Received: 25 May 2020 | Revised: 10 July 2020 | Accepted: 19 July 2020

DOI: 10.1002/jcla.23510

RESEARCH ARTICLE

WILEY

MicroRNA-146b correlates with decreased acute respiratory distress syndrome risk, reduced disease severity, and lower 28-day mortality in sepsis patients

Wenfeng Chen | **Lili Liu** | **Junhui Yang** | **Yujun Wang**

Department of Intensive Care Unit, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Correspondence
Yujun Wang, Department of Intensive Care Unit, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, 26 Shengli Street, Wuhan 430014, China.
Email: yuxun6740@163.com

Abstract

Objective: This study aimed to investigate the predictive value of microRNA-146b (miR-146b) on acute respiratory distress syndrome (ARDS) risk, and the correlation of miR-146b with disease severity and 28-day mortality in sepsis patients.

Methods: A total of 104 sepsis patients and 100 healthy controls (HCs) were consecutively enrolled, and miR-146b relative expression in their plasma samples was detected by reverse transcription-quantitative polymerase chain reaction. In sepsis patients, disease severity was assessed using Acute Physiology and Chronic Health Evaluation II (APACHE II) score and Sequential Organ Failure Assessment (SOFA) score. ARDS occurrence and 28-day mortality were recorded.

Results: MiR-146b was decreased in sepsis patients compared to HCs. ARDS occurred in 30 (28.8%) sepsis patients, and miR-146b was reduced in ARDS sepsis patients compared to non-ARDS sepsis patients. Meanwhile, miR-146b distinguished ARDS sepsis patients from non-ARDS sepsis patients (area under the curve (AUC): 0.728, 95% confidence interval (CI): 0.627-0.829). Subsequent multivariate logistic regression showed that miR-146b, age, smoke, respiratory infection, and serum creatinine predicted ARDS risk independently, and their combination well-discriminated ARDS sepsis patients from non-ARDS sepsis patients (AUC: 0.863, 95% CI: 0.792-0.934). Additionally, miR-146b was negatively correlated with serum creatinine, white blood cell, C-reactive protein, APACHE II score, and SOFA score, while positively correlated with albumin. Regarding prognosis, miR-146b was decreased in 28-day sepsis deaths compared to 28-day sepsis survivors, and it discriminated 28-day sepsis deaths from 28-day sepsis survivors (AUC: 0.785, 95% CI: 0.680-0.890).

Conclusion: MiR-146b might serve as a potential biomarker for ARDS prevention and prognostic reflection in sepsis.

KEYWORDS

28-day mortality, acute respiratory distress syndrome, disease severity, microRNA-146b, sepsis

Wenfeng Chen and Lili Liu contributed equally to this work.

1 | INTRODUCTION

Sepsis is characterized by the dysregulation of the immune response against infection such as bacteria, fungi, viruses, and mycoplasma.[1] In China, it is estimated that the prevalence of sepsis is about 437 cases per 100 000 populations, and the crude mortality rate is about 73.76 per 100 000 populations annually, indicating sepsis might be a heavy burden of the public health system.[2,3] Sepsis-induced acute respiratory distress syndrome (ARDS) is a common acute lung inflammatory injury secondary to sepsis, which affects about 30% of sepsis patients in China and increases patients' mortality risk.[4-6] Recent studies have recognized several microRNAs (miR) as potential biomarkers for sepsis and ARDS secondary to sepsis; however, more studies should be conducted for verification and digging before the clinical application of these miRNAs.[7,8]

MiR-146b, a member of the miR-146 family, is located on human chromosome 10q24.32.[9] It is reported that miR-146b is highly involved in regulating the inflammation, thus participating in the progression of various diseases including cancers, cardiovascular diseases, and notably, sepsis.[10-12] According to previous studies, miR-146b could modulate the Notch1 pathway to suppress inflammation, thus ameliorating sepsis-induced multiple organ injury such as myocardial injury and acute kidney injury.[12-14] Meanwhile, it is suggested that miR-146b might regulate the nuclear factor-κB (NF-κB) pathway to inhibit the inflammatory response, thereby acting as a protective regulator in the progression of acute lung injury and pneumonia.[15,16] Additionally, miR-146b was reduced in sepsis patients compared with healthy individuals in our pilot study. Based on the information mentioned above, we hypothesized that miR-146b might act as a potential biomarker in sepsis and ARDS secondary to sepsis; however, relevant study is rare, and only one previous study investigates the clinical role of miR-146b in sepsis, but not sepsis-induced ARDS.[17] In this study, we detected miR-146b relative expression in 104 sepsis patients, investigated the correlation of miR-146b with ARDS risk, disease severity as well as 28-day mortality in sepsis patients, and aimed to provide evidence for the clinical application of miR-146b in sepsis-induced ARDS and mortality.

