Peer Review File

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Reviewer A

In this study, Wang and colleagues presents a proteomic analysis of plasma from lung and spleen deficiency in HIV-infected immunological nonresponders. They identified 22 DEPs between INR with LSD and healthy controls, and validated A2M and SELL expression via ELISA. However, the study is not innovative, the study design and the data description need to be improved.

Major:

1. The author stated that the evidence for identification of the TCM syndromes in INR is lacking. This would guide an effective TCM prescription. But the author only analyzed the differentially expressed proteins between SLD-INR with HC. The DEG between SLD-INR and NS-INR should be analyzed, and the DEPs between two groups will guide the TCM prescription for SLD.

Reply 1: The comparison between LSD-INR and NS-INR samples was not completed because of strict inclusion criteria, difficulty in collecting samples, and limited blood samples. We will continue the unfinished experiment in our follow-up study.

2. The study has limited sample size, only 9 LSD-INR and 10 NS-INR were included. More samples should be included.

Reply 2: Because of the limited blood samples, we did not include too many cases, and we will further expand the sample size for the study in the subsequent experiments.

3. The inclusion criteria for INR should be describe in the method, not in the supplementary files. The definition should be concise and clear, and well-accepted by the international academic community.

Reply 3: The research on INR is relatively scarce and has not yet been unified internationally. We rely on national major projects to conduct research that is exploring the causes of INR and potential therapeutic targets.

4. The author stated that lung and spleen deficiency syndrome is a typical TCM syndrome of INR. The references from other researchers should be cited.

Reply 4: Lung and spleen deficiency (LSD) syndrome is a classic TCM syndrome of HIV/AIDS INRs, as established by the epidemiological questionnaire we used in our previous work, see reference 16.

5. For table S1, the clinical parameters including duration of ART, ART regimens, nadir CD4, CD4 count before treatment, CD8, CD4/CD8 ratio, viral load before treatment should be included.

Reply 5: Due to the small number of cases and some missing data, systematic statistics have not been performed, and we will focus on the baseline data in the follow-up study.

6. The correlation between the aberrant proteins with clinical parameters should be analyzed.

Reply 6: We will further analyze the relationship between the clinical data and the protein in a subsequent study report.

7. For the proteomic assay, how many proteins were identified in your experiments

Reply 7: A total of 272 proteins were identified in the LSD group, of which 18 were up-regulated and 4 were down-regulated. 272 proteins were identified in the NS group, of which 23 were up-regulated and 2 were down-regulated. See Figure S1 (A) and (B).

8. The writing of this article must be improved; I suggest your manuscript be revised by a native speaker.

For example, Line 68-69. “However, some patients with HIV/AIDS continue to have undetectable virus after HAART, and their CD4+T-cell counts never rebound.”

Reply 8: Thank you for your advice.

Reviewer B

Can authors explain why the ELISA validation was performed comparing LSD patient’s vs Controls if the authors have the objective to identify biomarkers for LDS wouldn´t be appropriate to compare INR-LSD vs NS-INR?

Reply: Because of the limited blood samples, we did not include too many cases, and we will further expand the sample size for the study in the subsequent experiments.

It will be important to include the HAART regimen of the participants and the period of time that participants have under a HAART regimen. As its known HAART cause a metabolic disorder in some HIV-infected subjects, are any of the participants have any metabolic disorder?

Reply: We performed blood tests at the time of inclusion and found no metabolic disorders.

Since INRs are defined generally as aviremic people that live with HIV that have CD4+ cell counts <300/μL for 2 years (with no HIV rebounds), authors could include the period of INR status of the participants and the time they have living with HIV.
Reply: Thank you for your suggestion and we will look at the correlation between the time of HIV infection and clinical presentation during the INR period in a follow-up study.

It will also be appropriate to include the nadir HIV load and the nadir TCD4 cell count, in order to evaluate if the groups had any difference when HIV diagnostic was performed, also it will be important to include co-infections of any other comorbidity accompanying the HIV infection since immune changes start at the beginning of the infection and most of these changes remain during the chronic phase of the infection.
Reply: We have excluded patients with co-infection at the time of inclusion, thank you for your suggestion, and we will look at the relationship between co-infection and INR in our follow-up study.