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Exploration of Values of MiR-7110-5p and MiR-223-3p in Predicting Sepsis

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ABSTRACT

The study aimed to explore the values of micro ribonucleic acid (miR)-7110-5p and miR-223-3p in predicting sepsis secondary to pneumonia. MiRNAmicroarray was utilized to analyze the expression difference of miRNA in patients with pneumonia and sepsis secondary to pneumonia. A total of 50 patients with pneumonia and 42 patients with sepsis secondary to pneumonia were included. Then quantitative polymerase chain reaction (qPCR) was carried out to detect the expression level of circulating miRNAs in patients and analyze its correlations with clinical characteristics and prognosis. 9 miRNAs, namely, hsa-miR-4689-5p, hsa-miR-4621-5p, hsa-miR-6740-5p, hsa-miR-7110-5p, hsa-miR-765, hsa-miR-940,hsa-miR-213-5p, hsa-miR-223-3p and hsa-miR-122, met the screening criteria of fold change ≥2 or <0.5 and p<0.01. The expression levels of miR-4689-5p and miR-4621-3p were different between the two groups of patients, which were up-regulated in the plasma of patients with sepsis secondary to pneumonia. The expression levels of miR-7110-5p and miR-223-3p in patients with pneumonia and sepsis were higher than those in healthy controls. Besides, the area under curve (AUC) of the receiver operating characteristic (ROC) curve of miR-7110-5p for predicting pneumonia and sepsis secondary to pneumonia was 0.78 and 0.863, respectively, while that of miR-223-3p for predicting pneumonia and sepsis secondary to pneumonia was 0.879 and 0.924, respectively. However, there were no significant differences in the levels of miR-7110-5p and miR-223-3p in plasm between survived and dead patients with sepsis. MiR-7110-5p and miR-223-3p can serve as potential biological indicators for predicting sepsis secondary to pneumonia.

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Introduction

Sepsis is a common complication secondary to severe trauma, shock, infection and surgery (1). It may lead to septic shock and multiple organ dysfunction syndrome (MODS), and it is also the main cause of death in critically ill patients. Owing to the high incidence and death rates, sepsis is still a great challenge in clinical medicine. In developed countries, the incidence rate of sepsis reaches 100/100,000 (2,3), and about 2% of hospitalized patients are diagnosed during admission (4,5). As medicine advances, evidence-based medicine has been extensively applied to treat sepsis and related diseases in clinical practice. However, approximately 1/5-1/2 of the patients still suffer from MODS sepsis (6, 7).

Micro ribonucleic acids (miRNAs) are endogenous non-coding small RNAs with a length of about 21-25 nucleotides (8). Researchers discovered the first miRNA, namely, (lin-4)miRNA, in a new species of Caenorhabditis elegans in 1993, since which numerous miRNAs have been discovered. MiRNAs are able to suppress post-transcriptional gene expression or facilitate targeted mRNA degradation, but they cannot encode proteins. Besides, they can be detected in different liquids (such as blood, sweat and urine). It has been reported that many miRNAs are abnormally expressed and regulated in the blood of patients with inflammatory/infectious diseases (9), indicating that circulating miRNAs may be suitable biomarkers for sepsis.

Nevertheless, there are still few reports on the significance of miRNAs in patients with respiratory tract infection, especially in patients with sepsis secondary to pneumonia. Therefore, this study aimed to determine the difference in the miRNA expression between patients with pneumonia and those with sepsis secondary to pneumonia, to investigate the clinical value of miRNA in predicting sepsis secondary to pneumonia.

Materials and Methods

Clinical data

To compare miRNA expression differences between patients with pneumonia and those with sepsis secondary to pneumonia, the plasmas of 3 patients with pneumonia and 3 patients with sepsis secondary to pneumonia were screened using miRNA microarray (Aksomics Inc., Shanghai). Then miRNA level differences between sepsis secondary to pneumonia group (sepsis group) and control group were examined via fluorescence quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Pneumonia was diagnosed according to the guidelines for the diagnosis and treatment of hospital-acquired pneumonia (HAP) and community-acquired pneumonia (CAP) drafted by the American College of Chest Physicians and the American Society of Infectious Diseases in 2007 and 2016, and sepsis secondary to pneumonia was diagnosed

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Table 1. MiRNA and primer sequences.

