

# Decreased microRNA 103 and microRNA 107 predict increased risks of acute respiratory distress syndrome and 28-day mortality in sepsis patients

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## Abstract

We aimed to investigate the predictive value of microRNA 103 (MIR103) and microRNA 107 (MIR107) for acute respiratory distress syndrome (ARDS) risk, as well as their correlations with overall disease severity and prognosis in sepsis patients.

Plasma samples were collected from 196 sepsis patients within 24 hours after enrollment and from 196 healthy individuals (as healthy controls (HCs)) at enrollment. Plasma MIR103 and MIR107 were detected by reverse transcription-quantitative polymerase chain reaction.

MIR103 and MIR107 were both decreased in ARDS sepsis patients and non-ARDS sepsis patients compared to HCs, and were reduced in ARDS sepsis patients than non-ARDS sepsis patients. Decreased MIR103 (area under curve (AUC): 0.727, 95% confidence interval (CI): 0.619–0.835) and MIR107 (AUC: 0.694, 95% CI: 0.577–0.811) predicted increased ARDS risk in sepsis patients. Meanwhile, MIR103 and MIR107 were negatively correlated with acute pathologic and chronic health evaluation (APACHE) II score, sequential organ failure assessment (SOFA) score, serum creatinine, C-reactive protein, tumor necrosis factor, interleukin 1 $\beta$ , interleukin 6 and interleukin 8, while positively correlated with albumin in sepsis patients. For prognosis, 28-day mortality was increased in ARDS sepsis patients compared to non-ARDS sepsis patients. Finally, MIR103 and MIR107 were reduced in deaths than survivors of sepsis patients, and decreased MIR103 (AUC: 0.704, 95% CI: 0.626–0.782) as well as MIR107 (AUC: 0.649, 95% CI: 0.569–0.729) predicted increased 28-day mortality risk in sepsis patients.

MIR-103 and MIR107 were predictive biomarkers for risks of ARDS and 28-day mortality in sepsis patients, which might improve the management of sepsis.

**Abbreviations:** ARDS = acute respiratory distress syndrome, AUC = area under curve, CI = confidence interval, HCs = healthy controls, HIV = human immunodeficiency virus, LPS = lipopolysaccharide, miR = microRNA, SOFA = sequential organ failure assessment, STAT = signal transducer and activator of transcription.

**Keywords:** 28-day mortality, acute respiratory distress syndrome, disease severity, MicroRNA-103 and MIR107, sepsis

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QW and QF contributed equally to this work.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## 1. Introduction

Sepsis is a life-threatening syndrome that may lead to multiple organ dysfunction, and the incidence of which ranges from 300 to 1000 per 100,000 populations.<sup>[1,2]</sup> Acute respiratory distress syndrome (ARDS), an acute lung inflammatory injury that affects about 30% of sepsis patients in China, is one of the deadly complications of sepsis.<sup>[3–5]</sup> The mortality risk of sepsis patients with ARDS is as high as 20% to 50% according to previous researches.<sup>[6,7]</sup> Therefore, it is vital to search for novel biomarkers to help the prediction of ARDS risk in sepsis patients, thus improving the management of sepsis patients and ameliorating the prognosis of them.

MicroRNA 103 (MIR103) and microRNA 107 (MIR107) are a pair of paralog microRNAs belong to the MIR103/107 group of microRNA genes, which are located in the intron of pantothenate kinase gene and regulate the metabolism of vertebrates.<sup>[8,9]</sup> Previous researches reveal that MIR103 and MIR107 are dysregulated in lipopolysaccharide (LPS)-induced sepsis cell models or animal models.<sup>[10,11]</sup> Moreover, it is proposed that MIR103 and MIR107 are regulators of inflammation<sup>[12,13]</sup> and play important roles in organ injury (including heart, kidney

and lung).<sup>[14–16]</sup> However, the roles of MIR103 and MIR107 in sepsis patients are unclear.

In the present study, we aimed to evaluate the predictive value of MIR103 and MIR107 for ARDS occurrence and 28-day mortality risk in sepsis patients.

## 2. Methods

### 2.1. Participants

A total of 196 sepsis patients were continuously enrolled in this study from our hospital, and the enrollment period was ranging from January 2016 to June 2019. The patients who met the following criteria were eligible:

1. diagnosed as sepsis in accordance with the International Guidelines for Management of Severe Sepsis and Septic Shock in 2012,<sup>[17]</sup> based on clinical manifestations, inflammatory index, hemodynamic index, organ dysfunction index, and tissue perfusion index;
2. age at least 18 years old;
3. no history of hematological malignancies (such as leukemia, megaloblastic anemia, hemolytic anemia, thalassemia, autoimmune hemolytic anemia, and pharmaceutical hemolytic anemia) or cancers;
4. without human immunodeficiency virus (HIV) infection.

The criteria of exclusion were:

1. died within 24 hours after they were admitted in hospital;
2. underwent immunosuppressive therapy (such as cyclosporin, glucocorticoids, adrenocortical hormone, and antilymphocyte globulin) in the past month;
3. no history of chemotherapy or radiotherapy exposure;
4. patients with burn;
5. pregnant or lactating women. Meanwhile, 196 healthy individuals were recruited as healthy controls (HCs).