2 | MATERIALS AND METHODS

2.1 | Participants

This study was approved by the Institutional Review Board of our hospital and conformed to the principles of the Declaration of Helsinki. Written informed consents were obtained from all patients or their family members. A total of 104 adult sepsis patients admitted in our hospital between April 2018 and September were consecutively enrolled in this study. All patients were confirmed as sepsis according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).[1]

TABLE 1 Patients' characteristics

Items	Sepsis patients (n = 104)
Age (y), mean ± SD	54.9 ± 10.9
Gender, no. (%)	
Female	42 (40.4)
Male	62 (59.6)
BMI (kg/m^2), mean ± SD	22.7 ± 3.5
Smoke, no. (%)	40 (38.5)
COPD, no. (%)	17 (16.3)
Cardiomyopathy, no. (%)	48 (46.2)
Chronic kidney failure, no. (%)	15 (14.4)
Cirrhosis, no. (%)	22 (21.2)
Primary infection site, no. (%)	
Abdominal infection	38 (36.5)
Respiratory infection	22 (21.2)
Skin and soft tissue infection	20 (19.2)
Bloodstream infection	12 (11.5)
CNS infection	6 (5.8)
Other infections	6 (5.8)
Primary organism, no. (%)	
G− bacteria	54 (51.9)
G+ bacteria	25 (24.0)
Anaerobes	12 (11.5)
Fungi	6 (5.8)
Mycoplasmas	4 (3.8)
Total culture negative	22 (21.2)
Biochemical indexes, median (IQR)	
Scr (mg/dL)	1.8 (1.3-2.6)
Albumin (g/L)	27.1 (23.2-35.4)
WBC (10^9/L)	18.2 (11.4-27.5)
CRP (mg/L)	90.7 (44.5-138.6)
Disease severity, median (IQR)	
APACHE II score	13.0 (8.3-17.0)
SOFA score	6.0 (4.0-8.0)

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; G−, Gram-negative; G+, Gram-positive; IQR, interquartile range; Scr, serum creatinine; SD, standard deviation; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell.

The exclusion criteria were as follows: (a) aged younger than 18 years; (b) complicated with hematologic malignancies, solid tumors, acquired immune deficiency syndrome, or other fatal diseases; (c) transferred from other hospitals or admitted to

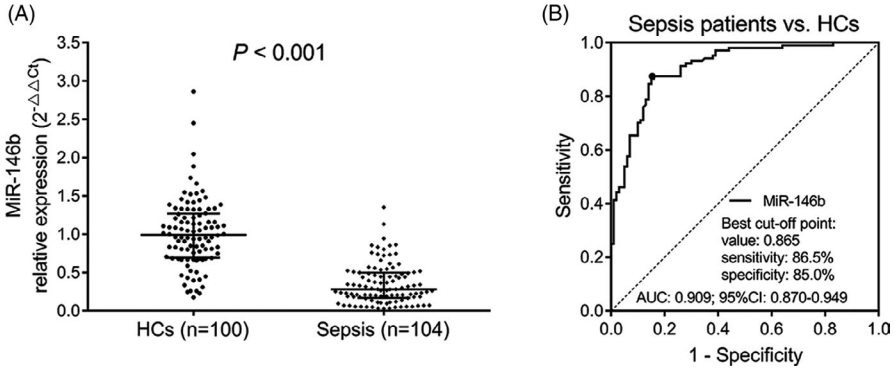

FIGURE 1 MiR-146b expression in sepsis patients and HCs, and its correlation with sepsis risk. A, Comparison of miR-146b expression between sepsis patients and HCs; B, correlation between miR-146b and sepsis risk by ROC curve. AUC, area under the curve; CI, confidence interval; HCs, healthy controls; MiR-146b, microRNA-146b; ROC, receiver operating characteristic

intensive care unit (ICU) more than 24 hours of sepsis onset; (d) received immunosuppressive therapy; and (e) pregnant or lactating women. Besides, 100 healthy subjects whose age and gender were matched with the sepsis patients and underwent health examination in our hospital from October 2019 to December 2019 were enrolled as healthy controls (HCs). The health status of the HCs was confirmed by the health examination, and all HCs had no history of sepsis, other severe infections, hematologic malignancies, or solid tumors.

2.2 | Sample collection and detection

The peripheral blood samples of sepsis patients were collected within 24 hours after admission, and the peripheral blood samples of HCs were obtained from health examination. Then, the peripheral blood samples were centrifuged at the condition of 3000 *g* for 15 minutes (4°C). Subsequently, the plasma samples were separated from peripheral blood samples and preserved at −80°C until further detection. The expression of miR-146b in plasma samples was detected using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) referring to our previous study.[18] The primers used in RT-qPCR were listed as follows (5′-3′): miR-146b forward primer, ACACTCCAGCTGGGTGAGAACTGAATTCCA, reverse primer, TGTCGTGGAGTCGGCAATTC; U6 forward primer, CT CGCTTCGGCAGCACATATACTA, reverse primer, ACGAATTTGCG TGTCATCCTTGC.

2.3 | Date collection

The clinical characteristics of sepsis patients were documented after admission, which included age, gender, body mass index (BMI), smoke, chronic obstructive pulmonary disease (COPD), cardiomyopathy, chronic kidney failure, cirrhosis, primary infection site (such as abdominal infection, respiratory infection, skin and soft tissue infection, bloodstream infection, central nervous system (CNS) infection, and other infections), primary organism (such as Gram-negative (G−) bacteria, Gram-positive (G+) bacteria, anaerobes, fungi, and mycoplasmas), serum creatinine (Scr), white blood cell (WBC), C-reactive protein (CRP), and disease severity.

2.4 | Disease severity and acute respiratory distress syndrome (ARDS) assessment

The severity of sepsis was assessed using Acute Physiology and Chronic Health Evaluation II (APACHE II) score and Sequential Organ Failure Assessment (SOFA) score within 24 hours after admission. During hospitalization, acute respiratory distress syndrome (ARDS) occurrence in sepsis patients was monitored intensively. The sepsis-related ADRS was assessed from three items according to Berlin definition of ARDS,[19] which included (a) timing, within 1 week of a known clinical insult or new or worsening respiratory symptoms; (b) chest imaging, bilateral opacities (not fully explained by effusions, lobar/lung collapse, or nodules); and (c) origin of edema, respiratory failure (not fully explained by cardiac failure or fluid overload).