MiRNA	MiRNA sequence	Forward sequence
hsa-miR-4689-5p	AGUGGACCGAGGAAGGAAGGA	AGTGGACCGAGGAAGGAAGGA
hsa-miR-4621-5p	CAGCAGGGGAGAGAGAGGAGUC	CAGCAGGGGAGAGAGAGGAGTC
hsa-miR-6740-5p	AGUUUGGGAUGGAGAGAGGAGA	AGTTTGGGATGGAGAGAGGAGA
hsa-miR-7110-5p	UGGGGGUGUGGGGAGAGAGAG	TGGGGGTGTGGGGAGAGAGAG
hsa-miR-765	UGGAGGAGAAGGAAGGUGAUG	TGGAGGAGAAGGAAGGTGATG
hsa-miR-940	AAGGCAGGGCCCCCGCUCCCC	AAGGCAGGGCCCCCGCTCCCC
hsa-miR-213-5p	UCUCCCAACCCUUGUACCAGUG	TCTCCCAACCCTTGTACCAGTG
hsa-miR-223-3p	UGUCAGUUUGUCAAAUACCCCA	UGUCAGUUUGUCAAAUACCCCA
hsa-miR-122	UGGAGUGUGACAAUGGUGUUUG	TGGAGTGTGACAATGGTGTTTG
Common miRNA primer		GATCCAGTCTCAGGGTCCGAG

based on Sepsis 3.0 guidelines. Briefly sepsis = infection + sequential organ failure assessment (SOFA)≥2. Three respiratory specialists diagnosed each case independently, and the cases with consistent diagnosis results were included for analysis. This study was approved by the Ethics Committee of The Third Hospital of Hebei Medical University, and all patients or their guardians signed the informed consent. Patients were excluded according to the following criteria:1) patients at the age <18 years old, 2) patients who died within 24 h after admission, 3) patients with neutrophil count $\leq 0.5\times10^9$/L, 4) patients with HIV/AIDS, or 5) patients unwilling to participate in this study.

MiRNA microarray analysis

MiRNA microarray (Aksomics) provided data from miRNA database (miRBase) 21.0, containing 1721 human-related miRNAs. Lateral evaluation of microarray quality indicated that it was stable. Besides, cluster analysis and biological repeated sample correlation analysis revealed that samples in pneumonia group and sepsis group exhibited good correlations.

Detection via qPCR

As per the manufacturer's instructions, total RNA was extracted using TRIzol (Life Technologies), and cDNA was synthesized using the miScript II RT kit (Qiagen) for miRNA analysis. During messenger RNA (mRNA) analysis, cDNA was synthesized using SuperScript III First-Strand Synthesis System (Life Technologies). Thereafter, iTaq Universal SYBR Green Supermix (Bio-Rad) and specific gene primers synthesized by Integrated DNA Technologies (Table 1) were used for qRT-PCR analysis on CFX Connect real-time PCR detection system (Bio-Rad). Finally, the relative quantitative expression of a single miRNA was determined in 3 independent wells using ΔΔCt method, and the miRNA expression was a multiple difference relative to U6.

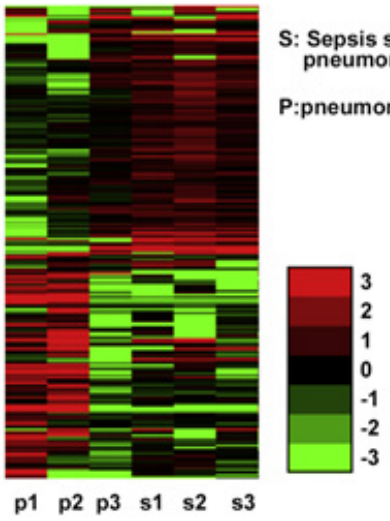

Figure 1. Heat map of miRNA differential expressions. In the sepsis group, the expression levels of miR-765, miR-7110-5p and miR-223-3p are up-regulated, while the expression level of miR-940 is down-regulated(p<0.01).

