Peer Review File

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Reviewer A:

The present study suggested the ability of KL-6 as biomarker for predicting mortality. This study has clinical significance but not novelty. Resolving the following issues is needed for acceptance of Journal of Thoracic Disease.

Major comments

Comment 1: Please analyze the influence of confounding factors to predict mortality in patients with ARDS reported previously such as age, initial oxygenation index, APACH II score, and pulmonary injury score on the results of the present study.

Reply 1: We are very sorry for our negligence of the influence of confounding factors to predict mortality in patients with ARDS reported previously, the follow-up studies will be carried out to clarify this point.

Comment 2: The cause of death should be shown. Is there subjects died due to disease other than ARDS.

Reply 2: The cause of death is respiratory failure due to ARDS.

Changes in the text: As reported in previous studies, the serum levels of KL-6 elevates in patients with ILDs, which may be due to an elevation in KL-6 production by increasing permeability following the destruction of alveolar capillaries and results in regenerating of alveolar type II pneumocytes in the affected lung.

Comment 3. The effectiveness of KL-6 as a biomarker is enriched in acute exacerbations of interstitial lung disease. The authors should mention about previous reports on this disease in the Introduction or Discussion section.

Reply 3: We have modified our text as advised (see Page 3, line 89).

Minor comments

Line 238

Is “0-7 months” wrong for “0-7 days”?

Reply: We were really sorry for our careless mistakes. Thank you for your reminder. We have modified it (see Page 7, line 238).

Reviewer B:

The paper titled “Expression and significance of serum KL-6 in patients with acute respiratory distress syndrome” is interesting. The serum KL-6 level is potentially a good indicator for

predicting the prognosis of patients with ARDS. However, there are several minor issues that if addressed would significantly improve the manuscript.

1)The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.
2) How to use KL-6 to stratify the disease outcomes or risk of death in ARDS? Suggest adding relevant content.
3) Besides KL-6, what other biomarkers are there for intrapulmonary and extrapulmonary ARDS? Suggest adding comparative analysis.
4) Suggest increasing the dynamics and prognostic significance of KL-6.
5) What is the future work plans? What is the guiding significance of this study?
6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Serum total bilirubin, an indicator for the impact of hemoglobin catabolism on outcome of patients with the acute respiratory distress syndrome, PMID: 36819562". It is recommended to quote the articles.
7) The number of patient samples in this study is too small, and a large sample study should be added for verification.
Reply: We feel great thanks for your professional review work on our article. As you are concerned, there are several problems that need to be addressed. We are currently carrying out follow-up studies to address these issues, such as comparative analysis between KL-6 and other biomarkers, and so on.

Reviewer C:

The authors studied the kinetic levels of serum KL-6 in 50 ARDS patients. They found that in the intrapulmonary ARDS group, patients who died had significantly higher serum KL-6 levels in the seven days following diagnosis than those who survived. In contrast, in the extrapulmonary group, the difference in KL-6 values between patients who survived and died was only significant on the first day after diagnosis. The peak levels of serum KL-6 in the death group were significantly higher than those in the survival group for both intra- and extrapulmonary ARDS.

Abstract

How were patients recruited? (e.g., consecutively admitted patients…).
Reply:We have modified our text as advised (see Page 1, line 25).
Changes in the text:Patients who met the ARDS Berlin criteria and were hospitalized in the intensive care unit of the *China-Japan Union Hospital of Jilin University* between September 2021 and September 2022 were recruited for analysis.

It says: "The peak levels of serum KL-6 in the death group were significantly higher than those in the survival group for both intra- and extrapulmonary ARDS (P value)". Please, indicate the specific p value, rather than "P value".

Reply: We have modified our text as advised (see Page 2, line 41).
Changes in the text: The peak levels of serum KL-6 in the death group were significantly higher than those in the survival group for both intra- and extrapulmonary ARDS (P =0.0253)

"The optimal cutoff value of the serum KL-6 level was 1,452.3 U/mL in intrapulmonary ARDS patients and 828.2 U/mL in extrapulmonary patients". At what point in time?
Reply:The cutoff value were calculated based on ROC curve analysis, and the data were derived from the peripheral venous blood of patients at the same time on days 0, 1, 3 and 7.
Changes in the text: none

Introduction

"Intrapulmonary edema typically occurs in the presence of…". This is confusing as edema is always "intrapulmonary", both in intrapulmonary and extrapulmonary ARDS.
Reply: We have modified our text as advised (see Page 3, line 71).
Changes in the text: ARDS is hypoxemic respiratory failure caused by non-cardiogenic pulmonary edema. It can be caused by intra- or extrapulmonary factors.Intrapulmonary ARDS typically occurs in the presence of diffuse pulmonary infections, aspiration, and pulmonary contusions.