2.5 | Treatment and follow-up

After admission, early goal-directed resuscitation was selectively given to the patients based on their disease severity. For all sepsis patients, intravenous antibiotic therapy must be carried out as early as possible, and blood cultures before antibiotic therapy were necessary if such cultures do not cause significant delay in antibiotic administration. Regular follow-up for the sepsis patients was continued to death or 28 days after admission. During follow-up, the survival status of the sepsis patients was recorded. All sepsis patients were further divided into 28-day survivors and 28-day deaths according to the survival status of 28-day follow-up. Meanwhile, accumulating mortality was calculated from the date of admission to the date of death or completion of the 28-day follow-up.

2.6 | Statistical analysis

Statistical analyses were performed using SPSS 24.0 software (IBM, Chicago, IL, USA), and figures were plotted using GraphPad

TABLE 2 Comparison of characteristics between non-ARDS sepsis patients and ARDS sepsis patients

Items	Non-ARDS sepsis patients (n = 74)	ARDS sepsis patients (n = 30)	*P*-value
Age (y), mean ± SD	53.6 ± 11.5	58.1 ± 8.5	.031
Gender, no. (%)			.169
Female	33 (44.6)	9 (30.0)	
Male	41 (55.4)	21 (70.0)	
BMI (kg/m^2), mean ± SD	22.5 ± 3.6	23.0 ± 3.4	.552
Smoke, no. (%)	22 (29.7)	18 (60.0)	.004
COPD, no. (%)	8 (10.8)	9 (30.0)	.017
Cardiomyopathy, no. (%)	34 (45.9)	14 (46.7)	.947
Chronic kidney failure, no. (%)	9 (12.2)	6 (20.0)	.303
Cirrhosis, no. (%)	17 (23.0)	5 (16.7)	.476
Primary infection site, no. (%)			
Abdominal infection	31 (41.9)	7 (23.3)	.075
Respiratory infection	9 (12.2)	13 (43.3)	<.001
Skin and soft tissue infection	14 (18.9)	6 (20.0)	.899
Bloodstream infection	10 (13.5)	2 (6.7)	.322
CNS infection	4 (5.4)	2 (6.7)	.803
Other infections	6 (8.1)	0 (0.0)	.108
Primary organism, no. (%)			
G− bacteria	37 (50.0)	17 (56.7)	.538
G+ bacteria	15 (20.3)	6 (20.0)	.975
Anaerobes	7 (9.5)	4 (13.3)	.561
Fungi	4 (5.4)	2 (6.7)	.803
Mycoplasmas	3 (4.1)	1 (3.3)	.863
Total culture negative	17 (23.0)	5 (16.7)	.476
Biochemical indexes, median (IQR)			
Scr (mg/dL)	1.8 (1.2-2.5)	1.8 (1.3-2.9)	.389
Albumin (g/L)	27.2 (23.0-35.9)	26.6 (23.1-35.5)	.714
WBC (10^9/L)	15.8 (11.2-27.4)	22.6 (13.0-28.5)	.120
CRP (mg/L)	68.1 (42.9-126.5)	122.4 (63.7-200.9)	.008
Disease severity, median (IQR)			
APACHE II score	12.0 (7.0-16.0)	14.0 (10.8-18.3)	.022
SOFA score	5.0 (4.0-7.3)	7.0 (5.0-10.0)	.007

Note: Comparison was determined by Student's *t* test, chi-square test, or Wilcoxon rank-sum test.

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; ARDS, acute respiratory distress syndrome; BMI, body mass index; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; G−, Gram-negative; G+, Gram-positive; IQR, interquartile range; Scr, serum creatinine; SD, standard deviation; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell.

Prism 7.01 software (GraphPad Software Inc, San Diego, California, USA). The comparison of normally distributed continuous variables between two groups was determined by Student's *t* test. The comparison of obviously skewed or unknown distributed variables and continuous variables between two groups was determined by Wilcoxon rank-sum test. The comparison of categorical variables between two groups was determined by chi-square test. The correlation was determined by Spearman's rank correlation test. The discriminated performance of variables was assessed using the receiver operating characteristic (ROC) curve and the area under the curve (AUC). Accumulating mortality was displayed by the Kaplan-Meier curve, and the comparison between two groups was determined by the log-rank test. Risk factors of ARDS in sepsis patients were analyzed by the univariate logistic regression model, and the independent risk factors of ARDS in sepsis patients were further analyzed using forward