Statistical methods

SPSS 18.0 was applied for statistical data analysis. In univariate analysis, data with normal and non-normal distributions and count data were evaluated by t-test, Mann-Whitney test and χ^2 test, respectively. p<0.05 indicated that the difference was statistically significant. According to the area under curve (AUC) of the receiver operating characteristic (ROC) curve, the specificity and sensitivity of miRNA in the diagnosis of sepsis were calculated.

Results

Microarray screening

MiRNA microarray analysis results showed that a total of 9 miRNAs, namely, hsa-miR-4689-5p, hsa-miR-4621-5p, hsa-miR-6740-5p, hsa-miR-7110-5p, hsa-miR-765, hsa-miR-940,hsa-miR-213-5p, hsa-miR-223-3p and hsa-miR-122, met the screening criteria of the multiple change ≥2 or <0.5 and p<0.01. Among these miRNAs, the expression of miR-940 was down-regulated in the sepsis group (Figure 1). Two miRNAs, namely miR-223-3p and miR-7110-5p, in the sepsis group also met the screening criteria and were up-regulated.

Expression levels of 9 miRNAs in patients with pneumonia and sepsis secondary to pneumonia

As shown in Table 2, there were 50 patients with pneumonia, including 21 males and 29 females, with an average age of (51.10±16.15) years old, of which 32 were diagnosed with CAP and 18 (34.1%) were diagnosed with HAP/ventilator-associated pneumonia (VAP). In addition, there were 42 patients with sepsis secondary to pneumonia, consisting of 24 males and 18 females aged19.83 years old through the diagnosis based on Sepsis 3.0 criteria, among which 38 (95.5%) had definite etiology. Specifically, sepsis was caused by bacteria, viruses, aspergillus and various pathogens in them. Furthermore, the ΔCt values of 9 miR-

Table 2. Clinical characteristics of patients with pneumonia and sepsis secondary to pneumonia.

	Pneumonia (n)	Sepsis secondary to pneumonia (n)	*p*
Gender male	21	24	0.072
Female	29	18	
Complicated with more than one basic disease	6	34	0.000
SOFA score		6.67±2.11	
SOFA score above baseline		5.54±2.52	
Prognostic survival	50	21	
Death	0	21	
Major potential complications Diabetes mellitus	0	11	
Acute cerebral infarction or cerebral hemorrhage	1	8	
Chronic renal insufficiency	2	7	
Coronary heart disease	1	5	
Autoimmune disease	0	3	

Table 3. Expression levels of 9 miRNAs in patients with pneumonia and sepsis secondary to pneumonia.

	Pneumonia (ΔCt)	Sepsis secondary to pneumonia (ΔCt)	*p*
hsa-miR-4689-5p	2.32±1.16	1.51±1.439	0.003
hsa-miR-4621-5p	4.32±2.26	2.17±2.54	0.002
hsa-miR-6740-5p	3.31±1.94	2.12±1.26	0.543
hsa-miR-7110-5p	6.28±2.71	6.15±2.43	0.247
hsa-miR-765	4.11±1.98	4.56±1.21	0.312
hsa-miR-940	-2.01±1.77	-2.13±1.10	0.412
hsa-miR-213-5p	2.50±2.13	2.15±2.17	0.212
hsa-miR-223-3p	5.12±3.10	6.21±3.41	0.123
hsa-miR-122	5.41±3.52	5.41±2.67	0.468

NAs in patients with pneumonia and sepsis secondary to pneumonia were analyzed. QRT-PCR results revealed that the expression levels of miR-4689-5p and miR-4621-3p were different between the two groups of patients, and they were up-regulated in the plasma of patients with sepsis secondary to pneumonia. Besides, the expression levels of miR-7110-5p and miR-223-3p in patients with pneumonia and sepsis were higher than those in healthy controls (Table 3).

