

RESEARCH ARTICLE

Prognostic value of pulmonary ultrasound score combined with plasma miR-21-3p expression in patients with acute lung injury

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Abstract

Purpose: The aim of this study was to explore the value of the combination between lung ultrasound score (LUS) and the expression of plasma miR-21-3p in predicting the prognosis of patients with acute lung injury (ALI).

Patients and methods: A total of 136 ALI patients were divided into survival ($n = 86$) and death groups ($n = 50$), or into low/middle-risk ($n = 77$) and high-risk groups ($n = 59$) according to APACHE II scores. Bioinformatics was used to explore the mechanism of action of miR-21-3p in humans. Real-time fluorescent quantitative PCR was used to detect the expression of miR-21-3p in plasma, and LUS was recorded. Receiver operator characteristic (ROC) curve and Pearson correlation were also used.

Results: The LUS and expression level of plasma miR-21-3p in the death and high-risk groups were significantly higher than those in the survival and low/middle-risk groups ($p < 0.01$ and $p < 0.05$). miR-21-3p expression leads to pulmonary fibrosis and promotes the deterioration of ALI patients by regulating fibroblast growth factor and other target genes. ROC curve analysis showed that the best cutoff values for LUS and plasma miR-21-3p expression were 18.60 points and 1.75, respectively. LUS score and miR-21-3p combined predicted the death of ALI patients with the largest area under the curve (0.907, 95% CI: 0.850–0.964), with sensitivity and specificity of 91.6% and 85.2%, respectively. The expression level of plasma miR-21-3p was positively correlated with LUS in the death group ($r = 0.827$, $p < 0.01$).

Conclusion: LUS and expression level of miR-21-3p in plasma are correlated with the severity and prognosis of ALI patients, and their combination has a high value for the prognostic assessment of ALI patients.

KEYWORDS

acute lung injury, miR-21-3p, prognostic evaluation, pulmonary ultrasound score

Renyang Liu and Guoxu Zhao contributed equally to this work.

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1 | INTRODUCTION

Acute lung injury (ALI) is a severe respiratory disease, which is characterized by acute inflammatory response in the airway and lung parenchyma, sudden onset, dangerous condition, and high mortality, seriously endangering the life and health of patients.¹ Although ALI morbidity declined in recent years, it reached 200 thousand per year in the United States and it has a relatively high rate of hospitalization, and it also accounts for over 10% of all intensive care unit.² Early and effective assessment of ALI patients is the key to guiding treatment and improving prognosis. The current treatment approach for ALI involves general supportive care including infection control, nutritional support targeting early enteral nutrition, blood glucose control, prevention of stress ulcers and deep venous thrombosis, and appropriate protective mechanical ventilation strategies in all critically ill patients.^{3,4} These treatments have improved the survival rate of ALI patients to some extent, but overall, the treatment effect is not as expected, and the clinical mortality is still between 36% and 44%.⁵

In recent years, lung ultrasound has been widely applied in the examination for a variety of lung diseases, among which lung ultrasound score (LUS) can evaluate the changes in lung ventilation, and has important clinical significance in judging the severity and prognosis of respiratory distress syndrome.⁶ MicroRNA (miRNA), as a new class of gene regulatory molecules, can regulate inflammatory pathways and immune responses by affecting the expression of target genes and play an important role in the pathogenesis of ALI.⁷ Studies in recent years have found that miR-21-3p is differentially expressed in ALI and regulates inflammatory pathways and immune responses by affecting the expression of target genes, which is expected to become a potential target for the treatment of ALI.⁸ In this study, lung ultrasound examination was used to score the lung ultrasound of ALI patients, and the expression level of plasma miR-21-3p was detected in ALI patients, so as to explore the value of LUS score and plasma miR-21-3p expression levels for the prognostic evaluation of ALI patients and to provide a reference for the treatment of ALI.

2 | MATERIAL AND METHODS

2.1 | Research subjects

A total of 136 ALI patients, including 89 males and 47 females, aged from 31 to 84 years old, were enrolled in Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College from May 2019 to December 2020. The inclusion criteria were as follows: (1) met the diagnostic criteria for ALI formulated by the respiratory society of Chinese medical association, and the diagnosis is confirmed based on clinical history, symptoms, and signs; (2) have received treatment and can cooperate with the researcher. The exclusion criteria were as follows: (1) patients with onset over 24h

prior and unclear clinical diagnosis; (2) presence of complications such as severe infection, malignant tumor, diseases of the blood system, autoimmune diseases, viral hepatitis, tuberculosis, bronchial asthma, and other respiratory diseases; and (3) incomplete medical records or inability to cooperate with the researcher.

