Peer Review File

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Reviewer A

I have reviewed your manuscript titled "Krebs von den Lungen-6 as a Biomarker for Distinguishing between Interstitial Lung Disease and Interstitial Lung Abnormalities." Your investigation into the differences in serum KL-6 levels between ILD and ILA patients is intriguing; however, there are significant issues that require further clarification and improvement.

Major Comments:

1. Definitive Diagnosis of ILA

Is it possible to make a definitive diagnosis of ILA with only short-term follow-up chest CT? In this study, the observation period is approximately two years from the start of the study. Generally, within this time frame, progressive ILDs like IPF may become apparent on CT images, but for certain PF-ILDs or early-stage IPF lesions, a two-year observation period might be insufficient for definitive diagnosis.

This limitation introduces a significant bias, especially given the small sample size and the lack of consideration for disease progression behavior. Furthermore, the absence of histopathological diagnoses to address these limitations raises concerns about the study's accuracy.

If conclusions are to be drawn from the current findings, would it not be more appropriate to revise the title to emphasize that the study identifies differences in KL-6 levels between patients with ILA findings on CT images and those without such findings?

Reply 1: We agree with your valuable opinion and acknowledge the absence of pathological diagnoses as a substantial constraint within the scope of our study. This issue has been deliberated upon in the discussion section.

The objective of the study was to demonstrate the potential of KL-6 in facilitating differentiation with chest CT, despite the fact that a considerable proportion of the study participants were elderly, which complicates diagnosis through general anesthesia or cryobiopsy.

We also clarified the distinction between ILA and ILD in the abstract and manuscript description, as you suggested.

While it is widely acknowledged that a considerable portion of ILA can lead to ILD, this study was cross-sectional in nature with no follow-up data, making it challenging to ascertain serial changes in lung function and KL-6 levels. Further research is necessary to clarify this matter.

Changes in the text 2:

We have changed the title of our article as follows;

Krebs von den Lungen-6 as a Biomarker for Distinguishing Between Interstitial Lung Disease and Interstitial Lung Abnormalities Based on CT Findings

Page 3 line 55-57

Due to the ambiguity regarding the distinction between ILA and ILD in imaging findings, (16) we defined patients with ILA as those without clinically significant limiting dyspnea of mMRC grade 3 or higher and not undergoing pharmacologic therapy

2. Interpretation of Figure 1b and Diagnosis Criteria

In Figure 1b, KL-6 values are depicted for each diagnosis using a box-and-whisker plot. Notably, three IPF patients exhibit KL-6 values exceeding 3000. Such extremely high levels in IPF are often associated with conditions like hypersensitivity pneumonitis (CHP) or malignancy. Were these patients diagnosed through pathology?

If imaging alone was used for differentiation, how were CHP patients excluded? Additionally, the diagnostic criteria or methods applied for each ILD subgroup should be clearly described to support the accuracy of the findings.

I hope these points will be helpful in improving your manuscript.

Reply 2: Thank you for your valuable and meticulous feedback. A reference regarding the diagnosis of the ILD subgroup has been incorporated into the methods section of this study. The majority of the patients in our study were elderly and lacked a pathological diagnosis, as they could not tolerate video-assisted thoracoscopic surgery (VATS) for diagnostic purposes due to the risks associated with general anesthesia. Of the study participants, only two of ILD patients had a pathological diagnosis. This represents a critical limitation of the study, as differentiating IPF from CHP is challenging without pathological testing and comprehensive epidemiological investigation. We have added a mention of this issue in the Discussion section. As authors of this study, the attending clinicians diagnosed and treated IPF in cases where there was no occupational or environmental exposure to potential hypersensitivity pneumonitis triggers.

Changes in the text 2:

Page 3-4 Line 47-52

. IPF was diagnosed according to the Official ATS/ERS/JRS/ALAT Clinical Practice Guidelines (2022) (13). NSIP (14,15), COP (14,15), PPFE, CPFE, and fibrosing medistinitis were diagnosed through discussion with a multidisciplinary team that included a radiologist, and CTD-ILD was diagnosed by rheumatologists who made the concomitant clinical diagnosis of ILD in conjunction with rheumatoid arthritis, Sjogren's syndrome, and microscopic polyangiitis (MPA).

Page 8 Line 139-145

The limitations of our study include diagnosis of most patients through imaging without pathological confirmation and a lack of central consensus-based diagnostic criteria, the

retrospective nature of the study, and the inclusion of only four women with ILD. This is a limitation related to the nature of clinical practice in this field, where older patients are often referred. The patients enrolled in the study were older adults, with a median age of 77.1 years, and included patients for whom the use of invasive diagnostic procedures, such as video-assisted thoracoscopic surgery or cryobiopsy, was difficult. Additionally, this study included a heterogeneous population of patients with ILD.