Identification of the indicators for the diagnosis and prognosis of sepsis

The AUC of miR-7110-5p ROC for predicting pneumonia was 0.781 (Figure 2). 4). When the threshold was 3.54, the sensitivity and specificity of miR-7110-5p were 62.3% and 100%, respectively. The AUC of miR-7110-5p ROC for predicting sepsis secondary to pneumonia was 0.863 (Figure 2). In the case of the cutoff value of 4.39, the sensitivity and specificity of miR-7110-5p were 81.2% and 89.6%, respectively. The AUC of miR-223-3p ROC for predicting pneumonia was 0.879 (Figure 3). When the threshold was 3.152, the sensitivity and specificity of miR-223-3p were 82.7% and 90.8%, separately. Moreover, the AUC of miR-223-3p ROC for the prediction of sepsis secondary to pneumonia was 0.924 (Figure 3). Under the threshold of 2.532, the sensitivity and specificity of miR-223-3p were 81.9% and 98.4%, respectively.

Comparison of curative effects of patients

Of the 42 patients with sepsis secondary to pneumonia, 20 survived (54.3%) and 18 died (45.7%). Besides, there were no significant differences in the levels of miR-7110-5p and miR-223-3p in plasma between survived and dead patients with sepsis (Table 4).

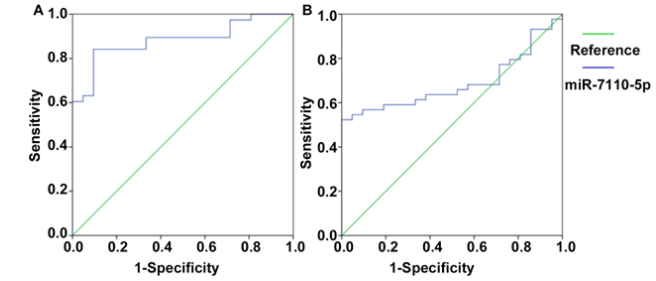

Figure 2. ROC curve of miR-7110-5p. A: AUC of miR-7110-5p ROC used to predict pneumonia is 0.78. B: AUC of miR-7110-5p ROC for predicting sepsis secondary to pneumonia is 0.863.

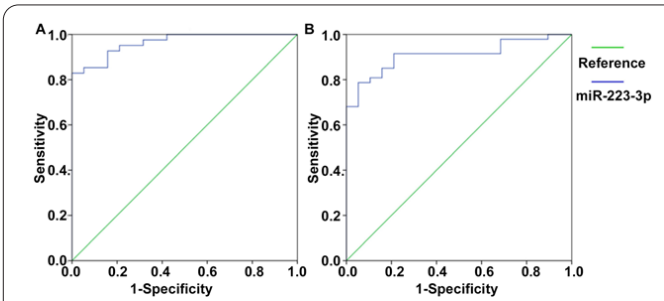

Figure 3. ROC curve of miR-223-3p. Left: AUC of miR-223-3p ROC that predicts pneumonia is 0.879. Right: AUC of miR-223-3p ROC for predicting sepsis secondary to pneumonia is 0.924.

Discussion

MiRNAs circulating in human peripheral blood have been utilized as biomarkers for various cancers since dis-

Table 4. △CT of miR-223-3p and miR-7110-5p between dead and survived patients with sepsis.

△Ct	Survived (n=20)	Dead (n=18)	*p*
miR-223-3p	1.23±1.12	0.91±0.31	0.076
miR-7110-5p	2.49±1.12	2.11±0.67	0.521

covery (10). Currently, it has been identified that several circulating miRNAs act as potential biomarkers for sepsis, but their values in infectious diseases have rarely been researched. The correlation between miRNAs and sepsis diagnosis has been studied, and some controversies also exist (11-13). Causes of sepsis vary, including severe wounds, burns, shock, infection and surgical operations, and these causes have similarities and differences. The functions of miRNAs in sepsis secondary to pneumonia and their relationship with sepsis diagnosis have not been investigated in any studies.