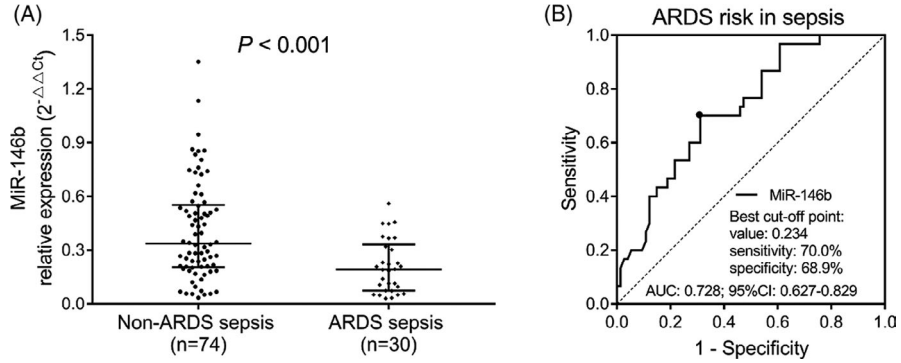

FIGURE 2 Comparison of miR-146b expression in ARDS sepsis patients and non-ARDS sepsis patients. A, Comparison of miR-146b expression between ARDS and non-ARDS sepsis patients; B, Predictive value of miR-146b on ARDS risk by ROC curve. ARDS, acute respiratory distress syndrome; AUC, area under the curve; CI, confidence interval; MiR-146b, microRNA-146b; ROC, receiver operating characteristic

stepwise multivariate logistic regression model. *P*-value < .05 was considered statistically significant.

3 | RESULTS

3.1 | Description of patients' clinical characteristics

The mean age of sepsis patients was 54.9 ± 10.9 years, and there were 42 (40.4%) females as well as 62 (59.6%) males. As to primary infection site, the number of patients with abdominal infection, respiratory infection, skin and soft tissue infection, bloodstream infection, CNS infection, as well as other infection was 38 (36.5%), 22 (21.2%), 20 (19.2%), 12 (11.5%), 6 (5.8%), and 6 (5.8%), respectively. Regarding primary organism that caused infection, 54 (51.9%) patients were infected by G− bacteria, 25 (24.0%) patients were infected by G+ bacteria, 12 (11.5%) patients were infected by anaerobes, 6 (5.8%) patients were infected by fungi, 4 (3.8%) patients were infected by mycoplasmas, and 22 (21.2%) patients were culture negative. For biochemical indexes, the median level of Scr, albumin, WBC, and CRP was 1.8 (1.3-2.6) mg/dL, 27.1 (23.2-35.4) g/L, 18.2 (11.4-27.5) × 10^9/L, and 90.7 (44.5-138.6) mg/L, respectively. Meanwhile, the median APACHE II score was 13.0 (8.3-17.0), and the median SOFA score was 6.0 (4.0-8.0) in sepsis patients. The detailed patients' characteristics are shown in Table 1.

3.2 | MiR-146b expression in sepsis patients and HCs

MiR-146b expression was decreased in sepsis patients compared to HCs (*P* < .001) (Figure 1A). Meanwhile, ROC curve showed that miR-146b could well recognize sepsis patients from HCs (AUC: 0.909, 95% CI: 0.870-0.949) with miR-146b expression at the best cut-off point (at which the sum of sensitivity and specificity was maximized) of 0.865, and the sensitivity and specificity at the best cut-off point of 86.5% and 85.0%, respectively (Figure 1B).

3.3 | Differences in clinical characteristics of ARDS sepsis patients and non-ARDS sepsis patients

During 28 days of follow-up, ARDS occurred in 30 (28.8%) sepsis patients. The mean age (*P* = .031), history of smoke (*P* = .004), COPD complication (*P* = .017), respiratory infection (*P* < .001), CRP (*P* = .008), APACHE II score (*P* = .022), and SOFA score (*P* = .007) at baseline were increased in ARDS sepsis patients compared to non-ARDS sepsis patients (Table 2). However, no difference was found in gender distribution, BMI, cardiomyopathy, chronic kidney failure, cirrhosis, abdominal infection, skin and soft tissue infection, bloodstream infection, CNS infection, and other infections, and primary organism that caused infection, Scr, albumin, or WBC between ARDS sepsis patients and non-ARDS sepsis patients (all *P* > .05) (Table 2).

3.4 | Correlation of miR-146b with sepsis-induced ARDS in sepsis patients

MiR-146b expression was reduced in ARDS sepsis patients compared to non-ARDS sepsis patients (*P* < .001) (Figure 2A). Additionally, ROC curve revealed that miR-146b could distinguish ARDS sepsis patients from non-ARDS sepsis patients (AUC: 0.728, 95% CI: 0.627-0.829) with miR-146b expression at the best cut-off point of 0.234, and the sensitivity and specificity at the best cut-off point of 70.0% and 68.9%, respectively (Figure 2B).

Meanwhile, univariate logistic regression analysis showed that miR-146b (*P* = .001, OR = 0.012) was correlated with reduced ARDS risk, while history of smoke (*P* = .005, OR = 3.545), COPD (*P* = .021, OR = 3.536), respiratory infection (*P* = .001, OR = 5.523), CRP (*P* = .002, OR = 1.009), and SOFA score (*P* = .005, OR = 1.241) were correlated with increased ARDS risk. Further forward stepwise multivariate logistic regression revealed that miR-146b (*P* = .048, OR = 0.030) was an independent predictive factor for reduced ARDS risk, while age (*P* = .048, OR = 1.060), history of smoke (*P* = .003, OR = 5.887), respiratory infection (*P* = .011, OR = 4.931), as well as CRP (*P* = .042, OR = 1.009) were independent predictive factors for

