Role of serum miR-122 and miR-21 for predicting early acute lung injury after pediatric liver transplantation: A retrospective case-control study

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Abstract

Background: Acute lung injury (ALI) is a complicated disease with high morbidity and mortality for pediatric after living donor liver transplant (LDLT), the identification of effective prediction model involved in ALI is urgent and highly demanded.

Methods: Perioperative data of patients were obtained through the electronic medical records system. Patients were divided into non-ALI group and ALI group according to whether ALI occurred in the first week after surgery. The main measure was serum miR-122 and miR-21 levels after anesthesia induction (T1), 10min of anhepatic phase (T2), 30min of neohepatic phase (T3) and directly after surgery (T4). Lung ultrasound examination was performed three times at 24h before LDLT, 24h and 72h after LDLT. Multiple logistic regression analysis of preoperative factors was conducted to screen the risk factors of ALI, and the predictive value of risk factors was evaluated by ROC curve. Lung ultrasound score (LUS) after LDLT was used to compare the pulmonary complications between two groups.

Results: A total of 90 patients were included, including 53 in the non-ALI group and 37 in the ALI group. Increased intraoperative blood transfusion volume, TNF-α at T3, miR-122 at T4 and miR-21 at T4 were related factors for the occurrence of ALI (P < 0.05), the AUC of the combination in predicting early ALI was 0.918 (0.876–0.987). The LUS of pulmonary consolidation and pulmonary edema were higher in ALI group. Conclusion: Increased intraoperative blood transfusion, TNF-α, miR-122 and miR-21 levels are independent risk factors for ALI after LDLT, the LUS of pulmonary complications was higher in ALI group, paying attention to these indicators can improve the efficacy of pediatric respiratory management after LDLT. Trial registration: www.chictr.org.cn/ identifier: ChiCTR2200059722.

1 Introduction

Acute lung injury (ALI) including acute respiratory distress syndrome (ARDS) after liver transplantation is a common and complex pulmonary complication, it is leading causes of disability and death, both in the acute and long-term postoperative period[1]. ALI after liver transplantation usually occurs within 1 week, and the earliest occurrence is 2h after surgery, with an incidence of more than 20%. It is more likely to occur in pediatric due to underdeveloped lungs[2]. Our previous work have shown that the incidence of postoperative ALI in children with living donor liver transplantation (LDLT) was 23.5%, and the intensive care unit (ICU) suspension time was significantly prolonged, which has seriously affected the post-operative quality of life and increased medical costs and family burden[3]. Accurate and early detection of pulmonary complication is conducive to early treatment, for these reasons, the identification of effective biomarkers involved in ALI is urgent and highly demanded for improving pediatric respiratory management after LDLT.

MicroRNA (miRNA), a class of non-coding single-stranded RNA molecules encoded by endogenous genes with a length of about 22 nucleotides, can play a regulatory role on target mRNA by disrupting its stability...
and inhibiting its translation. The dysregulation of multiple proteins by miRNAs was reported to contribute to the pathogenesis of various diseases[4-5]. Furthermore, miRNAs play an important role in the pathogenesis of ALI through influencing the expression of target genes to effect inflammatory pathways and immune responses[6]. However, whether and how miRNAs are involved in the progress of ALI after LDLT in pediatric is unknown.

Previous studies have reported that the serum miR-21 and miR-122 are highly expressed in lung inflammatory diseases, they are involved in lots of signaling pathways and various immune inflammatory reactions, and have been reported to regulate the production and development of ALI[7-8]. Hayato et al reported that the expression level of serum miR-21 changed in chronic lung disease(CLD) patients and was a potential biomarker for predicting CLD in premature infants[9]. The paper from Zilong demonstrated miR-122-5p protected against ALI via regulation of DUSP4/ERK signaling in pulmonary microvascular endothelial Cells[10]. The severity and prognosis of ALI can be determined by the lung ultrasound score (LUS), which assesses changes in lung ventilation. The LUS and the expression levels of miR-21 are correlated with the severity and prognosis of ALI and are expected to be used as new biomarkers for ALI in adults[11]. However, it remains to be seen whether the combination of miR-21 and miR-122 with LUS score can improve the accuracy of ALI prediction after liver transplantation.