In this study, the expression levels of miR-7110-5p and miR-223-3p differed in patients with pneumonia and sepsis secondary to pneumonia, and they are up-regulated in the plasma of patients with sepsis secondary to pneumonia. Besides, the AUC of miR-7110-5p and miR-223-3p ROCs was applied to predict the survived and dead patients with sepsis.

In addition, in this study, gene microarray analysis displayed that 9 miRNAs, namely, hsa-miR-4689-5p, hsa-miR-4621-5p, hsa-miR-6740-5p, hsa-miR-7110-5p, hsa-miR-765, hsa-miR-940,hsa-miR-213-5p, hsa-miR-223-3p and hsa-miR-122, met the screening criteria of $p<0.01$, multiple change ≥2 or <0.5, and copy number >50. To some extent, the data were limited. Reasons for determining the correlations of the 9 miRNAs with are as follows: 1) Causes of sepsis are various, so there is still a need to study the relationship between sepsis secondary to pneumonia and sepsis diagnosis (14, 15). 2) Different from previous studies, Sepsis3.0 was utilized for the diagnosis of sepsis in this study. 3) The sample size in gene microarray analysis is limited. 4 miRNAs related to sepsis previously reported were verified by qRT-PCR analysis to make up for the limitation of this research.

In this study, it was found that miR-223-3p was highly expressed in the circulating blood of patients with sepsis secondary to pneumonia. Wang et al (16) compared the levels of miR-223 in 50 patients with sepsis, those with systemic inflammatory response syndrome (SIRS) and healthy controls. They found that the expression level of miR-223 in the blood of patients with sepsis and SIRS caused by infection is increased, but it is decreased in the blood of patients with non-infectious SIRS. Therefore, miR-223 can be used as a biomarker to distinguish infectious SIRS from non-infectious SIRS. A cohort study(17) involving septic children and healthy children showed that the expression levels of miR-223 and miR-146a are remarkably raised, and the increased miR-223 level is positively correlated with the high level of tumor necrosis factor-α, disease severity and poor prognosis, but these conclusions are inconsistent with those in other published reports. Clinical research on non-infectious critical patients and septic patients reveals no significant difference exists in miR-223 level (18-20). A previous study on the efficacy of miR-223-3p in predicting sepsis secondary to pneumonia manifested that miR-223-3p expression level can be used as an accurate predictive indicator for the diagnosis of sepsis (21). In this study, the expression of miR-7110-5p that was rarely detectedwas up-regulated in the circulating blood of patients with sepsis secondary to pneumonia. However, the signal changes of miR-7110-5p are triggered by both pneumonia or sepsis and diseases caused by them. In addition, 14 miRNAs are differentially expressed in cancer stem cells, $CD133^{(+)}$ A549 cells and $CD133^{(-)}$ cells. Among these miRNAs, five (hsa-miR-23b-3p, -23a-3p, -15b-5p, -24-3p and -4734) are up-regulated, while nine (hsa- miR-1246, -30b -5p, 5096, 6510-5p, has-miR-7110-5p, 7641, 3197, 7108-5p and 6791-5p) are down-regulated (22). Although miR-7110-5p is down-regulated, its relationship with sepsis has not been studied.

There is no doubt that this study also has some limitations. In the verification of miRNAs via RT-PCR, 50 patients with pneumonia, 40 patients with pneumonia meeting the diagnostic criteria of Sepsis3.0 and 21 healthy controls were included. However, the total number of cases recruited in this study is limited. Therefore, a large sample size should be guaranteed for the further evaluation of miR-7110-5p and miR-223-3p values in predicting early sepsis secondary to pneumonia.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
XZ wrote the manuscript. XZ and SD helped with miRNA microarray analysis and PCR.All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the ethics committee of The Third Hospital of Hebei Medical Universityand written informed consents were signed by the patients and/or guardians.

Consent for publication
Not applicable.

Competing interests

The authors declare that they have no competing interests

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