"In contrast, extrapulmonary sepsis is caused by extrapulmonary sepsis…". Please, correct. Do the authors mean "extrapulmonary ARDS?.
Reply: We have modified our text as advised (see Page 3, line 74)
Changes in the text: In contrast, extrapulmonary ARDS is caused by extrapulmonary sepsis, severe nonthoracic injuries, and hyperperfusion

"Moreover, the annual ARDS prevalence in the ICU is 200 cases per 100,000 people". Do the authors really mean "prevalence in the ICU"?
Reply:We refer to the prevalence of ARDS patients requiring ICU care.
Changes in the text: none

"…with in-hospital and 90-day mortality rates of 68.5% and 70.4%, respectively (3)". This is much higher than the mortality generally reported in large series, e.g., in the Lung Safe study. Please, comment.
Reply:Because the sample size of our data is small, there may be bias, and we will gradually increase the sample size in future studies.
Changes in the text: none

Throughout the manuscript, I think it is more correct to speak about "concentration (serum or plasma)" rather than "content" of KL-6.
Reply:We have replaced the corresponding names in the article
Changes in the text: none

Methods

“…those who withdrew…” . Please, specify (e.g., treatment was withdrawn…).
Reply:By this we mean that a subset of patients who did not stay in the ICU for 7 days and could not collect complete data and would be excluded from the trial
Changes in the text: none

Was there IRB approval?
Reply:This experiment is based on a research project, which has completed ethical approval. For example, this article needs to be approved separately. We are sorry that we cannot complete it in a short time.
Changes in the text: none

Lung injury score. How was it calculated? Use the term consistently, as this is referred to later (Table 1) as "pulmonary injury score".
Reply:We used the Murry Lung Injury Scale to calculate lung injury score.(see Table 1)
Changes in the text: none

Results

"We observed no marked differences in the circulating KL-6 dynamic contents among deceased versus surviving patients with intrapulmonary ARDS at any time". Do not the authors mean rather "extrapulmonary"?
Reply:We are really sorry for our careless mistakes. Thank you for your reminder. We have modified it (see Page 6, line 189).
Changes in the text:We observed no marked differences in the circulating KL-6 dynamic concentration among deceased versus surviving patients with extrapulmonary ARDS at any time point throughout the study

"We revealed that the circulating KL-6 content among deceased patients reached its peak on Day 3.0±1.5, at 1,985.8±649.1 U/mL. The peak was achieved on Day 2.4±2.4 among patients who survived, with 1,005.6±595.6". Was this difference in the time to reach a peak statistically significant?
Reply: Yes,We have modified our text as advised (see Page 6, line 198).
Changes in the text:and differences compared between the survival and death groups at each time point and found to be statistically significant.

It is later said: "The circulating KL-6 contents reached their peak on Day 3.5±3.0 among deceased patients and on Day 2.2±2.0 among surviving patients". Does this refer now to extrapulmonary, and the previous values to intrapulmonary? Please, clarify.
Reply:Yes, this refer to extrapulmonary, and the previous values to intrapulmonary.(see Page 6, line 201)
Changes in the text:Among extrapulmonary ARDS patients, the circulating KL-6 concentration reached its peak on Day 3.5±3.0 among deceased patients and on Day 2.2±2.0 among surviving patients.

Discussion

"KL-6 is ubiquitously expressed following AT-II damage and regeneration". Is it KL-6 ubiquitously expressed, or only in AT-II? Please, correct.
Reply:KL-6 is ubiquitously expressed, and it obviously expressed when AT-II was damaged or regenerated.(see Page 8,line 257)
Changes in the text:KL-6 is ubiquitously expressed, and it obviously expressed when AT-II was damaged or regenerated.

Figures and Tables

Provide asterisks to indicate statistical significance.

Reply: **We feel very sorry that we can't reply the following comments immediately.**

It is not necessary to indicate p values with 4 decimals.

"Figure 3 Determination of the optimal cutoff value of the circulating KL-6 concentration among intrapulmonary ARDS patients (A) and extrapulmonary ARDS patients (B) using ROC curve analysis. The highest circulating KL-6 concentration in an individual patient, whereby the vertical axis indicates the true positive rate (sensitivity), the horizontal axis presents the false-positive rate (100% − specificity), and the AUC represents the proportion of patients who died at the time of a positive test (elevated serum KL-6 level)".

To my knowledge, in the ROC curve, the Y axis does not indicate the true positive rate, but the sensitivity. True positive rate is not the same as sensitivity. Likewise, the X axis does not indicate the false-positive rate, but 1− specificity, which is different.

Also, the AUC does not represent "the proportion of patients who died at the time of a positive test" but rather the sensitivity and specificity of each individual each quantitative value of the determination, in this case KL-6.