In this retrospective study, we evaluated link between intraoperative miR-122 and miR-21 levels and ALI during the first week after LDLT, we also compared the LUS of pulmonary complications after LDLT in two groups.

2 Methods

2.1 Data source and study population

This was a single-center retrospective case-control study, ethical approval for it (2020N260KY) was provided by the Ethical Committee of Tianjin First Central Hospital,Tianjin,China.on October 27, 2020. The data are anonymous, and the requirement for informed consent was therefore waived.

We reviewed the medical records of all pediatric liver transplantation recipients from January 2019 to May 2021 in Tianjin First Central Hospital. The inclusion criteria were as follows: (I) the diagnosis was biliary atresia; and (II) grafts received from the family members of the pediatric recipients. Exclusion criteria were as follows: (I) children with congenital airway or respiratory malformations, congenital cardiopulmonary deformity, or acute respiratory infection before surgery; (II) retransplantation; and (III) the serum miR-21 and miR-122 measurements were unavailable.

Patients were divided into non-ALI group and ALI group according to whether ALI occurred in the first week after surgery based on the criteria of ALI in our earlier study [3]. The operation was performed by experienced surgeons and the procedure was a piggyback liver transplant.

2.2 Data collection

We collected data in the preoperative, intraoperative, and postoperative periods through electronic medical record information management system and postoperative follow-up group data. Clinical data of the children were collected, including age, sex, preoperative serum bilirubin, alanine aminotransferase(ALT), aspartate aminotransferase(AST), international standardized ratio (INR), and pediatric end-stage liver disease score (PELD). PELD score was calculated using online calculators. Intraoperative fluid infusion volume, bleeding volume, blood transfusion volume and ALT, AST, serum bilirubin peak values within 7 days after LDLT and ICU stay time, postoperative hospitalization time and postoperative pulmonary complications were analyzed and recorded. Laboratory data including serum miR-122 and miR-21 levels and the concentration of TNF-α and IL-1β after anesthesia induction(T1), 10min of anhepatic phase(T2), 30min of neohepatic phase (T3) and directly after surgery(T4). Lung ultrasound examination for all children, patients were assessed three times during hospitalization: 1) “baseline”(24 h before operation); 2 “control”(24h after operation; and 3) “remission”(72h after operation). The LUS was assessed using the 36-point ultrasound score for pulmonary
consolidation, pulmonary edema, and pleural effusion, as previously described[12], shown in supplement figure 1.

2.4 Statistical analysis

Sample size:
The sample size was calculated using PASS, the AUC of ARDS predicted by miR-21 was 0.83 based on the published literature[9]. We predicted that the AUC could be increased to 0.9 when miR-21 was combined with miR-122, and we assumed that the number of people in the non-ALI group was twice the number of people with ALI, and concluded that 34 people were needed in the ALI group and 68 people were need in the non-ALI group at $\alpha=0.05, 1-\beta=0.9$. Considering the 10% dropout rate, the ALI group required at least 37 participants. After the ALI group reached 37 people, we conducted data analysis, obtained meaningful results, and stopped the experiment.

SPSS26.0 software was used for data analysis. Normally distributed data were shown as mean ± standard deviation ($x\pm s$) and used T-tests to compare differences between groups. Abnormally distributed data were represented as median(IQR), and the rank-sum test was used for comparison between groups, t test and the Fisher exact test were used for the categorical variables. Univariate and multivariate logistic regression models were used to identify the risk factors associated with ALI to determine the impact of early post-transplant complications using clinical variables. ROC curves were used to explore the value of serum miR-21 and miR-122 expression levels for predicting ALI. Statistical significance was set at $P < 0.05$.

3. Results

3.1 Characteristics of patients

A total of 200 children with LDLT were retrospectively analyzed, excluding 34 patients with incomplete data, 3 patients with secondary liver transplantation, 42 patients with non-biliary atresia undergoing LDLT surgery, 11 patients who withdrew from the trial after LDLT, and 20 patients older than 12 months, 90 children were included, including 53 patients in the non-ALI group, 37 patients in the ALI group. The intraoperative blood transfusion volume, postoperative serum total bilirubin peak, and postoperative Hospitalization time were higher in the ALI group ($P < 0.05$). There were no differences in other data between the two groups (Table 1).