The HCs were required to have no history of sepsis or other severe infections (such as HIV, cytomegalovirus or tuberculosis), no history of hematological malignancies or cancers and no obvious abnormalities in biochemical indexes. This study was approved by the Institutional Review Board of our hospital. Each participant or their guardian provided the written informed consent before enrollment.

### 2.2. Data collection and assessment

For all participants, the demographic characteristics and the levels of biochemical indexes were recorded after enrollment. In addition, for the sepsis patients, disease and organ dysfunction severity was assessed within 24 hours after admission. The severity of sepsis was evaluated using the acute pathologic and chronic health evaluation (APACHE) II score (ranging from 0 to 71), and the higher score indicated the higher severity of the sepsis patient.<sup>[18]</sup> Meanwhile, organ dysfunction severity was assessed by sequential organ failure assessment (SOFA) score. The total SOFA score was assigned 0 to 24 points, and a higher score was associated with a higher severity of organ dysfunction.<sup>[19]</sup>

### 2.3. Blood sample collection

Peripheral blood samples of sepsis patients were collected in vacuum tubes within 24 hours after admission, and the peripheral

blood samples of HCs were collected in vacuum tubes at enrollment. Then plasma was isolated from the blood samples by centrifugation at 3000g for 20 minutes (4°C). Afterward, the plasma samples were stored at –80°C for measurement.

### 2.4. Enzyme-linked immunosorbent assay (ELISA)

The levels of inflammatory cytokines (tumor necrosis factor (TNF), interleukin 1 $\beta$  (IL1B), interleukin 6 (IL6), and interleukin 8 (IL8)) in plasma for sepsis patients alone were measured by ELISA, and all commercial ELISA Kits were purchased from Abcam (Cambridge, MA, USA). The procedures were conducted with reference to the instructions.

### 2.5. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

The relative expressions of MIR103 and MIR107 in plasma of sepsis patients and HCs were detected by RT-qPCR. In brief, total RNA was extracted by QIAamp RNA Blood Mini kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, German); digestion of genomic DNA was performed with RNase-Free DNase Set (Qiagen, Duesseldorf, Nordrhein-Westfalen, German); reverse transcription was conducted by ReverTra Ace qPCR RT kit (Toyobo, Osaka, Kansai, Japan) (reverse transcription primers: MIR103: 5'-CTCAACTGGTGTCTCGTGGAGTCGGCAATTCAGTTGAGCAAGGCAG-3'; MIR107: 5'-CTCAACTGGTGTCTCGTGGAGTCGGCAATTCAGTTGAGTGATAGCC-3'<sup>[20]</sup>; U6: 5'-ACGAATTTGCGTGTGCATCCTTGC-3'<sup>[21]</sup>); and PCR was performed with KOD SYBR qPCR Mix (Toyobo, Osaka, Kansai, Japan). All kits were used under the manufacturers instructions. U6 was set as the internal reference. The specific PCR procedure was set as follows: 98°C 2 minutes for pre-denaturation, 98°C 10 seconds for denaturation and 61°C 30 seconds for annealing. 40 cycles of denaturation and annealing were conducted. The relative expressions of MIR103 and MIR107 were calculated by the  $2^{-\Delta\Delta C_t}$  formula. Primers for qPCR: MIR103 forward primer: 5'-ACACTCCAGCTGGGGGCTTCTTTACAGTGC-3', reverse primer: 5'-TGTCGTGGAGTCGGCAATTC-3'; MIR107 forward primer: 5'-ACACTCCAGCTGGGAGCAGCATTGTACAGG-3', reverse primer: 5'-TGTCGTGGAGTCGGCAATTC-3'<sup>[22]</sup>; U6 forward primer: 5'-CTCGCTTCGGCAGCACATA-TACTA-3', reverse primer: 5'-ACGAATTTGCGTGTGCATCCTTGC-3'.<sup>[21]</sup>

### 2.6. ARDS assessment

During hospitalization, closely surveillance was performed for the sepsis patients, and diagnosis of ARDS was established if patients suffered from PaO<sub>2</sub>/FiO<sub>2</sub>  $\leq$  200 mm Hg in accordance with the American-European Consensus Conference definition.<sup>[23]</sup> The sepsis patients who developed ARDS were recorded, and all sepsis patients were divided into ARDS sepsis patients and non-ARDS sepsis patients according to whether ARDS occurred or not.

### 2.7. Follow-up

All sepsis patients were daily followed up to death in hospital or to 28 days after enrollment. The patients who died during 28-day follow-up were recorded to calculate 28-day mortality. And according to the survival status within 28 days, the sepsis patients were grouped as deaths and survivors.

## 2.8. Statistical analysis

All tests were 2-sided, and the threshold of statistical significance was  $P$  value  $<.05$ . One-way analysis of variance (ANONA) and Kruskal–Wallis H rank-sum test were used to compare the continuous variables among 3 groups. The student  $t$  test and Wilcoxon rank-sum test were used for comparing the continuous variables between 2 groups. Chi-Squared test was used to compare the proportions between/among groups. Spearman rank correlation test was used to analyze the correlation between 2 continuous variables. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) with 95% confidence interval (CI) were used to assess the performance of variables in predicting ARDS risk for sepsis patients or in predicting 28-day mortality risk for sepsis patients. SPSS 24.0 software (IBM, Chicago, IL, USA) was used for statistical analyses and GraphPad Prism 7.01 software (GraphPad Software, San Diego, California, USA) was used to plot the figures.