TABLE 3 Analysis of risk factors of ARDS in sepsis patients

	Logistic regression model			
			95% CI	
Items	*P*-value	OR	Lower	Higher
Univariate logistic regression				
MiR-146b	.001	0.012	0.001	0.167
Age	.058	1.041	0.999	1.085
Gender	.173	1.878	0.759	4.645
BMI	.548	1.037	0.920	1.170
Smoke	.005	3.545	1.464	8.585
COPD	.021	3.536	1.211	10.324
Cardiomyopathy	.947	1.029	0.440	2.410
Chronic kidney failure	.307	1.806	0.581	5.612
Cirrhosis	.477	0.671	0.223	2.019
Primary infection site (respiratory vs others)	.001	5.523	2.024	15.068
Primary infection organism				
G− vs others	.538	1.308	0.557	3.071
G+ vs others	.975	0.983	0.341	2.835
Anaerobes/fungi/mycoplasmas vs others	0.612	1.304	0.647	3.642
Scr	.534	1.100	0.815	1.485
Albumin	.599	0.987	0.941	1.036
WBC	.189	1.027	0.987	1.068
CRP	.002	1.009	1.004	1.016
APACHE II score	.102	1.059	0.989	1.135
SOFA score	.005	1.241	1.069	1.441
Forward stepwise multivariate logistic regression				
MiR-146b	.048	0.030	0.001	0.972
Age	.048	1.060	1.001	1.124
Smoke	.003	5.887	1.819	19.055
Primary infection site (respiratory vs others)	.011	4.931	1.439	16.894
CRP	.042	1.009	1.000	1.017

Note: Risk factors of ARDS were analyzed by univariate logistic regression model, and the independent risk factors were analyzed by forward stepwise multivariate logistic regression model.

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; ARDS, acute respiratory distress syndrome; BMI, body mass index; CI, confidence interval;COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; G−, Gram-negative; G+, Gram-positive; miR-146b, microRNA-146b; OR, odds ratio; Scr, serum creatinine; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell.

increased ARDS risk in sepsis patients (Table 3). More importantly, the combination of ARDS independent predictive factors (including miR-146b, age, history of smoke, respiratory infection, and CRP) (AUC: 0.863, 95% CI: 0.792-0.934) could well-discriminate ARDS sepsis patients from non-ARDS sepsis patients (Figure 3).

3.5 | Correlation of miR-146b with clinical characteristics in sepsis patients

No correlation was found in miR-146b with common complications of sepsis (including COPD, cardiomyopathy, chronic kidney failure, and cirrhosis), primary infection site (including abdominal infection, respiratory infection, skin and soft tissue infection, bloodstream infection, and CNS infection), and primary organism that caused infection (including G− bacteria, G+ bacteria, and anaerobes/fungi/mycoplasmas) (all $P > .05$) (Table 4). As to biochemical indexes, miR-146b was negatively correlated with Scr ($P = .018$, $r = -0.232$), WBC ($P < .001$, $r = -0.349$), and CRP ($P < .001$, $r = -0.555$) while positively correlated with albumin ($P < .001$, $r = 0.348$). Regarding general disease severity, miR-146b was negatively correlated with APACHE II score ($P < .001$, $r = -0.422$) and SOFA score ($P < .001$, $r = -0.512$) (Table 5).

3.6 | Correlation of miR-146b with 28-day mortality in sepsis patients

MiR-146b expression was decreased in 28-day deaths compared to 28-day survivors ($P < .001$) (Figure 4A). Meanwhile, ROC curve showed that miR-146b (AUC: 0.785, 95% CI: 0.680-0.890) could discriminate 28-day deaths from 28-day survivors with miR-146b expression at the best cut-off point of 0.192, and the sensitivity and specificity at the best cut-off point of 69.2% and 83.3%, respectively (Figure 4B). Moreover, sepsis patients were divided into patients with miR-146b low expression and patients with miR-146b high expression according to the median value of miR-146b; accumulating mortality was increased in patients with miR-146b low expression compared to patients with miR-146b high expression ($P = .002$) (Figure 4C).

4 | DISCUSSION

MiR-146b is known as a critical regulator of inflammation, thus participating in the progression of several diseases including sepsis.[9] For instance, miR-146b is down-regulated in the myocardium tissue of sepsis mouse model, and overexpression of miR-146b suppresses interleukin-1β expression and myocardium apoptosis in sepsis mouse model by targeting the Notch1 pathway.[15] Meanwhile, the up-regulation of miR-146b reduced Scr and blood urea nitrogen levels; additionally, the up-regulation of miR-146b inhibits the NF-κB pathway to suppress inflammation in sepsis-induced acute kidney injury mouse model.[14] Moreover, overexpression of miR-146b could reduce pro-inflammatory cytokines and chemokines through modulating Toll-like receptor 4 (TLR4), myeloid differentiation primary response (MyD88), TNF receptor-associated factor 6 (TRAF6), and interleukin-1 receptor-associated kinase 1 (IRAK-1) pathways in sepsis cell model.[20]