3.2 The expression levels of serum miR-122, miR-21, TNF-α and IL-1β

We evaluated the expression of miR-122, miR-21, TNF-α and IL-1β at different phase, RT-qPCR analysis showed higher expression levels of serum miR-122 in ALI group than in the non-ALI group at T2, T3, and T4 (Fig.1A, $P < 0.05$), and the expression levels of miR-21 were higher than that in the non-ALI group at T3 and T4 (Fig.1B, $P < 0.05$), ELISA analysis showed that the levels of TNF-α were higher at T2, T3, and T4 in the ALI group (Fig.1C, $P < 0.05$), and the expression levels of IL-1β increased at T3 and T4 in the ALI group (Fig.1D, $P < 0.05$).

3.3 Univariate and multivariate logistic regression analysis of factors associated with ALI after LDLT

Univariate and multivariate logistic regressions were conducted to determine which preoperative factors were independent predictors of ALI after LDLT. Intraoperative transfusion volume, the expression level of serum miR-122 and miR-21, the concentration of TNF-α were predictors of early ALI. Multivariate logistic regression analysis showed that intraoperative transfusions volume, the levels of miR-122 and miR-21 at T4 and TNF-α at T3, were the independent predictors of early post-transplant ALI (Table 2).

3.4 Predictive value of serum miR-21, miR-122, TNF-α, and intraoperative transfusion volume

We analyzed the predictive value of predictors for the occurrence of ALI, the results showed that cut-off values of intraoperative transfusion volume, miR-122, miR-21 and TNF-α for predicting early ALI were 1.9, 4.89, 0.17 and 1.82. The AUC of the these factors was 0.918(0.876–0.987) in predicting early ALI, with a sensitivity and specificity of 83.8% and 83.0%, respectively. The value was significantly higher than
that of intraoperative transfusion volume [0.677 (0.566–0.788)], miR-21 at T4 [0.634 (0.513–0.755)], miR-122 at T4 [0.666 (0.545–0.787)], and TNF-α at T3 [0.679 (0.566–0.792)] alone, (Fig.2).

3.5 The relationship of influential factors and ALI

In order to build a better ALI prediction model, we categorized the relevant factors according to quartile. The univariate analyses showed that the highest quartile of miR-122 at T4, miR-21 at T4, TNF-α at T3, intraoperative transfusion volume, and LUS score of consolidation in remission were associated with ALI. In multivariate logistic regression analyses, miR-122 at T4, miR-21 at T4, TNF-α at T3 remained a significant predictors of ALI, while no such association was found for intraoperative transfusion volume and LUS of pulmonary consolidation in remission (Table 3).

3.6 LUS score of pulmonary edema, pleural effusion and pulmonary consolidation

Bilateral lung ultrasound was performed three times for all patients, and the score of them increased in control, and gradually decreased in remission, but was still higher than that in baseline (Fig.3A). In the ALI group, the LUS of pulmonary edema, pulmonary consolidation, and pleural effusion increased in control ($P < 0.05$), and the LUS of pulmonary consolidation and pulmonary edema were still higher than those in the non-ALI group in remission ($P < 0.05$, Fig 3B).

4. Discussion

This is the first study to investigate the relationship between the expression level of miR-122 combined miR-21 at different stages and early ALI after LDLT. We found that high levels of miR-122 and miR-21 were predictors of early ALI in univariate and multivariate models, also when adjusting for TNF-α intraoperative transfusion volume and LUS.

