variables were expressed as count (percentage). Comparison of miR-21 relative expression between two groups was determined by Wilcoxon rank sum test. Correlation between two continuous variables was analyzed by Spearman's rank correlation test. The performances of continuous variables in predicting sepsis risk and 28-day mortality risk were performed using receiver operating characteristic (ROC) curve and the area under the curve (AUC) with 95% confidence interval (CI). P value < .05 was considered significant.

3 | RESULTS

3.1 | Sepsis patients' characteristics

The mean age of sepsis patients was 56.5 ± 10.3 years, and there were 76 (34.7%) females and 143 (65.3%) males. Smoke was found in

TABLE 1 Clinical characteristics of sepsis patients

Items	Sepsis patients (N = 219)
Age (y), mean ± SD	56.5 ± 10.3
Gender, N (%)	
Female	76 (34.7)
Male	143 (65.3)
BMI (kg/m^2), mean ± SD	23.3 ± 5.0
Smoke, N (%)	81 (37.0)
Chronic comorbidities, N (%)	
COPD	32 (14.6)
Cardiomyopathy	73 (33.3)
Chronic kidney failure	23 (10.5)
Cirrhosis	43 (19.6)
APACHE II score, mean ± SD	13.7 ± 6.1
SOFA score, mean ± SD	6.2 ± 2.8
Scr (mg/dL), median (IQR)	1.6 (1.2-2.3)
Albumin (g/L), median (IQR)	26.8 (21.6-35.6)
WBC (×10^9/L), median (IQR)	12.6 (2.9-26.8)
CRP (mg/L), median (IQR)	106.1 (61.4-154.7)
TNF-α (pg/mL), median (IQR)	212.5 (131.4-315.3)
IL-1β (pg/mL), median (IQR)	9.7 (4.3-19.5)
IL-6 (pg/mL), median (IQR)	90.5 (48.3-171.3)
IL-17 (pg/mL), median (IQR)	192.1 (95.0-288.3)

Abbreviations: APACHE II, acute pathologic and chronic health evaluation II; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; IQR, interquartile range; Scr, serum creatinine; SD, standard deviation; SOFA, sequential organ failure assessment; TNF-α, tumor necrosis factor-α; IL, interleukin; WBC, white blood cell.

81 (37.0%) sepsis patients. As for chronic comorbidities, 32 (14.6%), 73 (33.3%), 23 (10.5%), and 43 (19.6%) of sepsis patients had COPD, cardiomyopathy, chronic kidney failure, and cirrhosis, respectively. Furthermore, the mean APACHE II score and mean SOFA score of sepsis patients were 13.7 ± 6.1 and 6.2 ± 2.8, respectively. Other detailed characteristics were listed in Table 1.

3.2 | The predictive value of miR-21 for sepsis risk

The miR-21 relative expression in sepsis patients (0.277 [0.193-0.451]) was lower than that in HCs (0.967 [0.400-1.630]) (P < .001; Figure 1A). Further ROC analysis disclosed that miR-21 could well predict decreased sepsis risk (AUC: 0.801, 95% CI: 0.758-0.844; Figure 1B).
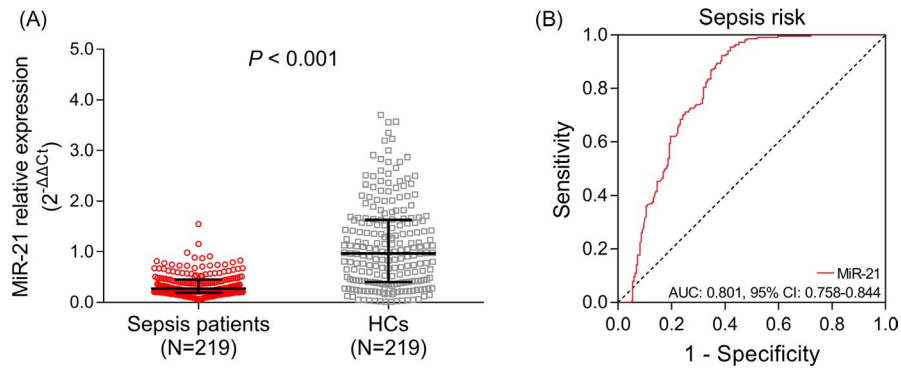
FIGURE 1 The value of miR-21 for predicting sepsis risk. Comparison of miR-21 relative expression between sepsis patients and HCs was analyzed by Wilcoxon rank sum test (A). The performance of miR-21 for predicting sepsis risk was assessed by ROC curve and AUC with 95% CI (B). $P < .05$ was considered significant. AUC, area under the curve; CI, confidence interval; HCs, healthy controls; MiR-21, microRNA-21; ROC, receiver operating characteristic
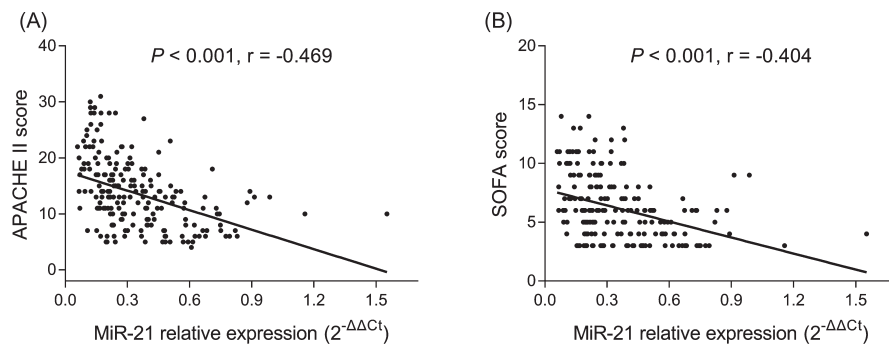