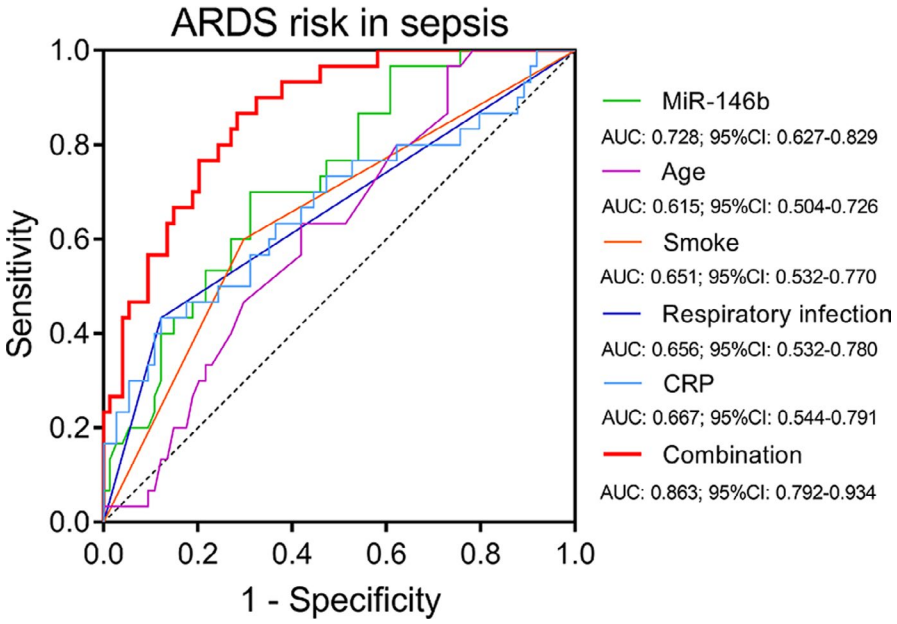

FIGURE 3 ROC curve analysis of miR-146b, age, history of smoke, respiratory infection, CRP for ARDS risk in sepsis patients. ARDS, acute respiratory distress syndrome; AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; ROC, receiver operating characteristic

Additionally, miR-146b is reported to reduce cell apoptosis through ameliorating inflammation in septic human airway smooth muscle cells and human lung fibroblasts,[16,21] indicating the potential regulatory role of miR-146b in sepsis-induced lung injury. Therefore, miR-146b might modulate several pathways to inhibit inflammation, thus participating in the regulation of sepsis and sepsis-induced lung injury.

According to previous studies, miR-146b might exert potential predictive value on the incidence of several diseases where inflammation is highly involved, such as coronary artery disease.[20] However, the relationship between miR-146b and sepsis risk is unclear. Based on the information mentioned above, we hypothesized that miR-146b could serve as a potential biomarker that correlated with sepsis risk. In this study, we found that miR-146b is down-regulated in sepsis patients compared to HCs, and it had a strong relation with sepsis risk. Possible explanations might be that (a) decreased miR-146b might activate several pro-inflammatory pathways such as Notch1 and NF-κB pathways to increase inflammation,[14,15] which could enhance the risk of sepsis; and (b) reduced miR-146b might exacerbate the injury in several organs such as kidney and heart.[16,20] Therefore, miR-146b low expression was correlated with higher sepsis risk.

ARDS is one of the common complications of sepsis, which might increase the mortality risk of sepsis patients. Currently, several miRs have been identified as potential predictive biomarkers for ARDS risk in sepsis patients such as miR-23a-5p and miR-155.[22,23] However, the predictive value of miR-146b on the risk of sepsis-induced ARDS was unclear. In the present study, we found that miR-146b low expression could discriminate ARDS sepsis patients from non-ARDS sepsis patients to a certain degree. Possible explanations might be that (a) reduced miR-146b expression might directly enhance injury in lung fibroblasts and airway smooth muscle cells[16,21]; and (b) decreased miR-146b expression might enhance inflammation by activating several inflammatory-related pathways such as the TRL4 and NF-κB pathway,[20] thus indirectly exacerbating injury in lung fibroblasts and airway smooth muscle cells.[24] Meanwhile, miR-146b was an independent predictive factor for ARDS risk in sepsis patients, and miR-146b, combined with other independent predictive factors (including age, history of smoke, respiratory infection, and CRP), showed high value on discriminating ARDS sepsis patients from non-ARDS sepsis patients, indicating the combination of independent predictive factors might potentially serve as a promising

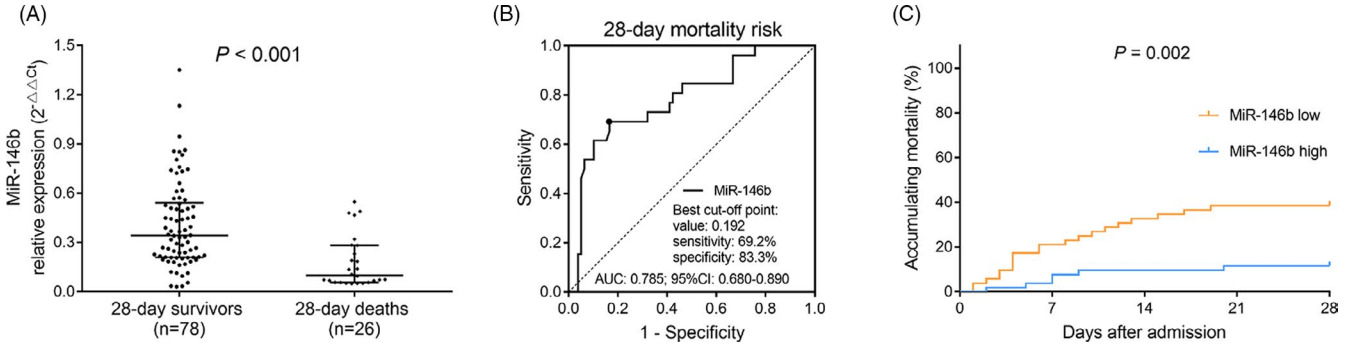

FIGURE 4 Prognostic value of miR-146b in sepsis patients. A, Comparison of miR-146b expression between 28-day deaths and 28-day survivors; B, Predictive value of miR-146b on 28-day mortality; C, Comparison of accumulating mortality between patients with miR-146b low expression and patients with miR-146b high expression. AUC, area under the curve; CI, confidence interval; MiR-146b, microRNA-146b

