Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-21-1973

Reviewer A

Introduction
Comment 1: State your hypothesis at the end of the Introduction.
Reply 1: We have added our hypothesis at the end of the Introduction section, as advised.
Changes in the text:
Page 7, lines 100–101.

Methods:
Comment 1: It's unclear how you selected 5 out of 25 possible patients with idiopathic multicentric Castleman's (line 104-105), raising concern for selection bias
Reply 1: The diagnoses of the five patients analyzed were confirmed with histological findings of lung or mediastinal lymph node specimens. Other 15 patients only had imaging findings but were not diagnosed based on histological findings. Because we could not distinguish pulmonary manifestation of iMCD and lung abnormal shadow of other comorbidities by imaging findings, we focused on histologically diagnosed iMCD patients with thoracic lesions.
However, as Reviewer B pointed out, pulmonary manifestation is not common with iMCD. Additionally, the patients with thoracic lesions who needed biopsy were rare, resulting in selection bias caused by the small sample size. We have described this limitation in the Discussion section.
Changes in the text: Page 7, lines 108- Page 8, lines 109. Page 15, lines 239-245.

Comment 2: Quantify the treatment response (complete, partial, stable, progressive, etc) in the 5 patients with Castlemans and clarify how treatment response was documented (biochemical, symptoms, radiographic response). Unless all 5 patients had a complete response, it's hard to put your results in perspective, as they could be confounded by residual or progressive disease. Did the change in biomarkers correlate with the degree of treatment response?
Reply 2: As you pointed out, the treatment response was described based on Castleman's Disease Collaborative Network (CDCN) response criteria. However, there was no significant change equal to PR or PD based on CDCN response criteria. Because all five patients had no complete response, we have modified our text as advised. (See Page 12,

line 185)

Severity and Treatment response based on CDCN response criteria

Patient No	Severity	Overall Response	Biochemical	Lymph node	Symptoms
No. 1	Not applicable	SD	SD	SD	SD
No. 2	Not applicable	SD	SD	SD	SD
No. 3	Not applicable	SD	SD	SD	SD
No. 4	Not applicable	SD	SD	SD	SD
No. 5	Applicable	SD	SD	SD	SD

In addition, the change for biochemical, symptoms, and radiographic findings were evaluated in detail. These are shown in Supplementary Table 1 and Supplementary Figure 1. LRG tended to correlate with biochemical values such as hemoglobin, albumin, and CRP, although not with changes of radiological findings and symptoms as observed in CRP.

Subsequently, we scrutinized the relationships with LRG and CHAP scores, proposed as scoring systems for disease activity (Fujimoto et al., 2018). “Tentative diagnostic criteria and disease severity classification for Castleman disease: A report of the research group on Castleman disease in Japan. Mod Rheumatology 28(1): 161-167).” The ΔLRG tended to correlate with ΔCHAP score, although not statistically significant due to the small sample size. (Polyserial correlation coefficient = 0.8795, p-value = 0.7818) (Supplementary Figure 2).

Changes in the text:

Page 12, line 185; Page 12, line 187-195.

Comment 3: You mentioned you used a complete case analysis to handle missing data. How often did you have missing data, which could be especially problematic in a study this small?

Reply 3: Because we used serum collected in the biobank, some parameters were missing. The data of hemoglobin, platelet, and neutrophil count of one of three healthy control patients were missing. These could underestimate the difference between the five

iMCD patients and healthy controls, as Reviewer B pointed out in Comment 1.
We have added this point to the Discussion section.
Changes in the text: Page 15, line 247-249.

Comment 4: Provide a classification of disease severity according to Castleman's Disease Collaborative Network Criteria. Did disease severity correlate with biomarker levels?
Reply 4: As you pointed out, we described patients' classification according to iMCD severity based on CDCN criteria.

Patient No	Severity
No. 1	Not applicable
No. 2	Not applicable
No. 3	Not applicable
No. 4	Not applicable
No. 5	Applicable

Only patient No. 5 was applicable because of renal dysfunction and pulmonary involvement. However, the LRG levels of this patient were not the highest. This may imply that LRG can be a useful individual biomarker but cannot reflect disease severity across individuals. We have added this point as text to the result and limitation.
Changes in the text:
Pages 12 and 13, lines 196-200.

Results
Comment 1: Did any patients develop progressive disease months or years after treatment? Did disease progression correlate with a rise in leucine-rich alpha-2 glycoprotein levels, following an initial decrease immediately following treatment? In other words, can leucine-rich alpha-2 glycoprotein levels be used to follow patients longitudinally over the course of their treatment and surveillance?
Reply 1: We could not evaluate whether LRG levels can be used to follow patients longitudinally because none of the five patients analyzed developed progressive disease

based on CDCN response criteria. Evaluating LRG levels longitudinally in future research is needed. We added this point in limitation.
Changes in the text: Pages 15, lines 250-252.