FIGURE 2 Negative association of miR-21 with APACHE II and SOFA score in sepsis patients. The association of miR-21 with APACHE II score (A) and with SOFA score (B) in sepsis patients, which were evaluated by Spearman rank correlation test. $P < .05$ was considered significant. APACHE II, acute pathologic and chronic health evaluation II; MiR-21, microRNA-21; SOFA, sequential organ failure assessment
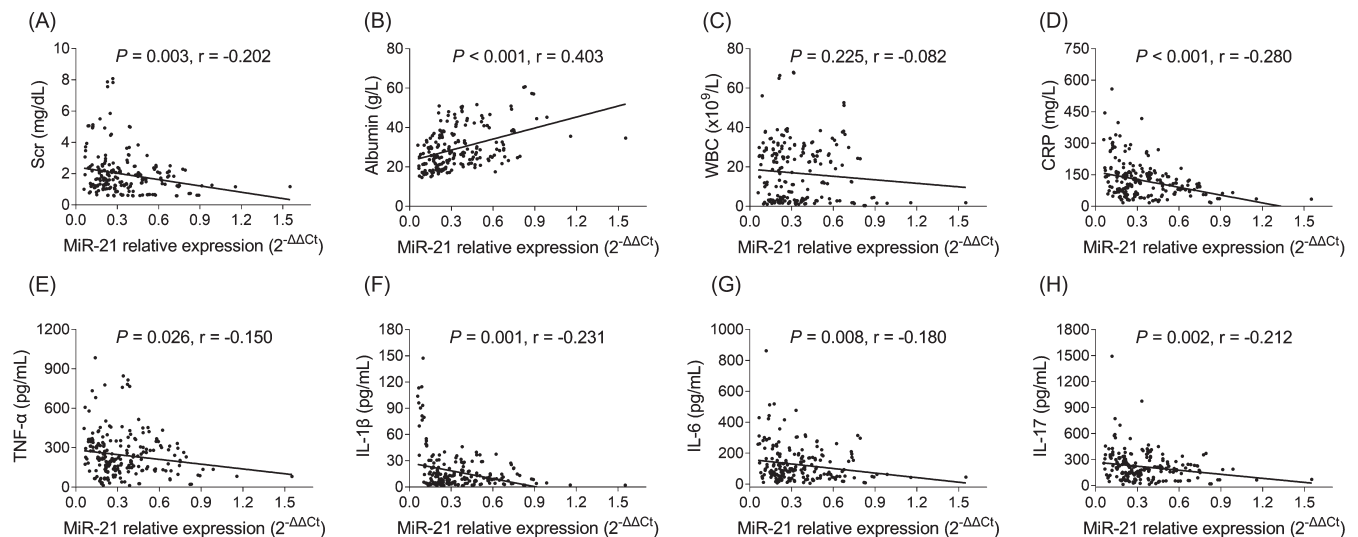

