Peer Review File

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Reviewer A

At the begining I would like to congratulate the authors very interseting study looking plasma metabolites as potential biomarker for predicting type B aortic dissection.

Aortic dissection is a disease with a very high mortality rate therefore the identification of factors that may predict it or determine the severity of the course is extremely important Currently,there are a few identified biomarkers unique to aortic dissection.

The study was designed correctly. The inclusion and exclusion criteria are clean and transparent. Clear presentation of results. The special value of the work is enhanced by the rich graphic designed that facilitates the reading of the work. The literature is up-to-date and well-chosen and the discussion is interesting.

I dont have many reservations for this article. One particular reservation may be the extremely small study group (16 patients) ,which means that the above study should be treated as a pilot of a large study-possibly a multicenter study-also with an extended period of observation of patients.

Reply: We thank the reviewer for these comments. In the Discussion section, we have emphasized that the small number of subjects is the main limitation of this study, and we will verify these differential metabolites in larger scale studies in the future. On page 17, lines 310-312 (clean Word version), we have modified the statement as “Nevertheless, this study is limited by a relatively small number of subjects included, and the changes of these metabolites need to be verified in larger scale studies in the future.”

Reviewer B

The authors used metabolomics tools to try to detect molecules that could help to improve not only diagnosis but also pathogenetic mechanisms that underlie aortic dissections. In my opinion, the study is well designed and well conducted. As the authors comment in the Discussion, the number of enrolled patients is small, but this fact does not invalid the findings as indicative to be focused in further studies. Thus in my opinion the paper may be published in Cardiovascular Diagnosis and Therapy, provided a few issues are explained:

1) Why only type B was focused? Why not the most common type A?

Reply: Type A aortic dissection (TAAD) is considered as an acute and severe life-threatening condition. Current guidelines consistently recommend immediate surgical intervention upon diagnosis of TAAD. In contrast, optimal medical therapy remains the treatment of choice for a great proportion of patients with type B aortic dissection (TBAD) , while endovascular repair is preserved for complicated TBAD, and used more widely for patients with high risks. However, identification of TBAD with high risks represents a challenge. Therefore, we believe it is more important to identify biomarkers, particularly those associated with the severity of TBAD, to help to make decision of treatment

strategies and predict prognosis.

2) What the authors define as complicated (and uncomplicated) aortic dissection type B?

Reply: Complicated TBAD is defined as type B dissection with evidence of rupture or end-organ malperfusion, while those without these complications are defined as uncomplicated TBAD, according to the guideline released by Society for Vascular Surgery (SVS) and Society of Thoracic Surgeons (STS) in 2020 (Lombardi et al. Journal of vascular surgery. 2020;71(3):723-47.). We have mentioned it on page 7, lines 102-105 (clean Word version), as “The severity of AD was also classified by the SVS/STS aortic dissection classification system (5). All 7 patients with complicated TBAD demonstrated malperfusion syndrome.”

Reviewer C

This manuscript is a nicely presented study looking at how metabolomics can be harnessed to predict type B aortic dissection in hypertensive patients. The results are presented clearly in the figures and described well in the text. The limitations of the study are also noted in the discussion.

Major comment:

The authors show that successively lower levels of 2 metabolites correlate with increasing severity of TBAD; however, claiming that these metabolites are protective against TBAD development is not justified.

Reply: This is a valid comment. We agree with the reviewer that the protective effects of the screening metabolites have not been proven, and further study is needed to confirm the causal effect. Thus, we have modified the statement throughout the manuscript, for instance, on pages 14-15, lines 258-260 (clean Word version), we have revised the sentences as “Therefore, we concluded that hydrocinnamic acid was independently correlated with the severity of TBAD, while glycine deoxycholic acid and glycochenodeoxycholic acid were inversely correlated with the severity of TBAD independently.”

Minor comments:

Figure 4 A,B,C, -- make the dots and graphs larger; consider writing out the categorical names slanted on the x-axis; what exactly is the y-axis showing?