Discussion
Comment 1:
It's known that tocilizumab can lead to spurious IL-6 levels for 18-24 months after treatment. How might this impact interpretation of your results? You hypothesized that IL-6 independent pathways may be important. On the contrary, could the early confounding effect of treatment on biomarker levels be impacting the interpretation of your findings?
Reply 1: The fact that tocilizumab can lead to spurious IL-6 levels did not particularly affect the results of this study because IL-6 was not treated as a biomarker. In fact, the respiratory function was not associated with IL-6 trends (Fig. 3). We cannot deny that treatment effects may have influenced the interpretation of the results. However, the fact that LRG levels behaved differently from CRP does not change. Thus, this point does not influence the interpretation of our results.
Changes in the text:
None.

Reviewer B

Comment 1: The major limitation of this article is the sample size. Only 5 and 3 cases are included for iMCD and healthy control, respectively. The small sample size precludes the authors to draw any solid conclusions. In addition, the non-significant difference of CRP as well as hemoglobin levels between treatment naive iMCD and healthy control further indicates the inadequency of sample size.
Reply 1: We focused on Castleman's disease complicated by pulmonary lesions. The sample size was small because patients not diagnosed based on histological findings of the mediastinal lymph node or lung specimen and not distinguishing pulmonary manifestation of iMCD and lung abnormal shadow of other comorbidities by imaging findings were excluded. As you pointed out, the lack of significant differences in CRP and hemoglobin values may be because of the sample size. We have added this point as a limitation.
Changes in the text: Page 15, line 239-245.

Comment 2: Pulmonary involvement is not a classic manifestation of iMCD, and its therapeutic response is not well studied. The authors should discuss why they specifically chose these patients as the study candidates. Although they mentioned that all five patients were biopsy proven with pulmonary involvement, no detailed information was provided in terms of the biopsy site, histological findings, baseline chest imaging as well as comprehensive pulmonary function test.

Reply 2: As you pointed out, we agree that pulmonary manifestation of iMCD is not common, but respiratory physicians occasionally see iMCD with pulmonary lesions in the foreground. It is difficult to discuss treatment effects, i.e., the disease activity evaluation is not well defined; respiratory physicians have no biomarkers for evaluating that. Therefore, this study investigated the biomarkers of iMCD with pulmonary manifestation. We focused on iMCD with pulmonary lesions and searched for biomarkers mainly by using pulmonary function data, which can only be quantified although with measurement error.

Table 1 was modified to describe biopsy site information, and it included the details of histological findings, baseline chest imaging, and comprehensive pulmonary function test in Supplementary text.

Changes in the text: Page 6, lines 86-87. Page 10, line 159-161.

Comment 3: LRG is a biomarker reflecting both IL-6 dependent and independent inflammation. I did not see its unique role specifically in iMCD patients with pulmonary involvement. The authors should also include iMCD cases without pulmonary involvement, and other typical mimickers, such as HHV-8 MCD, UCD, POEMS syndrome to justify the significance of LRG.

Reply 3: We agree with your opinion. We could not confirm LRG specificity to monitor pulmonary manifestation of iMCD because we did not compare iMCD with pulmonary involvement and other iMCD manifestation. The main purpose of this study was to investigate the candidate for the biomarker of iMCD with pulmonary involvement because respiratory physicians could see iMCD with pulmonary lesions in the foreground. However, there is no biomarker to evaluate disease activity. Thus, we did not mainly focus on LRG specificity, but on its utility for monitoring disease activity of iMCD with pulmonary involvement. However, we agree that this point is important and added the need to study this point in future research to the limitation. Other typical mimickers, HHV8-positive MCD, are very rare in Japan, and none was found among the patients in this study. UCD was not included in this study because the respiratory function was not assessed over time to evaluate disease activity. POEMS syndrome was observed in a few

cases but was not included in the LRG measurement since none of them had pulmonary involvement. However, this point is important to establish LRG levels as a biomarker. We have described this point in the Discussion.
Changes in the text: Page 15 and 16, lines 252-256.

Reviewer C

Comment 1: This study highly pertains to the specific status of iMCD in Japan rather than in other countries and regions. Given its small sample and limited data for a robust conclusion to be generalized elsewhere, the title should be ended with "a single-center case-control study from Japan". The authors should try to specify that this concept in the Discussion and somewhere in the main text as appropriate.
Reply 1: Thank you for your helpful comment. In accordance with your advice, we have changed the title from “Leucine-rich a-2 glycoprotein as a potential biomarker of idiopathic multicentric Castleman disease with pulmonary involvement: a case-control study” to “Leucine-rich a-2 glycoprotein as a potential biomarker of idiopathic multicentric Castleman disease with pulmonary involvement: a single-center case-control study from Japan”.
Furthermore, we have added text to the Discussion section indicating that this study greatly pertains to the specific status of iMCD in Japan, rather than in other countries and regions, as one of the limitations of the study.
Changes in the text: Page 16, lines 268–271: Fifth, this study was a single-center cohort study conducted in Japan; thus, the results may not completely represent the entire population of patients with iMCD with pulmonary involvement. A multicenter, international prospective study will be needed in future research to resolve these limitations.

Comment 2:
The affiliation of Tetsuji Naka is misspelt. "interactable" should be "Intractable".
Reply 2: Thank you for pointing out this oversight. We have corrected the affiliation of Tetsuji Naka accordingly.