## 3. Results

### 3.1. Clinical parameters

Among ARDS sepsis patients, non-ARDS sepsis patients and HCs, no difference was found in age ( $P=.183$ ), gender ( $P=.289$ ) or body mass index (BMI) ( $P=.523$ ). However, the median value of serum creatinine (Scr), white blood cell (WBC) and C-reactive protein (CRP) were highest in ARDS sepsis patients and lowest in HCs, whereas albumin was lowest in ARDS sepsis patients and highest in HCs (all  $P<.001$ ). Notably, between ARDS sepsis patients and non-ARDS sepsis patients, no difference was found in age ( $P=.059$ ), gender ( $P=.603$ ) or BMI ( $P=.338$ ); meanwhile, CRP ( $P=.006$ ), smoking behavior ( $P=.005$ ), chronic obstructive pulmonary disease ( $P=.027$ ), chronic kidney failure ( $P=.015$ ), APACHE II score ( $P<.001$ ), SOFA score ( $P<.001$ ), TNF ( $P=.006$ ) and IL1B ( $P<.001$ ) were increased in ARDS sepsis patients; whereas no difference was found in Scr ( $P=.351$ ), albumin ( $P=.331$ ), WBC ( $P=.364$ ), cardiomyopathy ( $P=.296$ ), cirrhosis ( $P=.502$ ), IL6 ( $P=.133$ ) or IL8 ( $P=.078$ ). As to antibiotic therapy, no difference was found in the administration of any antibiotic therapies (including cephalosporin, gentamicin, tobramycin, penicillin, amikacin, fluconazole, flucytosine, netilmicin, vancomycin, fluoroquinolone and oxacillin) between non-ARDS sepsis patients and ARDS sepsis patients (all  $P>.05$ ). The detailed clinical parameters were listed in Table 1.

### 3.2. MIR103 and MIR107 expressions in HCs, non-ARDS sepsis patients and ARDS sepsis patients

MIR103 expression was decreased in ARDS sepsis patients (median value: 0.152 (0.070–0.342)) ( $P<.001$ ) and non-ARDS sepsis patient (median value: 0.357 (0.195–0.590)) ( $P<.001$ ) compared to HCs (median value: 0.997 (0.662–1.265)), and was decreased in ARDS sepsis patients compared to non-ARDS sepsis patients ( $P<.001$ ) as well (Fig. 1A). Similarly, MIR107 expression was reduced in ARDS sepsis patients (median value: 0.159 (0.092–0.209)) ( $P<.001$ ) and non-ARDS sepsis patient (median value: 0.303 (0.158–0.470)) ( $P<.001$ ) compared to HCs (median value: 1.000 (0.732–1.377)), and was reduced in ARDS sepsis patients compared to non-ARDS sepsis patients ( $P<.001$ ) as well (Fig. 1B). Moreover, MIR103 expression was positively correlated with MIR107 expression in HCs ( $P<.001$ ,  $r=0.303$ ) (Fig. 1C), non-ARDS sepsis patients ( $P<.001$ ,

$r=0.422$ ) (Fig. 1D) and ARDS sepsis patients ( $P=.001$ ,  $r=.587$ ), respectively (Fig. 1E).

### 3.3. Predictive value of MIR103 and MIR107 for ARDS in sepsis patients

Reduced MIR103 expression predicted increased ARDS risk in sepsis patients (AUC: 0.727, 95% CI: 0.619–0.835). The sensitivity and specificity were 64.3% and 78.6%, respectively at the best cut-off point (the point on the curve where the sum of sensitivity and specificity was maximized) with MIR103 expression of 0.178 (Fig. 2A). Meanwhile, decreased MIR107 expression also predicted enhanced ARDS risk in sepsis patients (AUC: 0.694, 95% CI: 0.577–0.811). The sensitivity and specificity were 85.7% and 51.2%, respectively at the best cut-off point with MIR107 expression of 0.295 (Fig. 2B). Additionally, the specificities of MIR103 and MIR107 were 54.8% and 60.1% respectively when the sensitivities were adjusted to 75.0% (data not shown).

### 3.4. Correlation of MIR103 and MIR107 expressions with clinical parameters in sepsis patients

MIR103 expression was negatively correlated with APACHE II score ( $P<.001$ ,  $r=-0.384$ ), SOFA score ( $P<.001$ ,  $r=-0.340$ ), Scr ( $P<.001$ ,  $r=-0.305$ ), CRP ( $P<.001$ ,  $r=-0.457$ ), TNF ( $P<.001$ ,  $r=-0.325$ ), IL1B ( $P<.001$ ,  $r=-0.256$ ), IL6 ( $P<.001$ ,  $r=-0.366$ ) and IL8 ( $P<.001$ ,  $r=-0.346$ ), while positively correlated with albumin ( $P=.032$ ,  $r=0.153$ ). Similarly, MIR107 expression was negatively correlated with APACHE II score ( $P<.001$ ,  $r=-0.506$ ), SOFA score ( $P<.001$ ,  $r=-0.426$ ), Scr ( $P<.001$ ,  $r=-0.264$ ), CRP ( $P<.001$ ,  $r=-0.368$ ), TNF ( $P<.001$ ,  $r=-0.247$ ), IL1B ( $P<.001$ ,  $r=-0.346$ ), IL6 ( $P<.001$ ,  $r=-0.260$ ) and IL8 ( $P<.001$ ,  $r=-0.302$ ), while positively correlated with albumin ( $P<.001$ ,  $r=0.402$ ). However, no correlation was found in MIR103 or MIR107 expression with WBC (both  $P>.05$ ) in sepsis patients (Table 2).