Many studies about the possible role of miRNAs including miR-122 and miR-21 in ALI have been emerged in recent years. In lipopolysaccharide (LPS) induced ALI model, the expression level of miR-122 in lung tissues and pulmonary microvascular endothelial cells is increased, silencing of miR-122 can eliminate LPS-induced inflammatory response, reduce excessive oxidative stress, and alleviate ALI[13]. MiR-122 was initially found to play a role in the maintenance of normal liver function and liver disease, and considered to be a promising biomarker of liver injury[14]. The expression of miR-122 is correlated with the severity and prognosis of ARDS patients. MiR-122 and APACHE II scores have a high value in evaluating the prognosis of patients with ARDS[15]. However, the function of miR-122 and its modulation of ALI after liver transplantation has not been investigated. Our study demonstrated that as surgery progressed, the increasing level of miR-122 strong correlation with ALI, which may have stimulated inflammatory responses and oxidative stress. Therefore, miR-122 has the potential to be a novel and valuable biomarker for pediatric undergoing LDLT.

MiR-21 is involved in the occurrence and development of various lung diseases[16], and plays a role in the inflammatory response, reducing the expression of inflammatory cytokines, including TNF-α, IL-1β, and IL-6, and attenuating ALI by inhibiting the PTEN/FoxO1-TLR4/NF-κB signaling cascade[17-18]. Sheedy et al[19] reported that miR-21 is not merely a feature of proinflammatory or immunosuppressive states, but acts as a key signal regulating the balance and transition between the two states. The activation of miR-21 can be regarded as a "molecular varostat” regulating the inflammatory switch, which is an important switch for inflammation relief and homeostasis maintenance. When inflammation occurs, miR-21 is activated to resist the progression of inflammation. When the body lost the balance of inflammatory responses, the expression of miR-21 decreases. Perdiguero et al[20] also confirmed that the increase of miR-21 did not inhibit the development of inflammation. In our study, the expression level of miR-21 gradually increased with the progress of surgery, peaked at T3, and began to decline at T4, however, it was still higher than that in the non-ALI group. Logistic regression analysis suggested that the expression of miR-21 at T4 was correlated with early postoperative ALI.
In recent years, the advantages of ultrasound in the diagnosis of diseases with rapid development have become increasingly prominent. Numerous studies have demonstrated that LUS could predict COVID severity, and given the different scoring criteria used in LUS assessment[21-22]. De et al[23] suggested that some parameters could be used as indicators or predictors of diagnostic probability and disease severity. In screening, small peripheral consolidation was associated with a high probability of disease, in hospitalized patients, peripheral and parenchymal consolidation were associated with the severity of lung damage. In a pig model of neonatal ALI, the LUS was proven to be a sensitive indicator of ARDS, and there was a moderate correlation between the LUS and the degree of lung histological injury[24]. Previous work has shown that the LUS and expression level of miR-122 and miR-92 are related to the severity and prognosis of ARDS, and their combination has a high value in the prognosis assessment of patients with ALI[25]. However, the types of ALI in most patients are mixed, and multiple forms of lung injury clinically can coexist with different degrees of changes.

In our study, we use LUS to access different pulmonary complications including pulmonary edema, consolidation and pleural effusion, and explore the relationship between pulmonary complications and ALI. The results showed that the scores of the three complications increased at 24 hours after LDLT, and decreased during the remission period. The LUS of pleural effusion increased in the ALI group, but could not predict the occurrence of early ALI. Justin K et al. [26] also confirmed that the incidence of pleural effusion in children after liver transplantation was high, but it had no significant impact on the mortality. The LUS for pulmonary consolidation and edema had a significant influence on early ALI. In addition, the sensitivity and specificity of the LUS scores of pulmonary consolidation in remission were better than those of the others, therefore, we concluded that the LUS of pulmonary consolidation in the remission period had a good correlation with ALI.

The study had some limitations. First, the expression levels of miR-122, miR-21 were only measured before the end of LDLT, while ALI mostly occurred one week after LDLT. Measurements closer to the time of ALI onset may lead to better prognostic performance. Second, the sample size was small and the collection of clinical data of patients was not comprehensive, which may lead to a large deviation in multivariate analysis.

5. Conclusion

To sum up, the prediction of early ALI was correlated with the increase serum miR-21 and miR-122 expression levels at different periods during pediatric undergoing LDLT, combined with intraoperative blood transfusion volume, the expression levels of TNF-α can have higher diagnostic efficiency. The LUS of pulmonary consolidation in the remission period had a good correlation with ALI. Reducing the amount of intraoperative blood transfusion, targeting the expression levels of miR-122 and miR-21, and performing pulmonary ultrasound examinations as soon as possible may improve the efficacy of pediatric respiratory management after LDLT.