TABLE 4 Correlation of miR-146b with complications, primary infection site, and primary organism

Items	MiR-146b expression	*P*-value
Complications, median (IQR)		
COPD		.888
No	0.268 (0.168-0.509)	
Yes	0.341 (0.140-0.462)	
Cardiomyopathy		.365
No	0.323 (0.184-0.524)	
Yes	0.264 (0.125-0.480)	
Chronic kidney failure		.265
No	0.302 (0.165-0.507)	
Yes	0.210 (0.194-0.366)	
Cirrhosis		.417
No	0.251 (0.156-0.508)	
Yes	0.337 (0.218-0.456)	
Primary infection site, median (IQR)		
Abdominal infection		.649
No	0.284 (0.157-0.470)	
Yes	0.259 (0.177-0.613)	
Respiratory infection		.155
No	0.298 (0.185-0.520)	
Yes	0.230 (0.090-0.394)	
Skin and soft tissue infection		.488
No	0.283 (0.147-0.491)	
Yes	0.293 (0.191-0.508)	
Bloodstream infection		.669
No	0.267 (0.164-0.504)	
Yes	0.339 (0.218-0.466)	
CNS infection		.727
No	0.275 (0.177-0.503)	
Yes	0.388 (0.034-0.465)	
Primary organism, median (IQR)		
G− bacteria		.815
No	0.298 (0.111-0.483)	
Yes	0.255 (0.185-0.529)	
G+ bacteria		.981
No	0.268 (0.180-0.505)	
Yes	0.317 (0.118-0.458)	
Anaerobes/fungi/mycoplasmas		.496
No	0.268 (0.140-0.489)	
Yes	0.302 (0.206-0.514)	

Note: Comparison was determined by Wilcoxon rank-sum test.

Abbreviations: CNS, central nervous system; COPD, chronic obstructive pulmonary disease; G−, Gram-negative; G+, Gram-positive; IQR, interquartile range.

TABLE 5 Correlation of miR-146b with biochemical indexes, APACHE II score, and SOFA score in sepsis patients

	MiR-146b	
Items	Correlation coefficient (*r*)	*P*-value
Scr	−0.232	.018
Albumin	0.348	<.001
WBC	−0.349	<.001
CRP	−0.555	<.001
APACHE II score	−0.422	<.001
SOFA score	−0.512	<.001

Note: Correlation was determined by Spearman's rank correlation test.

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; CRP, C-reactive protein; Scr, serum creatinine; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell.

tool for the surveillance of the ARDS occurrence in sepsis patients to improve the management toward them.

Meanwhile, we had investigated the correlation of miR-146b with the clinical characteristics of sepsis patients. Data showed that miR-146b was negatively correlated with Scr, WBC, CRP, APACHE II score, and SOFA score, while positively correlated with albumin in sepsis patients. Possible explanations might be that (a) reduced miR-146b might exacerbate apoptosis in renal tubular cells to enhance kidney injury in sepsis patients[14]; therefore, it was negatively correlated with Scr in sepsis patients; and (b) decreased miR-146b might activate several pro-inflammatory pathways such as the TRL4 and NF-κB pathway to enhance inflammation[20]; therefore, miR-146b was negatively correlated with WBC and CRP in sepsis patients. However, we did not use inflammatory cytokines as the indicator of inflammation, and the correlations of miR-146b with inflammatory cytokines could be investigated in further studies; (c) miR-146b low expression might enhance inflammation (as was mentioned above), which could improve liver injury in sepsis patients[24]; therefore, miR-146b was positively correlated with albumin in sepsis patients; and (d) miR-146b down-regulation might enhance inflammation and exacerbate injury of multiple organs (as was mentioned above[12,14,20]), thus increasing disease severity of sepsis patients; therefore, miR-146b was negatively correlated with APACHE II score and SOFA score in sepsis patients.

The prognostic value of miR-146b in sepsis was quite unclear. In order to fill this vacancy, we performed this study and found that miR-146b was decreased in 28-day deaths compared to 28-day survivors, and ROC curve revealed that low expression could discriminate 28-day deaths from 28-day survivors to a certain degree; moreover, miR-146b was negatively correlated with accumulating mortality in sepsis patients. Possible explanations for our data might be that (a) decreased miR-146b might increase disease severity (as was mentioned above), thus directly worsened the prognosis of sepsis patients; and (b) reduced miR-146b could increase inflammation and multiple organ injury (as was mentioned above[12,14,20]), which directly reduced the prognosis of sepsis patients.

There were several limitations in this study, which should be clarified. First, the change of miR-146b relative expression in sepsis patients during the follow-up period was not investigated, and further studies could be conducted to explore that. Second, we did not enroll a verification cohort to verify the correlation of miR-146b with ARDS risk, disease severity, and prognosis in sepsis patients, which could be conducted further. Third, the potential downstream molecular target of miR-146b in sepsis-induced ARDS was not investigated, and further in vitro or in vivo studies could be conducted to explore that. Fourth, the number of patients with sepsis-induced ARDS was relatively small, which might cause low statistical power, and further studies with larger sample size could be conducted. Fifth, the role of miR-146b in severe sepsis or septic shock patients was not investigated in this study, which could be explored in further studies.