### 3.5. Correlation of ARDS with 28-day mortality in sepsis patients

In sepsis patients, there were 134 (68.4%) survivors and 62 (31.6%) deaths (Fig. 3A). The 28-day mortality rate was increased in ARDS sepsis patients (16 (57.1%)) compared to non-ARDS patients (46 (27.4%)) ( $P=.002$ ) (Fig. 3B).

### 3.6. Correlation of MIR103 and MIR107 expressions with 28-day mortality in sepsis patients

MIR103 expression was decreased in deaths (median value: 0.254 (0.074–0.374)) compared to survivors (median value: 0.375 (0.214–0.664)) ( $P<.001$ ) (Fig. 4A). And MIR107 expression was also decreased in deaths (median value: 0.184 (0.128–0.324)) compared to survivors (median value: 0.328 (0.156–0.499)) ( $P=.001$ ) (Fig. 4B). Moreover, decreased MIR103 expression (AUC: 0.704, 95% CI: 0.626–0.782) and MIR107 expression (AUC: 0.649, 95% CI: 0.569–0.729) predicted increased 28-day mortality risk in sepsis patients (Fig. 4C). In addition, for predicting 28-day mortality risk by ROC curves, the AUC values of MIR103 (0.704, 95% CI: 0.626–0.782) and MIR107 (0.649, 95% CI: 0.569–0.729) were numerically decreased compared to APACHE II score (AUC:

**Table 1**  
**Clinical characteristics.**

Items	HCs (N = 196)	Non-ARDS sepsis patients (n = 168)	ARDS sepsis patients (n = 28)	P value among groups	P value (ARDS vs non-ARDS)
Age (years), mean ± SD	57.5 ± 11.9	57.1 ± 11.0	61.4 ± 12.1	.183	.059
Gender, No. (%)				.289	.603
Female	79 (40.3)	55 (32.7)	9 (32.1)		
Male	117 (59.7)	113 (67.3)	19 (67.9)		
BMI (kg/m <sup>2</sup> ), mean ± SD	22.5 ± 3.1	22.6 ± 3.9	23.3 ± 4.0	.523	.338
Biochemical index, median (IQR)					
Scr (mg/dl)	0.8 (0.7–1.0)	1.7 (1.2–2.3)	1.8 (1.2–3.2)	<.001	.351
Albumin (g/L)	42.7 (39.6–45.8)	27.1 (22.4–36.4)	25.4 (20.2–34.9)	<.001	.331
WBC (×10 <sup>9</sup> /L)	6.6 (5.7–7.7)	11.7 (2.9–26.5)	18.3 (4.7–30.0)	<.001	.364
CRP (mg/L)	3.8 (2.5–6.3)	99.8 (55.5–151.4)	152.1 (65.6–299.8)	<.001	.006
Smoke, No. (%)	–	50 (29.8)	16 (57.1)	–	.005
Chronic comorbidities, No. (%)					
COPD	–	21 (12.5)	8 (28.6)	–	.027
Cardiomyopathy	–	55 (32.7)	12 (42.9)	–	.296
Chronic kidney failure	–	12 (7.1)	6 (21.4)	–	.015
Cirrhosis	–	33 (19.6)	4 (14.3)	–	.502
APACHE II score, mean ± SD	–	13.3 ± 6.1	18.1 ± 4.7	–	<.001
SOFA score, mean ± SD	–	6.0 ± 2.6	7.9 ± 2.8	–	<.001
Inflammatory cytokines, median (IQR)					
TNF (pg/ml)	–	202.7 (123.7–306.7)	299.4 (192.7–417.7)	–	.006
IL1B (pg/ml)	–	8.5 (3.9–16.9)	22.2 (8.3–40.3)	–	<.001
IL6 (pg/mL)	–	85.4 (51.9–168.2)	114.1 (52.6–240.6)	–	.133
IL8 (pg/ml)	–	118.5 (57.9–189.5)	178.4 (89.5–208.2)	–	.078
Antibiotic therapy, No. (%)					
Cephalosporin	–	77 (45.8)	9 (32.1)	–	.177
Gentamicin	–	61 (36.3)	11 (39.3)	–	.672
Tobramycin	–	41 (24.4)	4 (14.3)	–	.239
Penicillin	–	33 (19.6)	8 (28.6)	–	.282
Amikacin	–	33 (19.6)	6 (21.4)	–	.827
Fluconazole	–	17 (10.1)	2 (7.1)	–	.622
Flucytosine	–	17 (10.1)	2 (7.1)	–	.622
Netilmicin	–	16 (9.5)	5 (17.9)	–	.187
Vancomycin	–	16 (9.5)	3 (10.7)	–	.844
Fluoroquinolone	–	14 (8.3)	3 (10.7)	–	.679
Oxacillin	–	11 (6.5)	3 (10.7)	–	.428