2.2 | Research methods

A retrospective study was conducted to analyze the data of ALI patients at admission, including age, gender, body mass index, heart rate, respiratory rate, and underlying diseases. According to the diagnosis of ALI as the starting point of the study, and the recovery and discharge or death of ALI patients as the endpoint of the study, patients were divided into survival (86 cases) and death groups (50 cases). The acute physiology and chronic health evaluation II (APACHE II) scoring system software was used to calculate the APACHE II score value on the onset day of all ALI patients. The patients were divided into two groups: the high-risk group had APACHE II score >20 for 59 cases and, in the low/medium-risk group, APACHE II score ≤20 was present in 77 cases.

2.3 | Pulmonary ultrasound scoring

A Sono ultrasonic instrument with a phased array convex probe, which was 3.5–10.0 MHz, was used. All patients were in the supine position and examined by the same specially trained ultrasound observer, who focused on 12 lung areas, including bilateral anterior chest wall, lateral chest wall, and upper and lower posterior chest walls. The quantitative assessment standard for ultrasonic imaging provides a point score to the most serious performance evaluation: areas of consolidation of the lung (3 points), severely reduced lung ventilation area (2 points), pulmonary ventilation area (1 point), and normal ventilation area (0 points). The sum total of the score of the 12 lung areas is the LUS (score from 0–36 points); the higher the LUS, the more serious the illness.

2.4 | Bioinformatics analysis

The predictions for miR-21-3p downstream target genes obtained using online software Targetscan (<http://www.targetscan.org/>), miRDB (<http://www.mirdb.org/>), and miRWalk (<http://129.206.7.150/>) were intersected to obtain higher accuracy. The protein interaction network (PPI) based on the obtained target genes was combined with the STRING database (<https://string-db.org/>) to analyze its protein-protein interaction network (PPI network). The CytoHubba plug-in was used to screen 20 HUB genes. The HUB target genes obtained were enriched and analyzed using the clusterProfiler package of R software, and the ggplot2 package was used to visualize the results.

2.5 | miR-21-3p detection

After diagnosis, 5 ml of fasting venous blood was collected from all ALI patients and placed in an EDTA anticoagulant tube for centrifugal separation of blood components. Plasma was stored in a low-temperature refrigerator at -80°C . Real-time fluorescence quantitative polymerase chain reaction was performed on an ABI 7500 fluorescence quantitative PCR instrument. For a reaction system volume of 20 μl , we added 10.00 μl of TaqMan 2 \times Universal PCR Master Mix, 1.00 μl of TaqMan MicroRNA Assay, 1.33 μl of cDNA, and 7.67 μl of ddH₂O. The amplification was performed at 95°C for 10 min, and 45 cycles of 95°C for 15 s, and 60°C for 60 s. The experiment was repeated thrice. The relative expression level of miR-21-3p was calculated by the $2^{-\Delta\Delta C_t}$ method, where ΔC_t = the target gene of Ct - CTU6.

2.6 | Statistical methods

The SPSS19.0 statistical software was used for analysis, measurement data were represented by ($\bar{x} \pm S$), and group t-tests were used for comparison of two independent samples. The chi-squared test was used to compare groups. The value of LUS score combined with plasma miR-21-3p expression level in predicting the death of ALI patients was analyzed using receiver operating characteristic (ROC) curves. Areas under the curve (AUC) were compared using a Z-test. Pearson correlation was used to analyze the correlation between each index. The difference was statistically significant with $p < 0.05$.

2.7 | Ethical approval/patient consent

The present study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College (number: 011/2019, dated 25/02/2019). The colon cancer patients and healthy controls all provided informed consent. This investigation was conducted based on the principles of the declaration of Helsinki.

3 | RESULTS

3.1 | Comparison of demographic data

Compared with the survival group, the APACHE II score in the death group was significantly higher, and the difference was statistically significant ($p < 0.05$). There were no significant differences in gender, age, body mass index, underlying diseases, etiology, or respiratory rate and heart rate between the two groups ($p > 0.05$) (Table 1).