In summary, miR-146b predicts reduced ARDS risk independently and correlates with decreased disease severity and better prognosis in sepsis patients, which might improve the management toward sepsis patients by its in-time surveillance for the risk of sepsis-induced ARDS and mortality.

ORCID

Yujun Wang https://orcid.org/0000-0002-6198-7887

REFERENCES

1. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*. 2016;315(8):801-810.
2. Weng L, Zeng XY, Yin P, et al. Sepsis-related mortality in China: a descriptive analysis. *Intensive Care Med*. 2018;44(7):1071-1080.
3. Liao X, Du B, Lu M, et al. Current epidemiology of sepsis in mainland China. *Ann Transl Med*. 2016;4(17):324.
4. Englert JA, Bobba C, Baron RM. Integrating molecular pathogenesis and clinical translation in sepsis-induced acute respiratory distress syndrome. *JCI Insight*. 2019;4(2).e124061
5. Matthay MA, Zemans RL, Zimmerman GA, et al. Acute respiratory distress syndrome. *Nat Rev Dis Primers*. 2019;5(1):18.
6. Li S, Zhao D, Cui J, et al. Correlation of microRNA-125a/b with acute respiratory distress syndrome risk and prognosis in sepsis patients. *J Clin Lab Anal*. 2020;34(3):e23098.
7. Zhang TN, Li D, Xia J, et al. Non-coding RNA: a potential biomarker and therapeutic target for sepsis. *Oncotarget*. 2017;8(53):91765-91778.
8. Benz F, Roy S, Trautwein C, et al. Circulating MicroRNAs as biomarkers for sepsis. *Int J Mol Sci*. 2016;17(1):78.
9. Paterson MR, Kriegel AJ. MiR-146a/b: a family with shared seeds and different roots. *Physiol Genomics*. 2017;49(4):243-252.
10. Chou CK, Liu RT, Kang H-Y. MicroRNA-146b: a novel biomarker and therapeutic target for human papillary thyroid cancer. *Int J Mol Sci*. 2017;18(3):636.
11. Echavarria R, Mayaki D, Neel JC, et al. Angiopoietin-1 inhibits toll-like receptor 4 signalling in cultured endothelial cells: role of miR-146b-5p. *Cardiovasc Res*. 2015;106(3):465-477.
12. Wang X, Yu Y. MiR-146b protect against sepsis induced mice myocardial injury through inhibition of Notch1. *J Mol Histol*. 2018;49(4):411-417.
13. Pfeiffer D, Rossmanith E, Lang I, et al. miR-146a, miR-146b, and miR-155 increase expression of IL-6 and IL-8 and support HSP10 in an In vitro sepsis model. *PLoS One*. 2017;12(6):e0179850.
14. Zhang R, Zhu Y, Li Y, et al. Human umbilical cord mesenchymal stem cell exosomes alleviate sepsis-associated acute kidney injury via regulating microRNA-146b expression. *Biotechnol Lett*. 2020;42(4):669-679.
15. Zhang L, Dong L, Tang Y, et al. MiR-146b protects against the inflammation injury in pediatric pneumonia through MyD88/NF-kappaB signaling pathway. *Infect Dis (Lond)*. 2020;52(1):23-32.
16. He R, Li Y, Zhou L, et al. miR-146b overexpression ameliorates lipopolysaccharide-induced acute lung injury in vivo and in vitro. *J Cell Biochem*. 2019;120(3):2929-2939.
17. Chen L, Yu L, Zhang R, et al. Correlation of microRNA-146a/b with disease risk, biochemical indices, inflammatory cytokines, overall disease severity, and prognosis of sepsis. *Medicine (Baltimore)*. 2020;99(22):e19754.
18. Geng F, Liu W, Yu L. Potential role of circulating long noncoding RNA MALAT1 in predicting disease risk, severity, and patients' survival in sepsis. *J Clin Lab Anal*. 2019;33(8):e22968.
19. Force ADT, Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin definition. *JAMA*. 2012;307(23):2526-2533.
20. Takahashi Y, Satoh M, Minami Y, et al. Expression of miR-146a/b is associated with the Toll-like receptor 4 signal in coronary artery disease: effect of renin-angiotensin system blockade and statins on miRNA-146a/b and Toll-like receptor 4 levels. *Clin Sci (Lond)*. 2010;119(9):395-405.
21. Curtale G, Mirolo M, Renzi TA, et al. Negative regulation of Toll-like receptor 4 signaling by IL-10-dependent microRNA-146b. *Proc Natl Acad Sci U S A*. 2013;110(28):11499-11504.
22. Liu S, Liu C, Wang Z, et al. microRNA-23a-5p acts as a potential biomarker for sepsis-induced acute respiratory distress syndrome in early stage. *Cell Mol Biol (Noisy-le-grand)*. 2016;62(2):31-37.
23. Han Y, Li Y, Jiang Y. The prognostic value of plasma MicroRNA-155 and MicroRNA-146a level in severe sepsis and sepsis-induced acute lung injury patients. *Clin Lab*. 2016;62(12):2355-2360.
24. Woznica EA, Inglot M, Woznica RK, et al. Liver dysfunction in sepsis. *Adv Clin Exp Med*. 2018;27(4):547-551.

How to cite this article: Chen W, Liu L, Yang J, Wang Y. MicroRNA-146b correlates with decreased acute respiratory distress syndrome risk, reduced disease severity, and lower 28-day mortality in sepsis patients. *J Clin Lab Anal*. 2020;34:e23510. https://doi.org/10.1002/jcla.23510