APACHE II = acute pathologic and chronic health evaluation II, ARDS = acute respiratory distress syndrome, BMI = body mass index, COPD = chronic obstructive pulmonary disease, CRP = C-reactive protein, HCs = health controls, IL1B = interleukin 1 $\beta$ , IL6 = interleukin 6, IL8 = interleukin 8, IQR = interquartile range, Scr = serum creatinine, SD = standard deviation, SOFA = sequential organ failure assessment, TNF = tumor necrosis factor, WBC = white blood cell.

0.790, 95% CI: 0.724–0.856), SOFA score (AUC: 0.748, 95% CI: 0.671–0.825)) (Fig. 4D) and Scr (AUC: 0.778, 95% CI: 0.710–0.847), while were similar to that of albumin (AUC: 0.606, 95% CI: 0.517–0.695), WBC (AUC: 0.614, 95% CI: 0.535–0.693), CRP (AUC: 0.724, 95% CI: 0.647–0.802)) (Fig. 4E), TNF (AUC: 0.641, 95% CI: 0.560–0.722), IL1B (AUC: 0.742, 95% CI: 0.672–0.813), IL6 (AUC: 0.633, 95% CI: 0.553–0.714), and IL8 (AUC: 0.647, 95% CI: 0.568–0.726)) (Fig. 4F).

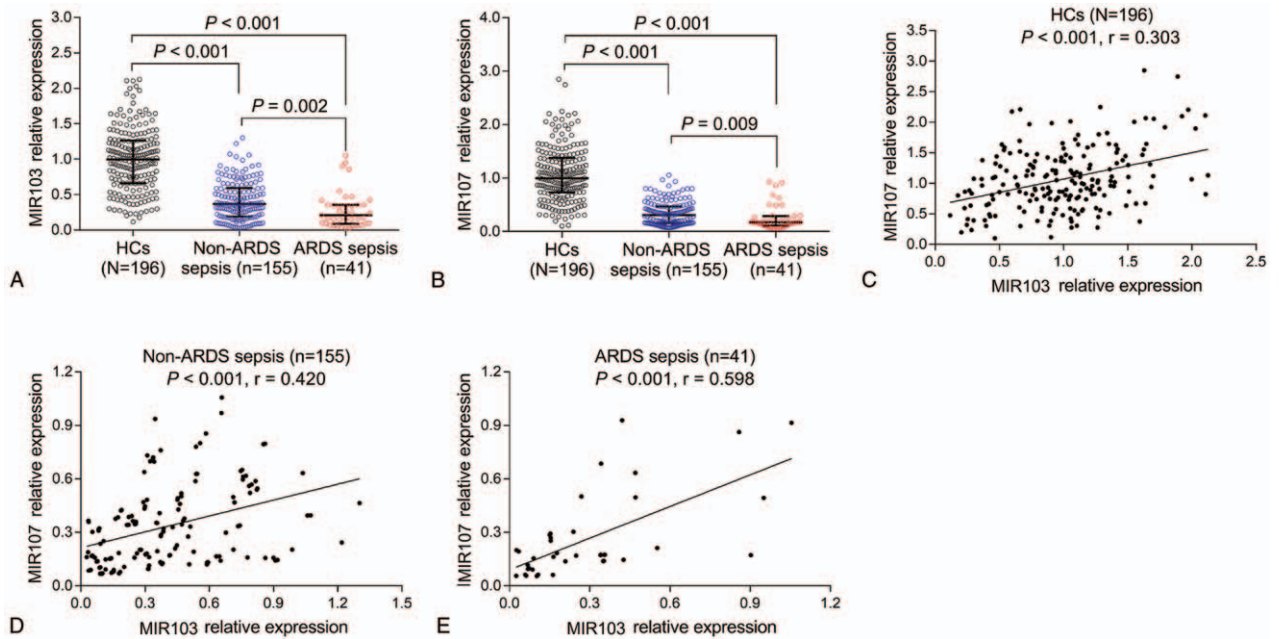
#### 4. Discussion

Several previous laboratory investigations have reported that MIR103 and MIR107 play important roles in LPS-induced septic organ injury and inflammation. For example, it is proposed that MIR103 expression is dysregulated in LPS treated human kidney proximal tubular cell line HK-2, and overexpression of MIR103 affects cell proliferation and apoptosis by regulating the nuclear factor-kappa B (NF- $\kappa$ B) pathway and signal transducer and activator of transcription (STAT) pathway.<sup>[10]</sup> Meanwhile, in septic acute kidney injury mouse model, MIR107 is abnormally

expressed and affect the kidney injury through the regulation of TNF secretion.<sup>[14]</sup> Moreover, it is proposed that MIR103 and MIR107 are vital regulators in inflammation via several inflammation-related pathways such as the mitogen-activated protein kinase (MAPK)/extracellular regulated protein kinases (ERK) pathway, the Notch signaling pathway and the NF- $\kappa$ B pathway.<sup>[12,13,24]</sup> Collectively, these studies reveal that MIR103 and MIR107 play important roles in septic organ injury and inflammation, implying that they might be critical regulators in sepsis.