3.2 | Bioinformatics analysis

From Targetscan, miRDB, and miRWalk, 3664, 12508, and 595 target genes were obtained, respectively. A total of 420 target genes were obtained with high accuracy through intersection (Figure 1). The protein-protein interaction of these target genes was analyzed using the STRING database, and the protein interaction network results of the interaction between target genes were obtained

TABLE 1 Comparison of two groups of general data

Parameters	Survival group (N = 86)	Death group (N = 50)	p
Male (N [%])	54 (62.8)	35 (70.0)	0.394
Age ($\bar{x} \pm S$)	58.60 \pm 9.84	60.15 \pm 10.28	0.173
BMI (kg/m ²)	23.25 \pm 2.40	22.80 \pm 2.26	0.538
History of diabetes (N [%])	14 (16.3)	6 (12.0)	0.497
History of hypertension (N [%])	24 (27.9)	11 (22.0)	0.447
History of coronary heart disease (N [%])	9 (10.5)	7 (14.0)	0.537
Etiology			
Infection (N [%])	59 (68.6)	32 (64.0)	0.420
Trauma (N [%])	18 (20.9)	14 (28.0)	0.104
Pancreatitis (N [%])	6 (7.0)	2 (4.0)	0.843
Other (N [%])	3 (3.5)	2 (4.0)	0.742
Respiration rate (times/min)	29.70 \pm 5.24	30.16 \pm 5.72	0.306
Heart rate (times/min)	106.20 \pm 16.75	108.40 \pm 18.63	0.275
Systolic blood pressure (mmHg)	125.60 \pm 13.84	126.82 \pm 14.26	0.236
Diastolic pressure (mmHg)	75.60 \pm 9.36	77.64 \pm 9.58	0.307
APACHE II score	13.70 \pm 4.50	26.80 \pm 6.40	<0.001

Abbreviations: APACHE II, acute physiology and chronic health evaluation II; BMI, body mass index.

(Figure 2). Cytohubba was used to obtain 20 hub genes by an MCC algorithm (Figure 3). The enrichment analysis of hub target genes showed that the cell sets of these hub target genes were divided into the spliceosomal complex, catalytic step 2 spliceosome, etc. The

molecular functions for these include disordered domain-specific binding, basal transcription machinery binding, etc., important in processes including the fibroblast growth factor-receptor signaling pathway, cellular response to fibroblast growth factor stimulus, etc. KEGG pathway analysis showed that these target genes might be involved in the regulation of endometrial cancer, breast cancer, etc. (Figure 4).