Reviewer B

This is a study which draws attention to an interesting point in the diagnosis and follow-up of patients with Interstitial Lung Disease (ILD), namely the possible role of Krebs von den Lungen-6 (KL-6) as a diagnostic and monitoring biomarker to evaluate patients in the real-world setting. Indeed, although a wide variety of innovative biomarkers (including KL-6) have and are being investigated, they are not largely employed to evaluate non-neoplastic lung diseases.

In the submitted paper, the Authors reported the results of measuring KL-6 in a population of 147 ILD patients and analyzed differences between clinically recognized and treated ILD patients and subjects with Interstitial Lung Abnormalities (ILA).

The aim of the study is of clinical importance: indeed, the paper may help to confirm that KL-6 can be utilized as an effective diagnostic and prognostic biomarker in individuals with ILD. Unfortunately, the aim is partially obscured by structural deficiencies that render the paper not acceptable in its present form.

Major comments

1. There is a major problem with regard to the study's methodology and I have a concern about this crucial point. In the “Methods” section (Classification of ILD and ILA), the Authors seem to make a clear distinction between patients with a diagnosis of ILD and those with ILA. However, in the “Results” section the impression is given that subjects with ILA may represent a subgroup of patients with ILD. This point needs clarification.

Reply 1: We are in full agreement with the aforementioned point. In response to your

comments, we have revised the wording and clarification of potentially confusing terms and classifications. In addition, among patients referred to our clinic with suspected ILD, cases categorized as ILA were limited to those who met the Fleischner Society's diagnostic criteria and did not have clinically significant dyspnea and were not receiving medication.

Changes in the text1:

Page 4 line 55-57

Due to the ambiguity regarding the distinction between ILA and ILD in imaging findings, (16) we defined patients with ILA as those without clinically significant limiting dyspnea of mMRC grade 3 or higher and not undergoing pharmacologic therapy

2. We suggest that the Authors mention the cut-off points of KL-6 within the Korean population to facilitate the understanding of the diagnostic efficacy of this biomarker in the real life.

Reply 2: We would like to express our gratitude for your valuable insight.

Changes in the text 2:

Page 11, Line 203-208

This study, which was conducted on a population of older Korean individuals diagnosed with interstitial lung disease (ILD) or idiopathic lung inflammation (ILA), consisted of mostly male subjects. Through clinical and radiological evaluation, the study suggests cut-off values for KL-6 and its application as a biomarker for patients requiring intensive treatment in real-world practice.

Reviewer C

The author showed the difference of KL-6 levels between patients with ILD and ILA. This paper has some problems for publishing in Journal of thoracic disease.

Major comments

This study result may be the first report of a difference in KL-6 between ILA and ILD patients, but this is an easily predicted result and has no clinical significance. Physician and researchers consider important what biomarkers involved in the progression of ILA.

Fibrotic ILA and non-fibrotic ILA are thought to have different characteristics and prognoses, so classification between the two should be included in this study as well.

Reply 1: We are grateful for the constructive feedback and have incorporated a note regarding subtypes of ILAs into the text.

Changes in the text 1:

Page 10 Line 152-155

Among the patients with ILA, 12 (70.6%) had fibrotic ILA, while 5 (29.4%) had nonfibrotic ILA.

Due to the small number of ILAs, the statistical accuracy of this study cannot be guaranteed.

Reply 2: We also agree with your opinion. Our analysis was constrained by the limited number of patients who underwent KL-6 and HRCT during the study period. This limitation is addressed in the discussion section. However, it is important to note that all patients with ILA in this study underwent KL-6 and PFT tests. Therefore, we analyzed the data from these tests, and the correlation between KL-6 levels and FVC and DLCO in the study subjects demonstrated an inverse relationship, which is consistent with previous studies, although these were not statistically significant.

Changes in the text 2:

Page 8-9 Line 146-150

Furthermore, the small number of patients with ILA included herein is one of the limitations of this study. However, it is important to note that all patients with ILA in this study underwent KL-6 and PFT tests. We analyzed the data from these tests, and the correlations between KL-6 levels and FVC and DLCO in the study subjects demonstrated inverse relationships, which is consistent with the findings of previous studies; however, these findings were not statistically significant.

The fact that the diagnosis of ILA was made by the attending physician rather than a central diagnosis significantly hampers the accuracy of this study.

Reply 3: We believe that this is very important opinion and difficult to overcome. A lack of central diagnosis is one of the limitation of our single center study and we included this discussion section. We defined ILA as more than 5% HRCT involvement, but there are no clear criteria for symptoms or lung function. In this study, ILA patients were asymptomatic or had no significant respiratory distress, and not candidates of treatment. However, in Korea, the number of HRCTs has increased due to National General Health Screening Program and the recent pandemic of COVID-19, so we tried to suggest evaluating the patient's condition with the KL-6 test. Of course, a careful history and examination by a clinician, as well as lung function testing, should be performed.

