

Dear Reviewer and Editors,

We appreciate your letter and the thoughtful comments provided for our manuscript titled "*Predictive Value of Angiopoietin-Like Protein 8 for Metabolic Dysfunction-Associated Fatty Liver Disease and its Progression: A Case-Control Study*" (ID 89342). Your insights have proven invaluable in refining our work and have significantly contributed to the overall quality of our research.

In response to the feedback, we have meticulously revised the manuscript. The revised/added content has been highlighted in yellow for your convenience. We are submitting the revised manuscript for your reconsideration of its suitability for publication, accompanied by detailed point-by-point responses to the reviewers' comments, as outlined below.

We hope these revisions align with your expectations and look forward to receiving your feedback at your earliest convenience.

Sincerely,
Zhe Dai

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Response: Thanks for your review and comments.

Specific Comments to Authors: Here, the Authors present and comment the results of a well conducted study exploring the value of ANGPTL8 for predicting MAFLD and its progression.

1. My main comment is: what could be the actual relevance of ANGPTL8 for clinical practice? to my knowledge, this is not a routine test and it requires specialized equipment. can the Authors comment on this?

Response:

Thanks for your review and comments. We underscore the clinical significance of ANGPTL8 for several compelling reasons.

Firstly, while liver biopsy is considered the gold standard for diagnosing MAFLD, its limitations, including high cost, inaccurate sampling, invasive procedures, and low acceptability, warrant exploration of alternative diagnostic markers. Imaging techniques such as Magnetic Resonance Imaging Proton Density Fat Fraction (MRI-PDFF) and Magnetic Resonance Spectroscopy (MRS) also have limitations and are not widely employed in clinical practice [1]. Previous studies have established a close association between ANGPTL8 and MAFLD, positioning it as a potential new marker capable of detecting MAFLD progression [2,3]. Animal experiments additionally indicate that gene knockout or inhibition of ANGPTL8 expression effectively inhibits hepatic steatosis [4]. Numerous studies have reported up-regulated expression of ANGPTL8 in

MAFLD patients [5]. Therefore, we assert that serum ANGPTL8 can serve as a simple reference marker for early prediction and timely management of MAFLD, thereby enriching the available non-invasive assessment methods in clinical settings.

Secondly, the detection method for serum ANGPTL8 is simple, reproducible, and well-received by patients. Hospital laboratories possess the requisite equipment and technology for its implementation.

Lastly, monitoring changes in ANGPTL8 levels facilitates early detection of liver fibrosis associated with MAFLD, enabling prompt intervention strategies to delay disease progression and reduce mortality risk. In our manuscript's conclusion, we have succinctly summarized the guiding significance of ANGPTL8 for clinical practice, highlighted in yellow for emphasis.

References:

[1] Jang W, Song JS. Non-Invasive Imaging Methods to Evaluate Non-Alcoholic Fatty Liver Disease with Fat Quantification: A Review. *Diagnostics (Basel)*. 2023 May 25;13(11):1852. [PMID: 37296703 DOI: 10.3390/diagnostics13111852]

[2] [2] Ke Y, Liu S, Zhang Z, Hu J. Circulating angiopoietin-like proteins in metabolic-associated fatty liver disease: a systematic review and meta-analysis. *Lipids Health Dis*. 2021 May 25;20(1):55. [PMID: 34034750 DOI: 10.1186/s12944-021-01481-1]

[3][Cengiz M, Ozenirler S, Kocabiyik M. Serum beta-trophin level as a new marker for noninvasive assessment of nonalcoholic fatty liver disease and liver fibrosis[J]. *Eur J Gastroenterol Hepatol*,2016,28(1):57-63 [PMID: 26513612 DOI: 10.1097/MEG.0000000000000502]

