Peer Review File

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Reviewer A

Comment 1: The objective of this study was to assess the diagnostic utility of hydroxyproline (HYP) in patients with pulmonary tuberculosis (PTB), both as a standalone marker and in conjunction with the T-cell spot assay (T-SPOT.TB). The combination of HYP and T-SPOT.TB demonstrated enhanced diagnostic accuracy in serum, yielding a sensitivity of 78.57%, specificity of 96.77%, and an area under the curve (AUC) of 0.8690. In bronchoalveolar lavage fluid (BALF), the sensitivity and specificity were 78.59% and 93.55%, respectively, with an AUC of 0.8606, indicating a marginal improvement compared to HYP alone. Although HYP is known to be involved in various pathological conditions, including hepatic fibrosis, dermatological disorders, graft-versus-host disease (GVHD), wound healing, and cardiovascular diseases, it does not appear to be specific for PTB. Thus, for a more comprehensive diagnostic evaluation of PTB, multivariate analysis incorporating HYP and IGRA, as well as demographic and clinical variables such as gender, age, and body mass index (BMI), is recommended.

Reply 1: Thanks to the advice of experts, TSPOT.TB in this paper is the enzyme-linked spot method for IFN-γ release, while IGRA is the quantitative detection method for IFN-γ release. The detection significance of the two methods is the same. In addition, there was no significant difference between the two groups in other indicators (such as gender, age, and body mass index (BMI)), so no multivariate analysis was conducted.

Changes in the text: None.

Reviewer B

Comment 2: The study addresses a significant and timely issue in public health by exploring new diagnostic markers for pulmonary tuberculosis (PTB). Given the persistent challenge of TB globally, this research is highly relevant.

Despite the positive co relations of HYP in all samples of PTB group, the author did not provide details regarding any other chronic illness the patients might be suffering from in the PTB group.

The sample size though adequate, a larger sample size might provide more robust data and increase the generalizability of the findings. The potential for selection bias in the chosen control groups should also be addressed.

However, comparing the values of HYP in different samples in HC, RC and PTB has not been done previously. The study shows significant results in regard to this.

The conclusion, while positive, feels somewhat brief. Expanding on the implications of the findings, potential limitations, and suggestions for future research would provide a more comprehensive summary of the study's contributions.

Reply 2: Thanks for the advice. In the patient inclusion information for materials and methods, we have excluded PTB patients with chronic disease (Line 137). In subsequent studies, we will continue to expand the sample size and conduct multi-center studies to continue to demonstrate the role of HYP in PTB and related diseases.
Changes in the text: We added the other chronic illness in method (Line 137). We added the context in the conclusion (Line 317-320).

Reviewer C

Comment 3:

1) I suggest the authors to indicate HYP alone or in combination with T-SPOT.TB.

Reply3 : We had indicate HYP alone or in combination with T-SPOT.TB for PTB diagnose in the study in Results and Discussion sections.
Changes in the text: None.

Comment 4:

2) In the abstract, the authors did not explain why HYP is potentially accurate for the identification of TB and why it needs to be used in combination with T-SPOT.TB in the background, did not describe how the golden diagnosis of TB was made and how the HYP and T-SPOT.TB were combined in the methods, did not describe the characteristics of the samples in the results, and did have comments on the limitations of this study in the conclusion.

Reply 4: In the abstract, The expression of HYP in serum of patients with TB was significantly increased as compared to that in controls, Thus as a accurate diagnosis marker for the identification of TB. T-SPOT.TB, as a commonly used body fluid test indicator, is the most common molecular marker used for joint examination in many studies.
Changes in the text: We added the context in the results (Line 254-261)and conclusion (Line 317-320).

Comment 5:

3) In the introduction of the main text, I suggest the authors to report the sensitivity and specificity of conventional diagnostic/screening methods for TB, as well as their combination. These data are important for the current study. Please also analyse the methodology limitations of prior studies. The authors need to explain

why they consider to combine HYP with T-SPOT.TB, not other available methods.

Reply 5: In the first paragraph of Introduction section , We had analyzed the methodology limitations of prior studies in line 79-92. T-SPOT.TB, as a commonly used body fluid test indicator, is the most common molecular marker used for joint examination in many studies. Thus we considered to combine HYP with T-SPOT.TB, not other available methods.

Changes in the text: None.

Comment 6:

4) In the methodology of the main text, please describe the sample size estimation procedures, specify how the HYP and T-SPOT.TB were combined, describe how the cut-off value of HYP was ascertained, and the threshold AUC, sensitivity and specificity values for a good diagnostic test method.

Reply 6: We had expound it in the statistical analysis in the methodology of our article. HYP and T-SPOT.TB were combined by the receiver operating characteristic (ROC). The cut-off value of HYP in the study was not conducted.

Changes in the text: None.

Comment 7:

5) Please consider to cite several related papers: 1. Kim KH, Jeong N, Lim JU, Lee HY, Lee J, Lee HY, Kim SC, Kang JY. Clinical relevance of false-negative interferon-gamma release assays in patients with tuberculous pleurisy in an intermediate tuberculosis burden country. J Thorac Dis 2022;14(4):1009-1019. doi: 10.21037/jtd-21-1723. 2. Yu R, Hu S, Wang C, Zhang H, Xiao Z, Ma L. Clinical diagnostic algorithm in defining tuberculous unilateral pleural effusion in high tuberculosis burden areas short of diagnostic tools. J Thorac Dis 2022;14(4):866-876. doi: 10.21037/jtd-21-1532. 3. Zhou W, Cheng G, Zhang Z, Zhu L, Jaeger S, Lure FYM, Guo L. Deep learning-based pulmonary tuberculosis automated detection on chest radiography: large-scale independent testing. Quant Imaging Med Surg 2022;12(4):2344-2355. doi: 10.21037/qims-21-676.

Reply 7: We replaced the references mentioned above (line 375-377, 409-411, 415-417).

Changes in the text: We replaced the references mentioned above (line 375-377, 409-411, 415-417).