Changes in the text 3:
(Page 8 Line 139-145)

The limitations of our study include diagnosis of most patients through imaging without pathological confirmation and a lack of central consensus-based diagnostic criteria, the retrospective nature of the study, and the inclusion of only four women with ILD. This is a limitation related to the nature of clinical practice in this field, where older patients are often referred. The patients enrolled in the study were older adults, with a median age of 77.1 years, and included patients for whom the use of invasive diagnostic

procedures, such as video-assisted thoracoscopic surgery or cryobiopsy, was difficult. Additionally, this study included a heterogeneous population of patients with ILD.

Reviewer D

Dear Authors,
regarding this interesting research I have the following comments:
- For me it is not clear if you are including patients with ILD or subclinical ILD, Because, as you are aware, following reference 8, we consider ILA " In individuals in whom interstitial lung disease is not suspected". But your patients were referred to an ILD Clinic. So it must be clarified why ILA was consider, including parameters as normal lung function or absence of symptoms. I think this is crucial for your study.

Reply 1: Thank you for your insightful comment. We have included the following clarification, which we did not formulate correctly.

Changes in text 1:

Page 4 line 55-57

Due to the ambiguity regarding the distinction between ILA and ILD in imaging findings, (16) we defined patients with ILA as those without clinically significant limiting dyspnea of mMRC grade 3 or higher and not undergoing pharmacologic therapy

- I am curious about the ILD population of this study. There is a significant proportion of patients with IPF, only a few woman. Is this the usual presentation in ILD Clinics in your country? Because this is significant bias for the external validation of this study.

Reply 2: We appreciate the insightful remark. The KICO registry data, which pertains to a Korean IPF cohort, (Tuberc Respir Dis (Seoul). 2021 Dec 13;85(2):185–194) indicates that approximately 76% of the patients are male. In contrast, the proportion of males in our study is notably high. Our hospital's focus on veteran patients might introduce a bias in our results. Furthermore, the prevalence of IPF has been documented

to be approximately 50% in Korean ILD study. (BMC Pulm Med. 2023 Mar 22;23(1):98) However, a detailed analysis of the characteristics of our patient population reveals a distinct profile, with a notable proportion of elderly male patients who have a history of smoking. A significant proportion of these patients were diagnosed with a UIP pattern on CT scans, a finding that may have contributed to the high prevalence of IPF observed in this study.

Changes in text 2:
Page 9 : line 161-166

Data from the KICO registry, a Korean IPF cohort (20) indicate that approximately 76% of patients are male. In contrast, the proportion of males in our study is remarkably high. Furthermore, the prevalence of IPF has been reported to be approximately 50% in the Korean ILD study. (2) However, a detailed analysis of the characteristics of our patient population reveals a distinct profile, with a notable proportion of older male patients with a history of smoking. The focus of our center on veteran patients may introduce a bias in our results.

- Both in the abstract and text avoid including results in methods (147 patients)

Reply 3 : We acknowledge your point and have made the requisite modifications.

Changes in the text 3:
Page 3 Line 32-33

This retrospective study included patients who visited the pulmonology clinic of a tertiary hospital between October 2022 and March 2023 and were examined for KL-6 levels.

- In the abstract please clarify the design of the study (retrospective)

Reply 4: We are grateful for the constructive feedback and have addressed the retrospective nature of this study in the abstract.

Changes in text (abstract)

The present study included patients who were referred to our pulmonology clinic for suspected ILD and had undergone KL-6 assessments between October 2022 and February 2023. Epidemiological and clinical details of the patients, including the outcomes of pulmonary function tests and computed tomography findings, were retrospectively extracted from electronic medical records.

- References need to be updated, please consider including PMID: 38644152, 39137538, 38943279

Reply 5: We appreciate the valuable suggestion to enhance the paper, and we have incorporated the reference as follows.

Changes in text 5:

Page 10 Line 171-174

. In a study by d'Alessandro et al., which assessed KL-6 levels in patients with fibrotic ILD, the KL-6 cut-off value was 885 U/mL, and KL-6 was reported to be elevated in patients IPF, fibrotic HP, and SSc-ILD; moreover, similar to this study, inverse relationships between KL-6 and FVC and DLCO were identified

Page 10 Line 180-183

Consistent with previous findings, guidelines suggest that KL-6 levels in patients with SSc-ILD and PM/DM-ILD can be used as a marker to assess the severity of the disease (27)

Page 10 Line 182-183

A European prospective cohort study comprising 179 patients reported a sensitivity of 82% and specificity of 86% for a cut-off value of 465 in 102 ILD patients compared with 77 controls (28).