Funding

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Conflict of interest

Yingli Cao and Mingwei Sheng contributed equally and are co-first authors. All authors disclosed no relevant relationships.

Authors’ contributions

Yingli Cao participated in research design, writing of the protocol, partial data collection, and writing the manuscript;

Mingwei Sheng designed the research, analyzed the data and assisted in the writing of the manuscript;

Chen Zhang analyzed the data, collected data and participated in the performance of the research
Hui Yu collected and analyzed the lung ultrasound data, designed the method;
Lili Jia was responsible for writing and editing the manuscript;
Yinghui Ren collected and analyzed the data, designed the method;
Hongyn Du made substantial contributions to the design of work, and revision of the article;
Wenli Yu was the overall advisor and sponsored the project. All authors read and approved the final manuscript.

Ethical Statement
This was a single-center retrospective case-control study, ethical approval for it (2020N260KY) was provided by the Ethical Committee of Tianjin First Center Hospital, Tianjin, China on October 27, 2020.

Data Sharing Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

References


Table 1. Characteristics of ALI and Non-ALI patients

<table>
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<th>ALL(n=37)</th>
<th>Non-ALL(n=53)</th>
<th>P</th>
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<tbody>
<tr>
<td>Preoperative</td>
<td></td>
<td></td>
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<tr>
<td>Age (mon)</td>
<td>8.19±2.259</td>
<td>7.58±2.098</td>
<td>0.196</td>
</tr>
<tr>
<td>SEX (M/F)</td>
<td>20/17</td>
<td>32/21</td>
<td>0.351</td>
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Weight (kg) 7.84±1.82 7.40±1.44 0.156
albumin, g/L 33.89±5.17 33.58±4.68 0.766
AST peak, IU/L 273.77±127.92 226.74±63.33 0.062
ALT peak, IU/L 141.17±60.84 159.72±65.42 0.177
Total bilirubin, g/L 239.66±80.37 232.40±102.95 0.720
Creatinine, μmol/L 17.05±6.00 10.83±5.425 0.071
INR 1.39±0.28 1.45±0.32 0.319
PELD score 12.48±5.00 10.56±4.54 0.062

**Intraoperative**
- Cold ischemia time, min 36.81±4.36 35.47±5.63 0.092
- Warm schema time, min 1.41±0.38 1.49±0.14 0.531
- Blood transfusions volume, U
  - Median [IQR]: 2(0.5) 2(0.5) 0.003*
- Frozen plasma, mL 120(100) 100(150) 0.083
- Crystalloids volume, mL 1456.30±443.06 1426.28±356.25 0.726
- Bleeding volume, mL 350(200) 300(200) 0.099
- Urinary volume, mL 537.57±184.82 621.42±235.60 0.074
- Operation time, min 529.84±92.60 520.98±89.03 0.649
- Anesthesia time, min 588.43±90.72 577.08±80.97 0.535

**Postoperative**
- AST peak, IU/L 319(281.7) 326.5(264.8) 0.382
- ALT peak, IU/L 390.8(341.3) 333(199.8) 0.566
- Total bilirubin peak, g/L 75.1(28.06) 56.31(22.24) 0.001*
- ICU stay time, day 3(2) 3(2) 0.175
- Hospitalization time, day 22(7) 20(5) 0.002*

ALT, aspartate aminotransferase; AST, aspartate aminotransferase alanine; INR, international standardized ratio; PELD, pediatric end-stage liver disease score. Data are presented as mean±SD, median [IQR]. *P < 0.05.

