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Review Comments:

The manuscript by Xie and coworkers present results of a prospective observational study with enteral nourished ICU patients. Patients were divided into two groups (diarrhea and no-diarrhea) and clinical patient data as well as several blood parameters were compared between both groups. The study determined significant differences between both groups in the amount of CD55 on granulocyte and erythrocyte membranes as well as in IL-10 in blood plasma. CD55 was shown to have a predictive value to suffer severe diarrhea.

Major changes:

Abstract:

• The background is too short. Please specify the objectives of the study and introduce your main prediction value (CD55).

• Methods: The number of beds, the hospital, and study period are not necessary in the abstract, but point out data processing analysis a little more.

• Results: In my opinion it is enough to provide p-values without raw data – then you can combine some sentences.

• Conclusion: Is "decrease of gCD55 and eCD55" the right wording? Do you take the differences (day 7 minus day 1) for you prediction model?

Reply 1: Thank you for your revision. We revised the abstract.

• The background :We specify the objectives of the study and introduce your main prediction value (CD55).

• Methods:We delete the number of beds, the hospital, and study period are not necessary in the abstract, and point out data processing analysis.

• Results:We provide p-values without raw data.

• Conclusion: We delete "decrease of".

Changes in the text: abstract

Introduction:

• Lines 73-78: Please add a reference for your statement “Severe diarrhea is the leading cause of electrolyte disturbance worldwide and the main cause of suspension of enteral nutrition” as well as for the statement “there is evidence that discontinuation of enteral nutrition and general antidiarrheal therapy is not ideal, and may cause nutritional deficiencies, delay recovery, and even increase hospital stay or mortality in some patients”.

• Line 80: If you say “CD55 is one of these regulatory membrane proteins…” you should mentioned regulatory membrane proteins before. Please add further information about the role of the regulatory membrane proteins/complement system and the function according regulating diarrhea.

• Lines 82-84: Please add references.

• Lines 89-94: Please rephrase the section “To solve this sequential decision-making problem, we designed a diagnostic model to extract relevant intestinal barrier indicators from cases. In a large validation cohort independent of training data, the clinician's diagnosis matched the model's patient sensitivity and specificity. Our model provides individualized and clinically interpretable treatment decisions for severe diarrhea that could improve patient outcomes.” and state your specific objectives of your study.

Reply 2: Thank you for your revision. We revised the introduction.

• Lines 73-78: we add a reference for statement “Severe diarrhea is the leading cause of electrolyte disturbance worldwide and the main cause of suspension of enteral nutrition” and “there is evidence that discontinuation of enteral nutrition and general antidiarrheal therapy is not ideal, and may cause nutritional deficiencies, delay recovery, and even increase hospital stay or mortality in some patients”.

• Line 80: We add further information about the role of the regulatory membrane proteins/complement system and the function according regulating diarrhea.

• Lines 82-84: we add references.

• Lines 89-94: We rephrase the section “To solve this sequential decision-making problem, we designed a diagnostic model to extract relevant intestinal barrier indicators from cases. In a large validation cohort independent of training data, the clinician's diagnosis matched the

model's patient sensitivity and specificity. Our model provides individualized and clinically interpretable treatment decisions for severe diarrhea that could improve patient outcomes." and state the specific objectives of our study.

Changes in the text: Introduction

Methods:

• Please remove the first sentence to avoid unnecessary repetition.

• Please merge sentence 2, 3 and 4: In example: This monocentric, prospective observational study was conducted from … to … with recruitments periods form … to ….

• Please remove the sentence "Exposure: Documented patients of enteral nutrition complicated with severe acute diarrhea."

• Please merge sentences "Patients follow up ended on January 31th 2020. Data collection periods were from January 31th 2019 to January 31th 2020."

• Line 107: Please provide the number of the ethic committee from lines 350-351.

• Line 107-108: Could you provide additional information about country regulatory? In some countries data collection is not allowed, not even if the data collection is anonymous.

• Lines 113- 118: Please rephrase the exclusion criteria (i.e. …were as follows: history of gastrointestinal surgery, chronic diarrhea, pregnancy, lactation period…etc.)

• Remove lines 119-121 (Source of participants). You can add the first sentence to the section "Patients".

• Add the sentence "The routine medical records system of the hospital was used to register the data for follow-up, if discharged, telephone follow-up was performed." within the section Data collection.

• Add the diagnostic criteria section in the section Data collection (i.e. after the first sentence). Please specify the definition of severe diarrhea. Means the comma between frequency ≥3per day and change of fecal characteristics that the patients must had both for assignment to the diarrhea group? How the change of fecal characteristics was documented? Do you use a classification system (i.e. Bristol Stool Chart)? Who documented this information? Were the subjects trained?

• Line 126: The sentence "In this series, there were 77 without severe diarrhea patients and 29

severe diarrhea patients." should be placed within the results section.

• Please remove the part of "Intervention" to avoid unnecessary information.

• Please place section Outcome before the statistics.