Clinically, the role of miRNAs as biomarkers in sepsis patients has been reported by previous researches.<sup>[25]</sup> For example, miR-25 shows great predictive value for increased sepsis risk,<sup>[26]</sup> and miR-122 predicts increased liver injury in sepsis patients.<sup>[27]</sup> However, the role of MIR103 and MIR107 in sepsis patients is yet to be clarified to the best of our acknowledge. Based on the above-mentioned studies that both MIR103 and MIR107 are vital regulators in septic organ injury and inflammation, we hypothesized that MIR103 and MIR107 might be important in sepsis patients. In the present study, both of the miRNA expressions were decreased in ARDS sepsis patients and non-ARDS sepsis patients





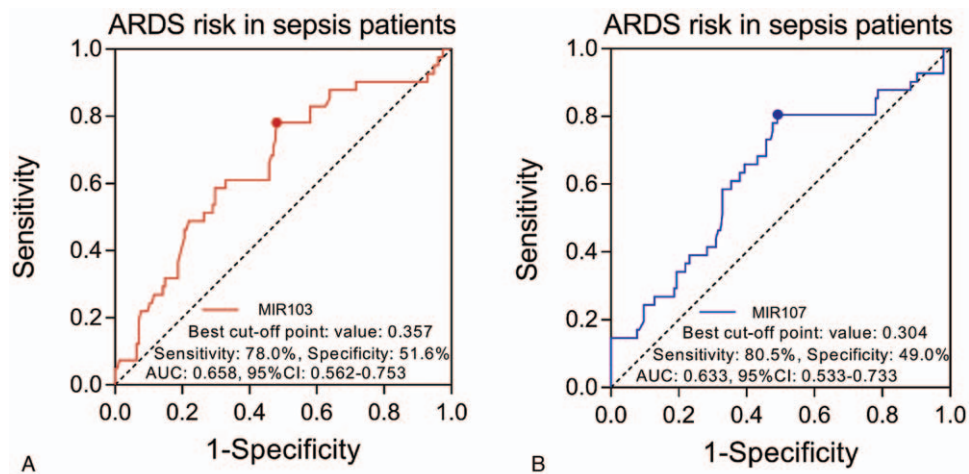
**Figure 1.** MIR103 and MIR107 expressions. A: MIR103 expression in ARDS sepsis patients, non-ARDS sepsis patients and HCs. B: MIR107 expression in ARDS sepsis patients, non-ARDS sepsis patients and HCs. C: Correlation between MIR103 and MIR107 expression in HCs. D: Correlation between MIR103 and MIR107 expression in non-ARDS sepsis patients. E: Correlation between MIR103 and MIR107 expression in ARDS sepsis patients. MiR, microRNA; HCs, healthy controls; ARDS, acute respiratory distress syndrome.

compared to HCs, and were further decreased in ARDS sepsis patients compared to non-ARDS sepsis patients. Moreover, both decreased MIR103 and MIR107 expressions predicted increased ARDS risk in sepsis patients. Possible explanations of these data might be that:

1. Reduced MIR103 and MIR107 might aggravate organ injury (as presented by previous researches<sup>[10,28]</sup>), thus increased the incidence of sepsis. Therefore, MIR103 and MIR107 expressions were decreased in sepsis patients compared to HCs.

2. Decreased MIR103 and MIR107 expressions might regulate several survival-related pathways such as MAPK/ERK pathway (as in PC12 cells<sup>[24]</sup>) to exacerbate the injury of epithelial and endothelial cells of multiple organs especially lung, which caused ARDS in sepsis patients.

3. Reduced MIR103 and MIR107 expressions might enhance systematic inflammation through activation of inflammation-related pathways such as the NF-κB pathway (as reported in HK-2 cells), which increased lung injury and caused ARDS in sepsis patients.



**Figure 2.** MIR103 and MIR107 expressions in discrimination of ARDS sepsis patients from non-ARDS sepsis patients. A: MIR103 expression in discrimination of ARDS sepsis patients from non-ARDS sepsis patients. B: MIR107 expression in discrimination of ARDS sepsis patients from non-ARDS sepsis patients. MiR, microRNA; ARDS, acute respiratory distress syndrome; AUC, area under curve; CI, confidence interval.

**Table 2**  
Correlation of MIR103/MIR107 with clinical/biochemical indexes in sepsis patients.

Items	MIR103		MIR107	
	P value	Correlation coefficient (r)	P value	Correlation coefficient (r)
APACHE II score	<.001	-0.384	<.001	-0.506
SOFA score	<.001	-0.340	<.001	-0.426
Scr	<.001	-0.305	<.001	-0.264
Albumin	.032	0.153	<.001	0.402
WBC	.264	-0.080	.108	-0.115
CRP	<.001	-0.457	<.001	-0.368
TNF	<.001	-0.325	<.001	-0.247
IL1B	<.001	-0.256	<.001	-0.346
IL6	<.001	-0.366	<.001	-0.260
IL8	<.001	-0.346	<.001	-0.302

APACHE II = acute pathologic and chronic health evaluation II, CRP = C-reactive protein, IL1B = interleukin 1 $\beta$ , IL6 = interleukin 6, IL8 = interleukin 8, MIR = microRNA, Scr = serum creatinine, SOFA = sequential organ failure assessment, TNF = tumor necrosis factor, WBC = white blood cell.