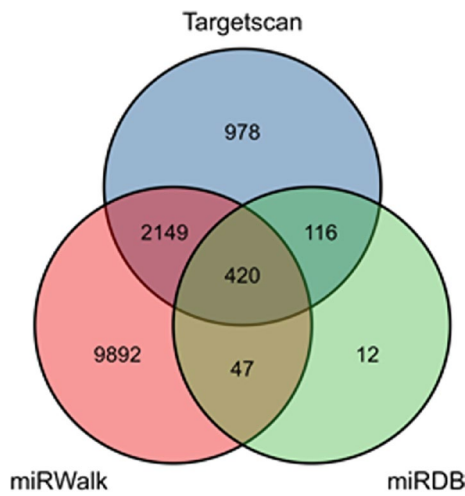


FIGURE 1 Wayne diagram of common genes in Targetscan, miRDB, and miRWalk

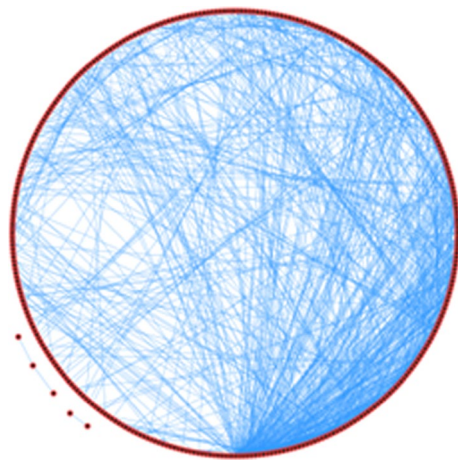


FIGURE 2 Protein-protein interaction (PPI) network of gene in target gene

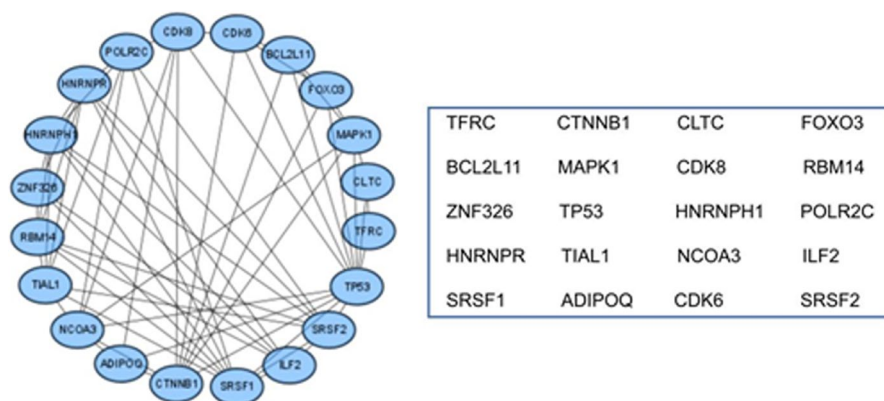


FIGURE 3 Protein-protein interaction (PPI) network of hub gene in target gene

3.3 | LUS score and plasma miR-21-3p expression levels

LUS score and plasma miR-21-3p expression levels in the death group were significantly higher than those in the survival group ($p < 0.01$, Table 2). Compared with the low/medium-risk group, LUS score, plasma miR-21-3p expression level, and mortality were significantly increased in the high-risk group ($p < 0.01$, Table 3).

3.4 | Value of LUS score combined with plasma miR-21-3p expression

The cutoff values for LUS score and plasma miR-21-3p expression level in predicting death in ALI patients were 18.60 points and 1.75 points, respectively. The sensitivity and specificity of the combination of the two methods for predicting death in ALI patients were 91.6% and 85.2%, respectively. The AUC (0.907, 95% CI: 0.850–0.964) was significantly higher than that of single LUS score (0.804, 95% CI: 0.746–0.865) and miR-21-3p (0.832, 95% CI: 0.771–0.890), with a statistically significant difference ($p < 0.05$, Figure 5).

3.5 | Correlation analysis

Pearson correlation analysis showed that plasma miR-21-3p expression level was positively correlated with the LUS score in the death group ($r = 0.827$, $p < 0.01$). There was no significant correlation between plasma miR-21-3p expression level and LUS score in the survival group ($r = 0.142$, $p > 0.05$).

FIGURE 4 Go analysis and KEGG analysis of target

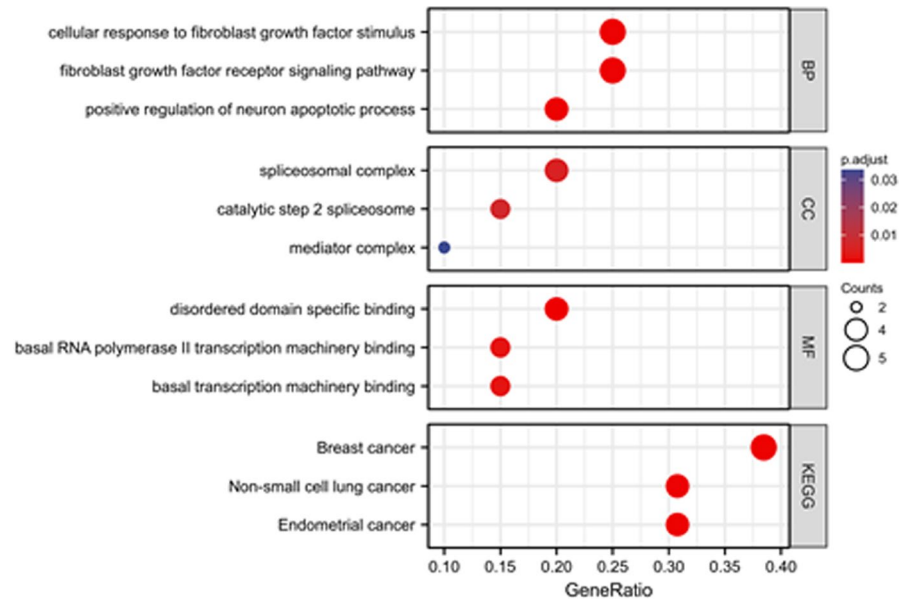


TABLE 2 Comparison of LUS score and plasma miR-21-3p expression between the survival group and death group ($x \pm S$)

Group	N	LUS score	miR-21-3p
Survival group	86	13.60 ± 3.50	0.97 ± 0.35
Death group	50	25.70 ± 4.60	2.84 ± 0.96
<i>p</i>	-	<0.001	<0.001

Abbreviation: LUS, lung ultrasound score.