• Line 141-149 (CD55 detection method):

o Do you first isolate neutrophils from whole blood?

o Line 144: 100um PBS – do you mean μM (mirco mol) or μl (micro liter)?

o Lines 145-147: do you mean monoclonal antibody from mice (Mouse IgG2a, $\varkappa$; BD Pharmingen™), who specifically binds to CD55, were added?

o Line 146: then you add RBC lysate (red blood cell lysat?) – do you make all further steps with whole blood?

• Line 150-151: Are there further efforts (i.e. training study personal, data management, defined procedures, same person who performed the flow cytometry)? Maybe you can add this information within the corresponding sections and not as a separate paragraph.

• Lines 152-154: Please add you statement to the study size estimation within the statistic section.

• Statistics:

o Line 157: quantitative indicators = quantitative values?

o Line 159: what do you mean with classification indexes?

o Which test do you used for the comparison of CD levels between both groups?

o Line 162-164: Please provide information which independent variables and dependent variable do you used for the regression.

o Line 167: area under the receiver operating characteristic curve = AUCOC

o Line 168: How the predictors were combined?

Reply 3: Thank you for your revision. We revised the method.

• We remove the first sentence to avoid unnecessary repetition.

• We merge sentence 2, 3 and 4: This monocentric, prospective observational study was conducted in one teaching hospital (Shanghai General Hospital, Shanghai,China)from January 1th, 2019 to January 31th, 2020 with recruitments periods form January 31th ,2019 to December 2th,2019.

• we remove the sentence "Exposure: Documented patients of enteral nutrition complicated with severe acute diarrhea."

• We merge sentences “Patients follow up ended on January 31th 2020. Data collection periods were from January 31th 2019 to January 31th 2020.”

• Line 107: We provide the number of the ethic committee from lines 350-351.

• Line 107-108: In China patients ’data collection is allowed if the data collection is anonymous.

• Lines 113- 118: We rephrase the exclusion criteria

• We remove lines 119-121 (Source of participants) and add the first sentence to the section “Patients”.

• We add the sentence “The routine medical records system of the hospital was used to register the data for follow-up, if discharged, telephone follow-up was performed.” within the section Data collection.

• We add the diagnostic criteria section in the section Data collection (i.e. after the first sentence). Please specify the definition of severe diarrhea. Means the comma between frequency ≥3per day and change of fecal characteristics that the patients must had both for assignment to the diarrhea group.The change of fecal characteristics was documented in nursing notes and medical history. We use a classification system (Bristol Stool Chart). Nurses and doctors documented this information. They trained before this trail.

• Line 126: The sentence “In this series, there were 77 without severe diarrhea patients and 29 severe diarrhea patients.”we placed it within the results section.

• We removed the part of “Intervention” to avoid unnecessary information.

• We place section Outcome before the statistics.

• Line 141-149 (CD55 detection method):

o We first isolate neutrophils from whole blood.

o Line 144: 100μl PBS

o Lines 145-147: We mean monoclonal antibody from mice (Mouse IgG2a, $\varkappa$; BD Pharmingen™), who specifically binds to CD55, were added.

o Line 146: We add RBC lysate (red blood cell lysat)

• Line 150-151: The further efforts (i.e. training study personal, data management, defined procedures, same person who performed the flow cytometry) We add this information within the corresponding sections .

• Lines 152-154: We add statement to the study size estimation within the statistic section.

• Statistics:

o Line 157: quantitative indicators = quantitative values, we revised it.

o Line 159: Classification indexes mean 'Enumeration data'

o Mann-Whitney U test we used for the comparison of CD levels between both groups.

o Line 162-164: independent variables (Age,gCD55, eCD55, IL-10, Diamine oxidase, D-lactic, Endotoxin, IL-1, IL-2, IL-6, IL-8, PCT) and dependent variable (diarrhea) we used for the regression.

o Line 167: area under the receiver operating characteristic curve = AUROC

o Line 168:The predictors were combined by AUROC.

Changes in the text: Methods

Results:

• Could you shorten the drop outs? i.e. 124 patients were eligible of the study, but 8 had to be excluded (Figure 1).

• Please remove the sentence "There was no participants with missing data." to avoid unnecessary repetition.

• Lines 182-185: The diarrhea and no-diarrhea group did not differ in age, gender and incidence of chronic disease (Table 1).

• Could you add main diagnoses of patients and the cause of need enteral nutrition in Table 1?

• Line 186: How you defined chronic mortality?

• Line 189: Delete "All the patients completed follow-up." To avoid unnecessary repetition.

• Line 190-197:

o You have determined the levels of each blood biomarkers on day 1 and 7. Please provide the values of both time points. Is there a significant change from day 1 to day 7 and a group difference?

o You only report the results from diamine oxidase, D-lactic acid and endotoxin – from witch time point are the group comparisons?

o The sentence "Continuous variables were analyzed by Student's t test, and categorical variables by the Chi-square test." Should be placed within the method section (statistics).

• Line 203: You wrote "Our previous study…" – are the results presented in Figure 2 from another study? If yes, you have to insert a reference!