FIGURE 3 Association of miR-21 relative expression with biochemical and inflammatory indexes in sepsis patients. The association of miR-21 relative expression with Scr (A), albumin (B), WBC (C), CRP (D), TNF-α (E), IL-1β (F), IL-6 (G), and IL-17 (H) in sepsis patients, which was examined by Spearman rank correlation test. $P < .05$ was considered significant. CRP, C-reactive protein; IL, interleukin; MiR-21, microRNA-21; Scr, serum creatinine; TNF-α, tumor necrosis factor-α; WBC, white blood cell
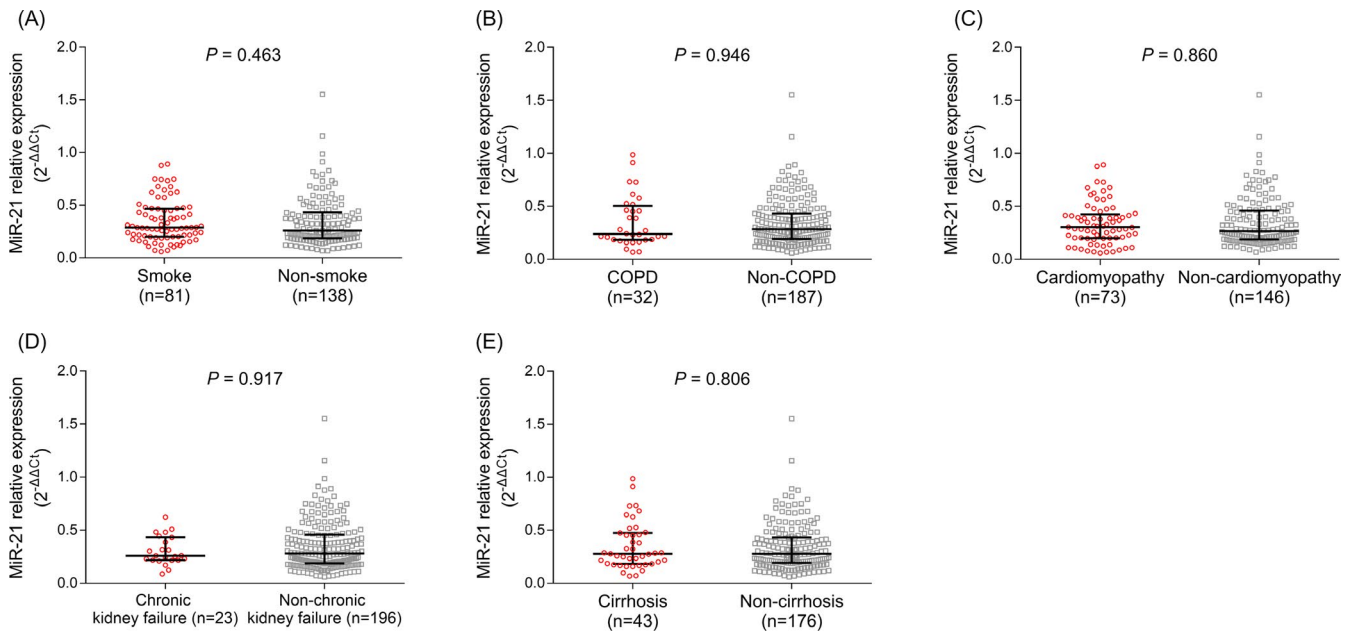
FIGURE 4 Association of miR-21 relative expression with smoke and comorbidities in sepsis patients. The comparison of miR-21 relative expression between smoke vs non-smoke (A), COPD vs non-COPD (B), cardiopathy vs non-cardiopathy (C), chronic kidney failure vs non-chronic kidney failure (D), cirrhosis vs non-cirrhosis (E) sepsis patients, which were assessed by Wilcoxon rank sum test. $P < .05$ was considered significant. COPD, chronic obstructive pulmonary disease; MiR-21, microRNA-21
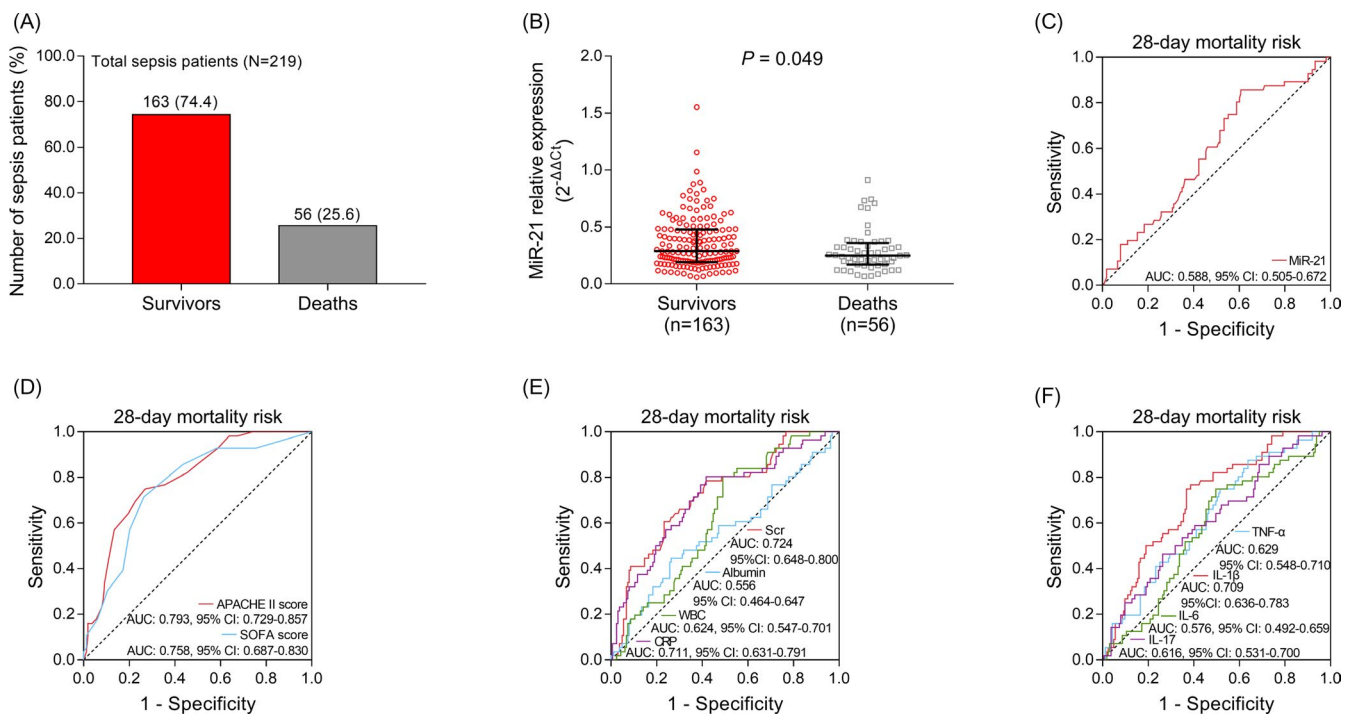