**Table 2.** Logistic regression analysis of factors associated with early posttransplant ALI.

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Univariate analysis</th>
<th>Mutivariate analysis</th>
<th>Mutivariate analysis</th>
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<tr>
<td></td>
<td>OR</td>
<td>P</td>
<td>OR</td>
<td>P</td>
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<tr>
<td>Pretransplant bilirubin</td>
<td>1.001(0.996-1.005)</td>
<td>0.717</td>
<td></td>
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<tr>
<td>Pretransplant INR</td>
<td>2.078(0.497-8.689)</td>
<td>0.316</td>
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<tr>
<td>Pretransplant PELD</td>
<td>1.090(0.995-1.195)</td>
<td>0.065</td>
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<tr>
<td>Transfusions volume</td>
<td>2.067(1.138-3.755)</td>
<td>0.017</td>
<td>6.856(1.481-14.970)</td>
<td>0.003*</td>
</tr>
<tr>
<td>miR-122 at T3</td>
<td>1.692(1.220-2.330)</td>
<td>0.001*</td>
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</tr>
<tr>
<td>miR-122 at T4</td>
<td>1.546(1.240-1.927)</td>
<td>0.001*</td>
<td>3.564(1.430-3.431)</td>
<td>0.006*</td>
</tr>
<tr>
<td>miR-21 at T4</td>
<td>7.415(1.333-41.253)</td>
<td>0.022*</td>
<td>52.297(2.946-928.325)</td>
<td>0.007*</td>
</tr>
<tr>
<td>TNF-α at T2</td>
<td>1.009(1.001-1.016)</td>
<td>0.002*</td>
<td>1.040(1.014-1.066)</td>
<td>0.002*</td>
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<tr>
<td>TNF-α at T3</td>
<td>1.006(1.002-1.010)</td>
<td>0.003*</td>
<td></td>
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<tr>
<td>TNF-α at T4</td>
<td>1.008(1.002-1.013)</td>
<td>0.008</td>
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**Table 3.** Relationship between different levels of predictors and early ALI

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<tr>
<td>First quartile(0.028-1.305)</td>
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<tr>
<th>Quartile Range</th>
<th>miR-21 at T4</th>
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<tr>
<td>Second quartile(0.021-0.050)</td>
<td>1.422(0.399-5.072)</td>
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<td>0.621(0.059-6.533)</td>
<td>41.418(1.680-1020.965)</td>
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<td>Third quartile(0.050-0.199)</td>
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<td>Forth quartile(0.199-2.221)</td>
<td>6.007(2.058-17.534)</td>
<td>0.001*</td>
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**Figure legend**

Φιγ 1. Τηε λεvεl οφ μιP-122, μιP-122,μιP-21,ΙΛ-1β και TNF-α ντυ τωο γρουπς. *P<0.05·** P<0.000.

Fig 2. ROC curve for predicting sensitivity and specificity by the combination of five methods in ALI patients. ROC, receiver operator characteristic.

Fig 3. LUS score of pleural effusion, pulmonary edema and pulmonary consolidation at baseline, control and remission. *P<0.05

Supplement figure 1. Lung ultrasound findings of consolidations, B-lines, and pleural effusion. The degree of consolidations(asterisks) was divided into four grades: C1, minimal juxtapleural consolidations; C2, small-sized consolidations; and C3, large-sized consolidations. The degree of B-lines(arrowhead) was divided into four grades: B1, multiple well defined B-lines; B2, multiple coalescent B-lines; and B3, white lung. The degree of pleural effusion(triangulum) was divided into four grades: P1, minimal pleural effusion; P2, small amount of pleural effusion; and P3, moderate amount of pleural effusion.
Fig 1. The level of miR-122, miR-122, miR-21, IL-1β and TNF-α in two groups. * $P < 0.05$; ** $P < 0.001$.

Fig 2. ROC curve for predicting sensitivity and specificity by the combination of five methods in ALI patients. ROC, receiver operator characteristic.

Fig 3. LUS score of pleural effusion, pulmonary edema and pulmonary consolidation at baseline, control and...
**Supplement figure 1.** Lung ultrasound findings of consolidations, B-lines, and pleural effusion. The degree of consolidations (asterisks) was divided into four grades: C1, minimal juxtapleural consolidations; C2, small-sized consolidations; and C3, large-sized consolidations. The degree of B-lines (arrowhead) was divided into four grades: B1, multiple well defined B-lines; B2, multiple coalescent B-lines; and B3, white lung. The degree of pleural effusion (triangulum) was divided into four grades: P1, minimal pleural effusion; P2, small amount of pleural effusion; and P3, moderate amount of pleural effusion.