• Please also add a sentence that both groups show no significant difference in diamine oxidase,

D lactic acid and endotoxin level (Figure 2 D-F).

• Diagnostic value of CD55/Figure 3: The curves of CD55 (blue and green) are under the references line, indicating a high false positive rate and a probable misinterpretation of data.

• Formulation and evaluation of the prediction model: Witch values (day 1?) were used for the prediction model?

• Line 220: Figure 5!

• Line 220: On what will you judge the good degree of calibration and differentiation?

Reply4: Thank you for your revision. We revised the Results.

• We shorten the drop outs. 124 patients were eligible of the study, but 8 had to be excluded (Figure 1).

• We remove the sentence "There was no participants with missing data."

• Lines 182-185: The diarrhea and no-diarrhea group did not differ in age, gender and incidence of chronic disease (Table 1).

• We add main diagnoses of patients and the cause of need enteral nutrition in Table 1.

• Line 186: We're misrepresenting it. It's mortality.

• Line 189: We delete "All the patients completed follow-up."

• Line 190-197:

o We provide the values on day 1 and 7. There is a significant change from day 1 to day 7 .(Table3)

o We report the results from diamine oxidase, D-lactic acid and endotoxin – from Day1 .

o we delete "Continuous variables were analyzed by Student's t test, and categorical variables by the Chi-square test."

• Line 203: We're misrepresenting it. – the results are presented in Figure 2 from this study.

• We add a sentence that both groups show no significant difference in diamine oxidase, D lactic acid and endotoxin level (Figure 2 D-F).

• Diagnostic value of CD55/Figure 3: The curves of CD55 (blue and green) are under the references line, indicating the lower the CD55 value, the higher the incidence of diarrhea.

• Formulation and evaluation of the prediction model: Witch values (day 1) were used for the prediction model.

• Line 220:We revised it to Figure 5

• Line 220: On AUC-ROC you judge the good degree of calibration and differentiation.

Changes in the text:Results

Discussion:

• The discussion provides several information, but is lengthy written. Could you shorten the discussion and/or combined sentences?

• In addition, you should discuss your results with results of other studies in more detail. Are there studies the other non-significant biomarkers? Are there similar studies like this or is this study the first study analyzing CD55 in ICU patients with/without diarrhea?

• Please do not provide numeric results within the discussion (i.e. line 251-253; 279-284).

• Line 284 to 291 is an exact repetition of the result part – please remove it.

• I think you have to add further limitations or provide additional data. Diarrhea could be induced by several factors i.e. the daily caloric intake, the flow rate of the tube feeding, the feeding composition, medication (in particular antibiotics), morbidity of the patient, infection with Clostridium difficile. You should mention this or better compare these factors between both study groups.

General minor changes:

• Please use a uniform wording (i.e. no diarrhea/non-severe diarrhea; D-lactic acid/D lactic acid/d lactic acid)

• The whole manuscript contain several mistakes of space characters (i.e. lines 81, 86,…., 199-202)

• Please control for spelling mistakes (i.e. line 111)

• Wording of sentence is confusing: lines 255-256

• Please control figure and table assignments within the text.

• Line 347: HLOS = LOS

• Figure 1: Rephrase the exclusions/drop outs.

• Figure 2: The quality of the figure is low and the text is blurred.

• Figure 3: The description of curves includes special symbols.

• Figure 4: The size is very large. Could you change the scaling of the y-axis?

• Table 1: the information behind the * is confusing – please delete “p>0.05, no significant difference”

• Table 2: IL-10 is not significant?

• Abbreviation PCT?

Reply5: Thank you for your revision. We revised the Discussion.

• We shorten the discussion and/or combined sentences.

• We discuss the results with results of other studies in more detail. There are no similar studies like this or is this study the first study analyzing CD55 in ICU patients with/without diarrhea.

• We delete numeric results within the discussion (i.e. line 251-253; 279-284).

•We remove Line 284 to 291 .

• We add further limitations : Diarrhea could be induced by several factors i.e. the daily caloric intake, the flow rate of the tube feeding, the feeding composition, medication (in particular antibiotics), morbidity of the patient, infection with Clostridium difficile.

General minor changes:

• We revised and use a uniform wording (i.e. no diarrhea/non-severe diarrhea; D-lactic acid/D lactic acid/d lactic acid)

• We revised mistakes of space characters (i.e. lines 81, 86,…., 199-202)

• We control for spelling mistakes (i.e. line 111)

• We delete the sentence: lines 255-256

• We control figure and table assignments within the text.

• Line 347: HLOS = LOS

• Figure 1: we rephrase the exclusions/drop outs.

• Figure 2: We revised the quality of the figure and the text.

• Figure 3: The description of curves includes special symbols.

• Figure 4: The size is very large. We change the scaling of the y-axis.

• Table 1: We delete “p>0.05, no significant difference”

• Table 2: IL-10 is not significant by multivariate analysis of diarrhea risk.

•We add Abbreviation PCT.

Changes in the text: Discussion