Therefore, MIR103 and MIR107 expressions were decreased in ARDS sepsis patients compared to non-ARDS sepsis patients and whose reduced expressions predicted increased ARDS risk in sepsis patients.

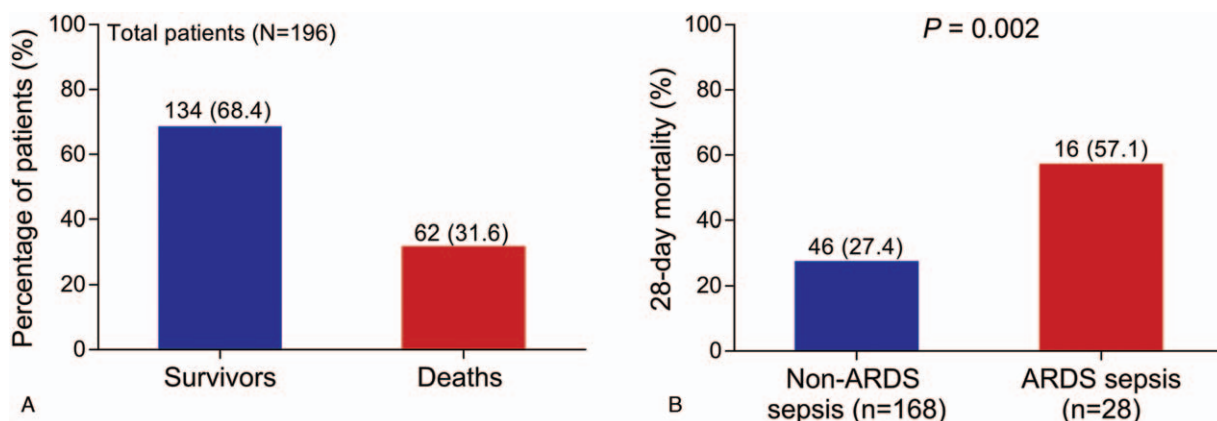
In the present study, the correlations of MIR103 and MIR107 expressions with clinical characteristics of sepsis patients were investigated. We found that both of the miRNA expressions were negatively correlated with APACHE II score, SOFA score, Scr, CRP, and pro-inflammatory cytokines including TNF, IL1B, IL6, and IL8, while positively correlated with albumin, indicating that MIR103 and MIR107 expressions were associated with elevated systematic inflammation, increased level of multiple organ injury and enhanced disease severity in sepsis patients. These data could be explained by that:

1. Decreased MIR103 and MIR107 expressions might activate the NF- $\kappa$ B pathway to enhance inflammation (as in HK-2 cells<sup>[10]</sup>), therefore, they were negatively correlated with CRP and pro-inflammatory cytokines in sepsis patients.
2. Reduced MIR103 and MIR107 might exacerbate multiple organ damage including liver and kidney (mentioned above), therefore, they were negatively correlated with SOFA score and Scr while positively correlated with albumin in sepsis patients.

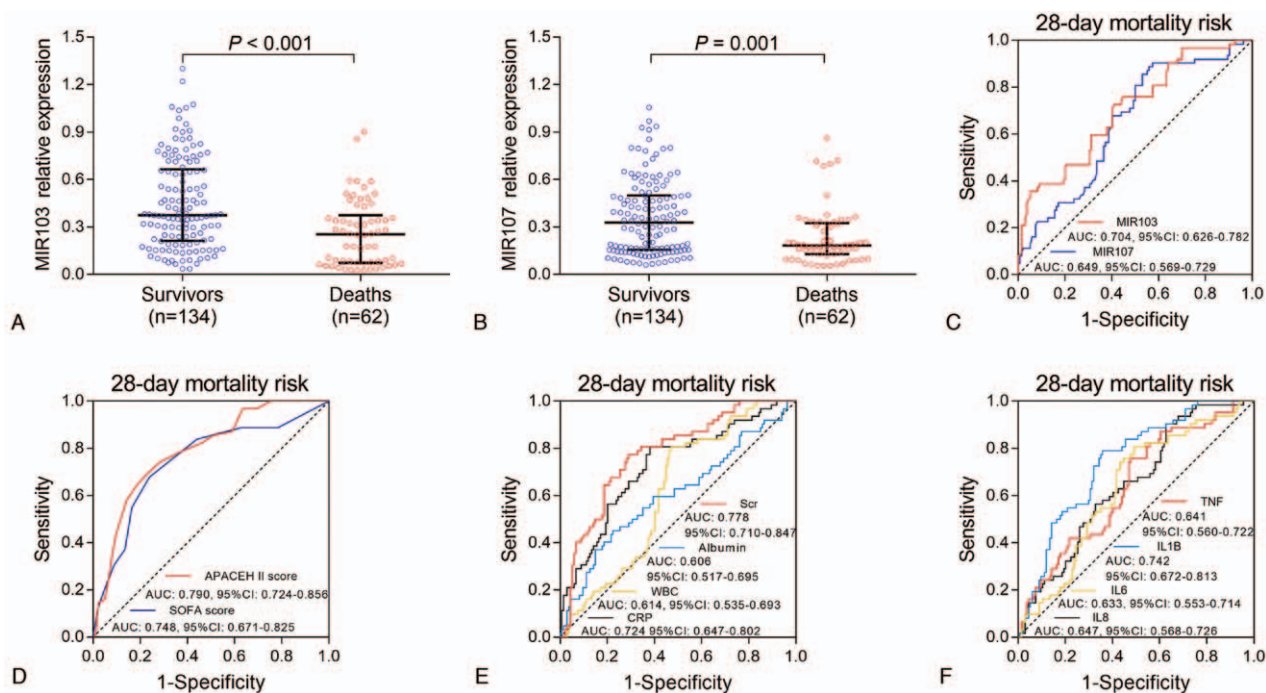
3. Decreased MIR103 and MIR107 expressions might aggravate systematic inflammation and accelerate organ damage (mentioned above) to enhance the disease severity of sepsis patients. Therefore, both MIR103 and MIR107 expressions were negatively correlated with APACHE II score in sepsis patients.