Reply: We have made the graphs and dots larger and slanted the column names below the x-axis for figures 4A, B, C. Moreover, we added the titles of y-axis for these figures as well as the description of the meaning of the titles in the figure legend section as “(A-C) Spearman correlation analysis between three groups and the abundance of hydrocinnamic acid (A), the abundance of glycine deoxycholic acid (B), as well as the abundance of glycochenodeoxycholic acid (C). The values of the metabolites were performed by log2 transformation.” on page 23, lines 445-449 (clean Word version).

p. 10, line 327: The phrasing here “we hypothesized that …” makes it sound like the start of your study, but I presume the authors are saying that this is their working hypothesis for a future study.

Reply: We thank the reviewer for this comment. We have modified the phrasing as “Therefore, we speculated that the increased severity of TBAD caused by decreased bile acids might be related to the reduction of bile acid receptor activation, which needs to be validated in future studies.” on page 17, lines 307-309 (clean Word version).

Check word choice and grammar throughout.

Reply: We have proofread the manuscript and polished the language throughout.

Reviewer D

The manuscript addresses an interesting and significant problem for which there is currently no diagnostic blood test. This study used LC-MS to identify many changes. The authors selected three analytes that when used in concert are predictive of type B dissection severity.

(1) The text should be edited for syntax and clarity.

Reply: We have proofread the manuscript and edited the language throughout.

(2) I recommend the addition of limitations of the methods used. Specifically, please provide details of the use of LC-MS in this capacity.

Reply: We appreciate this recommendation. In this study, we used widely targeted metabolomics to screen for disease-related metabolites. One of the limitations is that we cannot get the exact concentrations in the blood. We added this limitation as “Moreover, we cannot obtain the exact concentrations of the metabolites in the blood via widely targeted metabolomics, which should be addressed in the future.” on page 17, lines 312-313 (clean Word version). Moreover, another challenge of metabolomics is the functional analysis of differential metabolites. Therefore, we discussed the potential reasons and mechanisms for the discrepancy, for instance, on pages 16-17, lines 295-309 (clean Word version), as “Glycine deoxycholic acid and glycochenodeoxycholic acid are two types of bile acids ... Therefore, we speculated that the increased severity of TBAD caused by decreased bile acids might be related to the reduction of bile acid receptor activation, which needs to be validated in future studies.”

(3) The conclusions are based on computer modeling, and it would add to the significance if quantitative methods were used alongside to measure the concentrations of these metabolites in the three cohorts for confirmation. Perhaps the addition of enzymatic quantification that includes a standard curve of known concentrations for each metabolite could address this concern. If this is not possible, or available, the authors should detail further the limitations of computational analyses in this capacity and explain how this could

be used as a potential diagnostic without methods for direct quantification in blood.

Reply: We appreciate these critical comments. In this study, we used widely targeted metabolomics to qualify and quantify the metabolites. Specifically, we identified the metabolite via detecting the retention time, precursor/product ion pairs, and secondary mass spectrum data, and subsequently comparing the acquired information with the metabolite from the Metware database which includes reference compound information. Then, we calculated the peak area of the characteristic ions formed in the mass spectrometer for quantification. However, this strategy cannot obtain the exact metabolite concentration in the blood sample. Due to a lack of wide research on these screened metabolites, the current concentration measurement methods primarily rely on targeted metabolomics. But owing to time and budget constraints, we are unable to utilize this technique for sample analysis in this study. We added this statement in the discussion section as “Moreover, we cannot obtain the exact concentrations of the metabolites in the blood via widely targeted metabolomics, which should be addressed in the future.” on page 17, lines 312-313 (clean Word version).

(4) Also, the details of validation methodology must be described to ensure precision in proficiency testing and reproducible reporting. These details are also necessary for others to validate the use of these analytes in predicting type B dissection severity.

Reply: As suggested, we have added detailed qualitative and quantitative methods of widely targeted metabolomics in method section as “The ultra performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS) was performed to identify the metabolites in each sample by detecting the retention time, precursor/product ion pairs, and secondary mass spectrum data, and subsequently comparing the acquired information with the metabolite from the Metware database which includes reference compound information. Then, quantitative data were obtained via calculating the peak area of the characteristic ions formed in the mass spectrometer. Raw data were analyzed by Analyst 1.6.3 software.” on page 9, lines 131-138 (clean Word version). What’s more, we also added the tandem mass spectrometry figures of the metabolites which related to the TBAD severity in the manuscript as a supplementary figure for others to validate, and described it as “Tandem mass spectrometry figures of the metabolites were shown in the Supplementary figure.” on page 14, lines 249-250 (clean Word version).