[4]Vatner DF, Goedeke L, Camporez JG, Lyu K, Nasiri AR, Zhang D, Bhanot S, Murray SF, Still CD, Gerhard GS, Shulman GI, Samuel VT. Angptl8 antisense oligonucleotide improves adipose lipid metabolism and prevents diet-induced NAFLD and hepatic insulin resistance in rodents[J]. *Diabetologia*,2018,61(6):1435-1446 [PMID: 29497783 DOI: 10.1007/s00125-018-4579-1]

[5]Lee YH, Lee SG, Lee CJ, Kim SH, Song YM, Yoon MR, Jeon BH, Lee JH, Lee BW, Kang ES, Lee HC, Cha BS. Association between betatrophin/ANGPTL8 and non-alcoholic fatty liver disease: animal and human studies[J]. *Sci Rep*,2016,6:24013 [PMID: 27045862 DOI: 10.1038/srep24013]

Additionally, we acknowledge your perspective that serum ANGPTL8 is not a routine test. The detection of serum ANGPTL8 was conducted using ELISA, and accurate detection necessitates specific kit requirements. Notably, there are no special requirements for automatic enzyme labeling instruments. With the prevalence of automatic enzyme labeling instruments in hospital laboratories, these instruments have become commonplace, facilitating effective serum ANGPTL8 detection technology. Therefore, serum ANGPTL8 detection can be incorporated into routine clinical examination methods.

2. Two minor comments, in terms of style:

2.1- there is no need, in my opinion, to report a sentence like the following: Among the 160 patients, 80 patients were diagnosed with MAFLD, and 80 patients did not have MAFLD. it would better read as "Among the 160 patients, 80 patients (50%) were diagnosed with MAFLD". this can apply to several other sentences in the Results.

Response: Thanks for your review and comments. We have already made changes in the corresponding parts of the manuscript.

2.2- there is no need to state $p > 0.05$ if a comparison is not significant. Better to report the actual p-value instead

Response:

Thanks for your review and comments. we readily accepted your comments and suggestions.

When analyzing a variable in isolation, it is more informative to report the specific p-value for better understanding. However, when multiple variables are analyzed together, listing the p-value of each variable may make the sentence excessively long and hinder reading comprehension. Therefore, we believe that when multiple variables are found to be either insignificant or significant, they can be uniformly described as $P > 0.05$ or $P < 0.05$ respectively. However, specific p-values can still be referred to in the corresponding table.

Revised sentences for amendments:

a) Original: "while the AST level did not significantly differ between the 2 groups ($P > 0.05$)"

Revised: "while there was no significant difference observed in AST levels between the two groups ($P = 0.181$)"

b) Original: "while HDL-C was significantly lower in the MAFLD group than in the non-MAFLD group ($P < 0.05$)"

Revised: "while HDL-C levels were significantly lower in the MAFLD group compared to those in the non-MAFLD group ($P = 0.015$)"

We have added this information to the revised manuscript. Thanks again for your review and comments!

LANGUAGE POLISHING REQUIREMENTS FOR REVISED MANUSCRIPTS SUBMITTED BY AUTHORS WHO ARE NON-NATIVE SPEAKERS OF ENGLISH

Response: Thanks for your review and comments. The revised manuscript has been edited by a professional scientific editor.

ABBREVIATIONS

Response: Thanks for your review and comments. We have modified it according to the basic rules of the abbreviation and highlighted it in yellow.

EDITORIAL OFFICE'S COMMENTS

(1) Science editor:

1 Conflict of interest statement: Academic Editor has no conflict of interest.

2 Manuscript's theme: The topic is within the scope of the journal.

3 Academic misconduct: No academic misconduct was found.

4 Scientific quality and comments: The study aims to explore the value of angiotensin-like protein 8 (ANGPTL8) for predicting metabolic dysfunction-associated fatty liver disease and its progression. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1 Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide the original figure documents. Please add secondary title to your figure legends in order to explain the figures more clearly.

5 Language evaluation: Further language polishing is required in order to meet the publication requirement (Grade A).

6 Recommendation: Conditional acceptance.

Language Quality: Grade B (Minor language polishing)

Scientific Quality: Grade C (Good)

Response: Thanks for your review and comments.