**Peer Review File**

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**Reviewer A:**

Comment 1. First of all, the authors describe that they developed a diagnostic model for UC, however, the clinical significance of this research focus is very limited and the findings have little clinical implications. Because the authors made use of this model to make differential diagnosis between normal controls and UC patients, but this is not the clinical concern in real-world clinical practice. In general, we have interest in the differential diagnosis between UC and other conditions that have similar clinical presentations and may be misdiagnosed with UC such as dysentery and Crohn's disease. The other major methodology concern is the large proportion of UC (184/227) and the small proportion of normal controls (43/227) in the study sample, which artificially result in the high AUC values but in the real-world clinical practice, the proportion of UC cannot be so high. The authors need to analyze and report the diagnostic accuracy parameters such as sensitivity and specificity.

Reply: Thank you very much for your suggestion. It is really true as Reviewer suggested that the differential diagnosis of UC from Crohn's disease and tuberculous bowel disease is indeed a very difficult clinical problem. We will consider further studies on the differential diagnosis of UC in the future.

In view of the imbalance in the sample size between UC and normal controls, we divided 184 UC patients into 4 groups, with 46 patients in each group (corresponding to 43 patients in the normal control group), trained 4 sub-models respectively, and performed aggregation analysis on the results. I apologize for not making this clear in the article. I've added that in the "Method" section (see Page 6, line 167-170). Table 1 is supplemented with the AUC, sensitivity and specificity of the diagnostic models generated by the four algorithms (see Page 24, line 573-576 ). Special thanks to you for your good comments.

Changes in the text: We have modified our text as advised (see Page 6, line 167-170). We added a table in our text (see Page 24, line 573-576).

Comment 2. Second, please indicate the development and validation of a diagnostic model in

the title.

Reply: Thank you very much for your comments. The development and validation of the diagnostic model is described in more detail in the "Methods" and "Results" sections (see Page 6, line 167-174) (see Page 8, line 252-253).

Changes in the text: We have modified our text as advised (see Page 6, line 167-174) (see Page 8, line 252-253).

Comment 3. Third, the abstract needs further revisions. The background did not indicate the clinical difficulties in diagnosing UC and why ferroptosis-related genes can potentially and accurately diagnose UC. The methods need to describe the generation of training and validation samples, how the diagnostic accuracy was assessed and the calculation of accuracy parameters. The results need to describe how the diagnostic model was generated and report other diagnostic accuracy parameters. The conclusion is not strict and even misleading, please consider to tone down it and have comments on the limitations of this model.

Reply: Considering the Reviewer's suggestion, we added the difficulties in the diagnosis of UC and the relationship between ferroptosis-related genes and UC in the "Background" section of the abstract (see Page 1, line 30-34 ). The generation of training set and the number of samples in the validation set are added in the "Method" section (see Page 6, line 167-174). The AUC, sensitivity and specificity of the diagnostic model were supplemented in the results section (see Page 24, line 573-576). It is really true as Reviewer suggested that this study lacks further cell experiments and animal experiments. Therefore, we modify the conclusion of the article (see Page 11, line 333-334).

Changes in the text: We have modified our text as advised (see Page 6, line 167-174) (see Page 11, line 333-334). We added a table in our text (see Page 24, line 573-576).

Comment 4. Fourth, the introduction of the main text did not explain the clinical needs for this research such as the clinical difficulties in the diagnosing of UC, what has been known and unknown on the diagnostic models of UC, the limitations and limited diagnostic accuracy of available models, and explain why the ferroptosis-related genes can potentially and accurately diagnose UC.

Reply: Thank you very much for your comments. In the introduction, we summarize the difficulties in the diagnosis of UC and the shortcomings of existing models (see Page 3, line 82-84). We looked up more articles on the relationship between ferroptosis and UC. We referenced and supplemented diagnostic models developed based on ferroptosis-related genes in other diseases (see Page 4, line 86-99).

Changes in the text: We have made modification according to the Reviewer's comments (see Page 3, line 82-84) (see Page 4, line 86-99).

Comment 5. Fifth, in the methodology of the main text, please describe the research methodology of this study, the generation of training and validation samples, calculation of diagnostic accuracy parameters such as sensitivity, and importantly, the clinical significance of the use of normal controls for making differential diagnosis is very limited. Please clearly indicate whether external sample was used for the validation.