As to the correlation of MIR103 and MIR107 expressions with the prognosis of sepsis patients, we discovered that both miRNA expressions were decreased in deaths compared to survivors. Moreover, decreased MIR103 and MIR107 expressions displayed good predictive values for increased 28-day mortality risk of sepsis patients. These data could be explained by the negative correlation of MIR103 and MIR107 expressions with disease severity, which resulted in a worse prognosis of sepsis patients. Interestingly, we found that the predictive value of MIR103 and MIR107 expressions for 28-day mortality of sepsis patients were not inferior to that of several biochemical indexes (including albumin, CRP, WBC, and pro-inflammatory cytokines), indicating the potential of MIR103 and MIR107 as promising prognostic biomarkers in sepsis, which might improve the management and prognosis of sepsis patients.

This study existed several limitations:



**Figure 3.** 28-day mortality of sepsis patients. A: Deaths and survivors of sepsis patients. B: Comparison of 28-day mortality between ARDS sepsis patients and non-ARDS sepsis patients. ARDS, acute respiratory distress syndrome.



**Figure 4.** Evaluation of the prognostic value of MIR103 and MIR107 in sepsis patients. A: MIR103 expression in deaths and survivors of sepsis patients. B: MIR107 expression in deaths and survivors of sepsis patients. C: Predictive value of MIR103 and MIR107 expressions for 28-day mortality risk in sepsis patients. D: Predictive value of APACHE II score and SOFA score for 28-day mortality risk in sepsis patients. E: Predictive value of biological indexes for 28-day mortality risk in sepsis patients. F: Predictive value of inflammatory cytokines for 28-day mortality risk in sepsis patients. MIR, microRNA; AUC, area under curve; CI, confidence interval; APACHE, acute pathologic and chronic health evaluation; SOFA, sequential organ failure assessment; Scr, serum creatinine; WBC, white blood cell; CRP, C-reactive protein; TNF, tumor necrosis factor; IL1B, interleukin 1 $\beta$ ; IL6, interleukin 6; IL8, interleukin 8.

1. In the present study, decreased MIR103 and MIR107 predicted increased ARDS risk in sepsis patients; however, the molecular mechanisms of MIR103 and MIR107 in sepsis-induced lung injury were not explored, which needed further investigation.
2. Our data revealed that MIR103 and MIR107 expressions were negatively correlated with SOFA score and Scr, while positively correlated with albumin, which implied that these 2 miRNAs might play important roles in sepsis-induced kidney injury and liver injury. However, the specific mechanisms of MIR103 and MIR107 in sepsis-induced kidney or liver injury were not explored in this study.
3. The correlation of MIR103 and MIR107 expressions with septic shock was not investigated in this study for it was not the main objective.
4. In this study, the use of antibiotics might cause bias in the correlation of ARDS, MIR103 and MIR107 with 28-day mortality in sepsis patients.
5. In this study, we plotted the data calculated by the  $2^{-\Delta\Delta C_t}$  method rather than plotted them after logarithm transformation, which might cause bias in the upregulation of MIR103 as well as MIR107, thereby potentially overstating the clinical significance of MIR103 and MIR107 in sepsis and ARDS sepsis patients.

To be collective, decreased MIR103 and MIR107 predicted increased ARDS risk, and were correlated with unfavorable clinical parameters and worse prognosis in sepsis patients. This study identified MIR103 and MIR107 as potential biomarkers to predict the ARDS risk and mortality risk of sepsis patients, which

might improve the management of sepsis patients to enhance their prognosis.

### Author contributions

Conception and design: Qianqian Wang and Qiang Feng; Provision of study materials or patients: Yanmin Zhang, Shaoying Zhou and Huimin Chen; Collection and assembly of data: Yanmin Zhang, Shaoying Zhou and Huimin Chen; Data analysis and interpretation: Qianqian Wang and Qiang Feng; Manuscript writing: All authors. Final approval of manuscript: All authors.

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