FIGURE 5 The value of miR-21, APACHE II and SOFA score, biochemical indexes, and inflammatory cytokines for predicting 28-day mortality risk in sepsis patients. The number of survivors and deaths in sepsis patients (A), and the miR-21 relative expression of survivors and deaths in sepsis patients (B). Furthermore, the performance of miR-21 (C), APACHE II, SOFA score (D), Scr, albumin, WBC, CRP (E), TNF-α, IL-1β, IL-6, and IL-17 (F) in predicting 28-day mortality risk in sepsis patients. The comparison of miR-21 relative expression was evaluated by Wilcoxon rank sum test. $P < .05$ was considered significant. The ability of miR-21 (C), APACHE II, SOFA score (D), Scr, albumin, WBC, CRP (E), TNF-α, IL-1β, IL-6, and IL-17 (F) in predicting 28-day mortality risk in sepsis patients was identified by ROC curve and AUC with 95% CI. MiR-21, microRNA-21; APACHE II, acute pathologic and chronic health evaluation II; AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; IL, interleukin; Scr, serum creatinine; SOFA, sequential organ failure assessment; ROC, receiver operating characteristic; TNF-α, tumor necrosis factor-α; WBC, white blood cell

3.3 | Correlation of miR-21 with APACHE II and SOFA score in sepsis patients

MiR-21 relative expression was negatively correlated with APACHE II score ($P < .001$, $r = -.469$; Figure 2A) and SOFA score ($P < .001$, $r = -.404$; Figure 2B) in sepsis patients.