TABLE 3 Comparison of LUS score and plasma miR-21-3p expression between the high-risk group and low-middle-risk group ($x \pm S$)

group	N	LUS score	miR-21-3p
Low/medium-risk group	77	15.50 ± 3.70	1.20 ± 0.51
High-risk group	59	22.60 ± 4.20	2.58 ± 0.84
<i>p</i>	-	<0.001	<0.001

Abbreviation: LUS, lung ultrasound score.

4 | DISCUSSION

ALI refers to progressive hypoxic respiratory failure caused by trauma, infection, shock, and other reasons, and is a common and rapidly progressing critical inflammatory lung disease, in clinical practice. With the progress of modern medicine, the prognosis of ALI has improved, but its mortality rate is still high. Therefore, to predict the severity and prognosis of ALI patients early and take effective treatment measures in time, it is helpful to reduce the mortality of ALI patients. Pulmonary ultrasound has the advantages of simplicity, ease of operation, bedside operation, strong repeatability, low price, and reduced exposure to radiation; it is widely used in the diagnosis and treatment of acute and critical diseases. LUS score can not only better display the lung apex and intrapulmonary focal lesions, but also further evaluate the severity and prognosis of lung

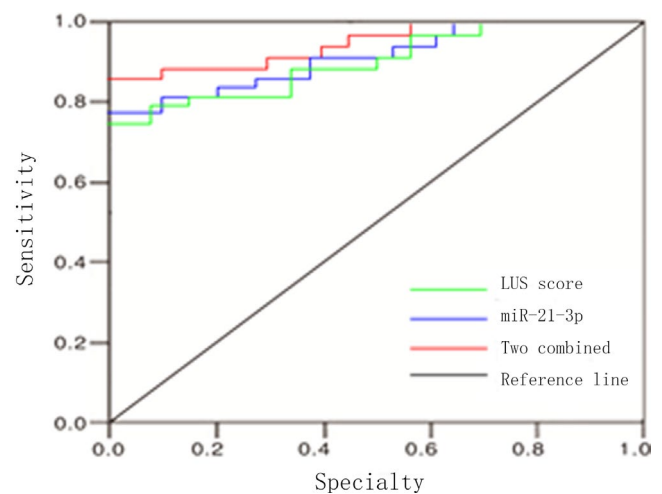


FIGURE 5 ROC curve for predicting sensitivity and specificity by the combination of two methods in ALI patients. ALI, acute lung injury; ROC, receiver operator characteristic

injury, which has strong advantages in the diagnosis and prognosis of lung diseases.⁹ Many research results show that bioinformatics analysis plays an important role in the identification of biomarkers and the prognosis of the disease. Qiu et al. Identified ABCC5 as a new biomarker of hepatocellular carcinoma by bioinformatics analysis.¹⁰ Li et al.¹¹ constructed a melanoma prognosis model based on apoptosis-related genes using bioinformatics. miRNA is a kind of endogenous, non-coding small molecule RNA that can regulate the expression of target genes and play an important role in cell proliferation, differentiation, apoptosis, angiogenesis, and inflammatory immune response.^{12,13} Zheng et al.¹⁴ showed that miRNA is involved in the occurrence and development of ALI and plays an important regulatory role in the inflammatory response and apoptosis of ALI, which is expected to become a new biomarker and therapeutic target for ALI. Rajasekaran et al.¹⁵ found that miRNA expression changes

during ALI, and miRNA regulates gene expression by acting on target mRNA, thus affecting the germination of pulmonary microvessels, the maturation of alveoli, and participating in the occurrence and development of lung injury related diseases.