Reply: Special thanks to you for your comments. The process of training set generation and the number of samples in the validation set are added in the "Method" section (see Page 6, line 167-174). The AUC, sensitivity and specificity of the diagnostic model were supplemented in the results section (see Page 8, line 252-253) (see Page 24, line 573-576). GSE75214 and GSE87466 are the training set for the model and GSE87473 is the validation set for the model (see Page 6, line 167-174).

Changes in the text: We have made modification according to the Reviewer's comments (see Page 6, line 167-174) (see Page 8, line 252-253). We added a table in our text (see Page 24, line 573-576).

Comment 6. Finally, please consider to cite the below papers to enrich the background of this study: 1. Yang X, Yin F, Liu Q, Ma Y, Zhang H, Guo P, Wen W, Guo X, Wu Y, Yang Z, Han Y. Ferroptosis-related genes identify tumor immune microenvironment characterization for the prediction of prognosis in cervical cancer. Ann Transl Med 2022;10(2):123. doi: 10.21037/atm-21-6265. 2. Yang BY, Zhao MS, Shi MJ, Lv JC, Tian Y, Zhu YC, Zhao FZ, Li XH, Song J. Establishment of a novel prognostic prediction model through bioinformatics analysis for prostate cancer based on ferroptosis-related genes and its application in immune

cell infiltration. Transl Androl Urol 2022;11(8):1130-1147. doi: 10.21037/tau-22-454. 3. Wang L, Chen Y, Zhao J, Luo D, Tian W. Analysis and prediction model of ferroptosis related genes in breast cancer. Transl Cancer Res 2022;11(7):1970-1976. doi: 10.21037/tcr-21-2686.

Reply: Thank you very much for the paper you recommended, which is of great help to us. We have added references to them in the introduction to enrich the background of this study (see Page 4, line 86-99) (see Page 13, line 416-242).

Changes in the text: We have made modification according to the Reviewer's comments (see Page 4, line 86-99) (see Page 13, line 416-242).

**Reviewer B**

1. Please a part Statistical Analysis in "Method" section.

Reply: Thank you very much for your suggestion. As you requested, we have added a part with a subtitle named "Statistical Analysis" in "Method" section.

Changes in the text: see Page 6, line 178-183.

2. For research involving human, authors should state that the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013), available at: https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2013-JAMA.pdf

Reply: Thank you very much for your suggestion. We have added the ethical statement in Footnote.

Changes in the text: see Page 12, line 364-367.

3. Abstract

Please defined WGCNA.

Reply: Thank you very much for your comments. The definitions of our abbreviations are annotated.

Changes in the text: We have modified our text as advised (see Page 2, line 37).

4. Figure 2

Please explain UC and FC in the legend.

Reply: Thank you very much for your suggestion. The abbreviations in the figure are

explained.

Changes in the text: We have modified our text as advised (see Page 19, line 532-533).

5. Figure 5

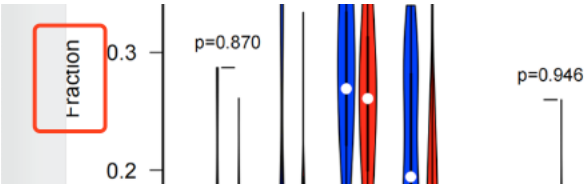
Please explain FC in the legend.

Reply: Thank you very much for your suggestion. The abbreviations in the figure are explained.

Changes in the text: We have modified our text as advised (see Page 21, line 557).

6. Figure 6

The description of the y-axis was covered.




Reply: I apologize for our negligence. We remade the image and sent the TIFF format as an attachment.

Changes in the text: We adjusted the figure (see Page 22, line 599-560).

7. References/Citations

Please double-check if more studies should be cited as you mentioned “studies”. OR use “study” rather than “studies”

375 studies of UC, logistic regression is acknowledged as an extensively applied machine
376 learning technique (58). The use of machine learning can realize gene grouping of the

Reply: I'm sorry about our oversight. I changed the noun into the singular form.

Changes in the text: We have modified our text as advised (see Page 11, line 325).