3.4 | Correlation of miR-21 with biochemical and inflammatory indexes in sepsis patients

MiR-21 relative expression was negatively associated with Scr ($P = .003$, $r = -.202$; Figure 3A), CRP ($P < .001$, $r = -.280$; Figure 3D), TNF-α ($P = .026$, $r = -.150$; Figure 3E), IL-1β ($P = .001$, $r = -.231$; Figure 3F), IL-6 ($P = .008$, $r = -.180$; Figure 3G), and IL-17 ($P = .002$, $r = -.212$; Figure 3H), while miR-21 relative expression was positively associated with albumin ($P < .001$, $r = .403$; Figure 3B) in sepsis patients. And no correlation of miR-21 relative expression with WBC was observed in sepsis patients ($P = .225$, $r = -.082$; Figure 3C).

3.5 | Correlation of miR-21 with smoke and comorbidities in sepsis patients

No correlation of miR-21 with smoke ($P = .463$; Figure 4A), COPD ($P = .946$; Figure 4B), cardiomyopathy ($P = .860$; Figure 4C), chronic kidney failure ($P = .917$; Figure 4D), or cirrhosis ($P = .806$; Figure 4E) was observed in sepsis patients.

3.6 | The predictive value of miR-21 for 28-day mortality risk in sepsis patients

There were 163 (74.4%) survivors and 56 (25.6%) deaths in sepsis patients (Figure 5A). And the miR-21 relative expression was decreased in deaths (0.251 [0.171-0.360]) compared with survivors (0.287 [0.193-0.479]) in sepsis patients ($P = .049$; Figure 5B). Subsequent ROC curve analysis displayed that miR-21 was of a poor value in predicting 28-day mortality risk (AUC: 0.588, 95% CI: 0.505-0.672) in sepsis patients (Figure 5C). Besides, APACHE II score (AUC: 0.793, 95% CI: 0.729-0.857) and SOFA score (AUC: 0.758, 95% CI: 0.687-0.830) exhibited relatively good predictive values for 28-day mortality risk in sepsis patients (Figure 5D). Furthermore, Scr (AUC: 0.724, 95% CI: 0.648-0.800), WBC (AUC: 0.624, 95% CI: 0.547-0.701), and CRP (AUC: 0.711, 95% CI: 0.631-0.791) could predict 28-day mortality risk, while albumin (AUC: 0.556, 95% CI: 0.464-0.647) failed to predict 28-day mortality risk in sepsis patients (Figure 5E). Additionally, TNF-α (AUC: 0.629, 95% CI: 0.548-0.710), IL-1β (AUC: 0.709, 95% CI: 0.636-0.783), and IL-17 (AUC: 0.616, 95% CI: 0.531-0.700) had potential values for predicting 28-day mortality risk, whereas IL-6 (AUC: 0.576, 95% CI: 0.492-0.659) could not predict 28-day mortality risk in sepsis patients (Figure 5F). These suggested that MiR-21 presented a poor predictive value for 28-day mortality risk in sepsis patients,

which was much less valuable compared with common prognostic factors such as APACHE II score, SOFA score, etc.

4 | DISCUSSION

In the present study, we have discovered that: (a) MiR-21 relative expression was reduced in sepsis patients compared with HCs, and it exhibited a good predictive value for sepsis risk. (b) MiR-21 was negatively correlated with APACHE II, SOFA score, Scr, WBC, CRP, and proinflammatory cytokines, whereas positively correlated with albumin in sepsis patients. (c) MiR-21 had a poor value for predicting 28-day mortality risk in sepsis patients.

Sepsis is devastating and lethal disorder with complex pathology, among which host immune responses and inflammation are major contributors.[3] As a major public health problem, sepsis is the leading cause of death among patients admitted to the intensive care units. And sepsis survivors experience physical limitations, cognitive impairment, and mental health impairment after discharge.[7] Therefore, it is of value to explore potential biomarkers for the early identification of sepsis risk and disease monitoring to improve the prognosis in sepsis patients.