Previous studies have shown that miRNA regulates diseases through a variety of receptors and signaling pathways. It was reported that miR-21-3p regulates AGE/RAGE signaling and improves diabetic atherosclerosis.¹⁶ There is a significant increase in the serum level of miR-21-3p in children with sepsis-induced acute kidney injury,¹⁷ and miR-21-3p promotes hepatocellular carcinoma progression via SMAD7/YAP1 regulation.¹⁸ In addition, miR-21-3p can regulate the prognosis of gastric cancer and colorectal cancer.^{19,20} As far as we know, this is the first study which specially investigates the miR-21-3p expression levels for prognosis in acute lung injury. Lung ultrasound score and expression level of miR-21-3p in plasma are correlated with the severity and prognosis of acute lung injury patients, and their combination has a high value for the prognostic assessment of acute lung injury patients. It is worth mentioning that Wang et al. have published a literature and they concluded that miR-21-3p aggravates injury in rats with acute hemorrhagic necrotizing pancreatitis by activating TRP signaling pathway.⁸

This study showed that LUS score and plasma miR-21-3p expression level in the death group were significantly higher than those in the survival group, while LUS score, plasma miR-21-3p expression level, and mortality in the high-risk group were significantly higher than those in the low/medium-risk group. These results suggest that the increased LUS score and plasma miR-21-3p expression level are related to the severity of the disease in ALI patients. ALI patients with high LUS score and plasma miR-21-3p expression level have a poor prognosis and a higher risk of death. Among them, those with high LUS score have poor oxygenation and ventilation function of the lung, or even lack of lung ventilation or lung failure, and the risk of death is greater. Caltabeloti et al. believed that LUS score can effectively evaluate the change in pulmonary ventilation area in respiratory distress syndrome and then detect the loss of pulmonary ventilation at an early stage, which plays a certain role in assessing the condition and prognosis of patients, by guiding clinicians to draw up treatment plans and implement therapeutic measures.²¹ Cao et al.²² showed that miRNA expression level increased significantly in ALI patients, and the change in miRNA expression level was closely related to the severity and prognosis of ALI patients. Other studies have shown that miRNA is highly sensitive and highly expressed in vivo and participates in the occurrence and development of lung injury, by regulating the innate immune response and inflammatory response of the body.²³ Through the prediction of miR-21-3p target genes and protein interaction network screening, 20 hub target genes were obtained. After enrichment analysis of these genes, polygenes were found to be closely related to the role of fibroblasts in biological process. Previous studies have shown that the formation of pulmonary fibrosis can significantly promote the formation of late ALI.²⁴ The discovery of miR-21-3p function is of great significance for improving the understanding of ALI disease progression. The KEGG pathway plays a role in the formation of a

variety of cancers. ROC curve analysis was applied in this study, and the results showed that LUS score ≥ 18.60 and plasma miR-21-3p expression level ≥ 1.75 had relatively high sensitivity and specificity for predicting the death of ALI patients, as the AUC was the largest. The combined detection of LUS score and plasma miR-21-3p expression level can improve the accuracy of predicting the death of ALI patients. Correlation analysis also showed that the expression level of plasma miR-21-3p in the death group was positively correlated with LUS score, which further suggested that the two combined tests had high clinical value in predicting the prognosis of ALI patients. Han et al. found that, compared with the control group, the level of miR-155 in patients with acute lung injury was significantly increased.²⁵ miR-155 has a certain value in predicting 30-day mortality of patients with ALI, and can be used as a biomarker for their prognosis assessment. Other studies have shown that miRNA is related to disease development and severity of ALI, can be used as a biomarker for the prognosis assessment of ALI, and provide new ideas for the clinical treatment of ALI.²⁶

5 | CONCLUSION

In conclusion, LUS score and plasma miR-21-3p expression level are correlated with the severity and prognosis of ALI patients and are expected to be used as new biomarkers for ALI. The two combined tests have high value for predicting the prognosis of ALI patients, and also provide a new idea for understanding the pathogenesis of ALI, as well as targeted therapy. However, this study is at a preliminary exploratory stage, and more prospective studies are needed to further confirm the value of miR-21-3p for ALI patients.

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CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

LR conceived study design. ZG and GF conceived the content concept. GF and ZG performed the data collection, extraction, and analyzed the data. LR and WQ interpreted and reviewed the data and drafts. WQ reviewed the final draft. All authors were involved in literature search, writing the study, and had final approval of the submitted and published versions. All authors contributed to data analysis, drafting, or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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