MiR-21 is ubiquitously expressed in multiple organs such as heart and kidney, which participates in the processes of inflammation process and organ malfunction associated with sepsis.[14-17] For instance, overexpression of miR-21 reduces TNF-α level, while the inhibition of miR-21 upregulates TNF-α and IL-6 levels.[16] Another study exhibits that knockdown of miR-21 facilitates the expression of programmed cell death protein 4 and phosphatase and tensin homolog deleted on chromosome 10, which results in an elevation in apoptosis and exacerbation of LPS-induced septic acute kidney injury.[14] Considering the association of miR-21 with inflammation suppression and organ protection, we hypothesized that miR-21 might have the potential for predicting lowered sepsis risk. The present study disclosed that miR-21 relative expression in sepsis patients was lower than that in HCs, and miR-21 was with a good value for predicting reduced sepsis risk (AUC: 0.801, 95% CI: 0.758-0.844). The possible explanations might be that: (a) MiR-21 suppressed the release of macrophage cytokines (such as TNF-α and IL-6) via downregulating NF-κB signaling, thus dampening inflammation and sepsis risk.[16] (b) MiR-21 might protect multiple organs (such as lung, kidney, and heart) from damage via reducing cell apoptosis and proinflammatory cytokines, thereby, alleviating organ injury and lowering sepsis risk.[9]

Further analysis of the correlation of miR-21 relative expression with inflammation and disease severity displayed that miR-21 relative expression was negatively correlated with APACHE II, SOFA score, Scr, WBC, CRP, and proinflammatory cytokines, whereas positively correlated with albumin in sepsis patients, which suggested that miR-21 was associated with attenuated disease severity in sepsis patients. The possible reasons were as follow: (a) MiR-21 might attenuate the inflammatory response via decreasing the production of inflammatory mediators and subsequent release of inflammatory cytokines, which was responsible for declined inflammation and disease severity in sepsis patients. (b) Higher miR-21 level might inhibit the necrosis,

injuries, and dysfunctions of several organs such as kidney, heart, and lung, thereby, decreasing the disease severity in sepsis patients.

Known that miR-21 was associated with decreased inflammation and disease severity in sepsis patients, we analyzed the effect of miR-21 in predicting 28-day mortality. And we observed that miR-21 presented a poor predictive value for 28-day mortality risk in sepsis patients (AUC: 0.588, 95% CI: 0.505-0.672), which was numerically inferior to that of APACHE II score (AUC: 0.793, 95% CI: 0.729-0.857), SOFA score (AUC: 0.758, 95% CI: 0.687-0.830), and CRP (AUC: 0.711, 95% CI: 0.631-0.791). These might be explained by that: (a) MiR-21 was negatively associated with APACHE II score, SOFA score, and CRP in sepsis patients. And APACHE II score, SOFA score as well as CRP could predict 28-day mortality risk in sepsis patients. Thus, miR-21 might affect the 28-day mortality risk indirectly via APACHE II score, SOFA score, and CRP, resulting in a poor predictive value. (b) The poor value of miR-21 for predicting 28-day mortality risk might also influenced by the relatively small sample size. (c) MiR-21 exhibited a dual effect in the regulation of inflammation and organ injuries. From a review, miR-21 inhibited inflammatory responses and alleviated organ injuries in some situations, whereas it enhanced inflammatory responses and exacerbated organ injuries in other situations.[18] Thus, the value of miR-21 for predicting 28-day mortality risk was poor in sepsis patients.

There were certain limitations in the present study. Firstly, the sample size was relatively small, which might reduce the statistic power. Secondly, only 28-day mortality was evaluated, thereby, the long-term effect of miR-21 on prognosis needed further study. Thirdly, miR-21 is impacted by many biological processes.[19,20] In our study, as the miR-21 expression of sepsis patients was only detected once within 24 hours after admission, the variance of miR-21 during the treatment of sepsis was not considered. The detection of miR-21 expression at different time point among the treatment of sepsis should be conducted in the future. Fourthly, circulating miR-21 was variously affected by many biological processes (such as metabolism and immunity) and diseases (such as tumors and cardiovascular diseases),[19,20] which might cause variability in the results. Lastly, the detailed mechanism of miR-21 in the development and progression of sepsis was not investigated. Thus, further related experiments should be carried out.

In conclusion, miR-21 exhibits the potential for predicting sepsis risk and correlates with alleviated inflammation and disease severity in sepsis patients, but it presents a poor value for prognosis.

ORCID

Jian Yu https://orcid.org/0000-0002-7958-6555
Yanjun Zhao https://orcid.org/0000-0003-1180-3135

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