Peer Review File
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Comment 1

In your methods, you mentioned that "when a patient had more than one episode of positive blood culture, only the last episode of positive blood culture was selected". So, which one is your case number in figure 1., the blood culture episode? Or the patient numbers? And what is the average interval between different blood culture episode in these patients? And if in each episode, they had also a new set of these 4 biomarkers?

Reply 1

Thank you for your concerns. We have made a point-by-point response as follows:

(1) Which one is your case number in figure 1., the blood culture episode? Or the patient numbers?

We are sorry that we failed to make us clearly in original manuscript. In figure 3 (I guess you mean the scatterplot of figure 3 instead of the flow chart of figure 1), when we were analyzing the relationship between blood culture results and the 4 sets of parameters (PCT, CRP, WBC, and N%), all the eligible blood culture episodes were included (not the number of patients). In contrast, when we were analyzing the relationship between patient prognosis and the 4 sets of parameters (PCT, CRP, WBC, and N%), only the last episode of positive blood culture was selected because the last episode of positive blood culture had more prognostic implications than other episodes. In this case, the number of blood cultures was identical to the number of patients.

We have revised the method section to clarify this issue:

"We included all the eligible blood culture episodes when analyzing the relationship between infection biomarkers and the blood culture results. However, when analyzing the relationship between infection biomarkers and patient prognosis, only the last episode of positive blood culture was selected for prognosis analysis because the last episode of positive blood culture had more prognostic implications than other episodes." (Page 7, line 128 to 132)

(2)What is the average interval between different blood culture episode in these patients?

For included patients, the median time interval with interquartile range between different episodes of blood cultures was 5 (2-12) days. For each patient, the minimum time interval between two episodes was 1 day, which precluded using duplicates data for analysis.

(3) If in each episode, they had also a new set of these 4 biomarkers?

For each episode of blood cultures, there were 1:1 matched 4-set of parameters (PCT, CRP, WBC, N%).

Comment 2

Since your study include variety of patients that admitted to ICU (cardiovascular, neurology, trauma), the etiology of mortality could be not related to sepsis. So how about the mortality prediction of blood culture negative group by using your method?

Reply 2

Thank you for your question. As you can see from the flow chart of this study (figure 1), when we were analyzing the relationship between infection biomarkers and patient prognosis using PCA method, the population was restricted to patients with positive blood cultures. We did not test the diagnostic performance of the derived components and primary biomarkers for predicting mortality in patients with negative blood cultures. For patients with negative blood cultures, the etiology of mortality may be related to other disease, rather than sepsis. In this context, indicators for mortality prediction should be selected based on the etiology of death. For example, if patients died from cardiovascular diseases, B-type natriuretic peptide and biomarkers for heart injury, rather than the infectious biomarkers used in this study, may be the better choice for mortality prediction. This is why we did not use infectious biomarkers for mortality prediction in patients with negative blood cultures.

Comment 3

What about the ROC curve or accuracy rate of these four biomarkers in distinguish positive blood culture from negative blood culture result by PCA directly?

Reply 3

Thank you for your inspiring comment. Based on your comment, we have plotted the ROC curves of the components and the four biomarkers for predicting blood culture results (see figure 9A). In addition, the diagnostic parameters for individual ROC curves were given in Table 3. As shown in figure 9A and Table 3, component 2 yield the largest AUC (0.81) in the discrimination of blood culture results, and 80.7% can be correctly classified at the optimal cut-off point.

Changes in the main text: we have added figure 9A, Table 3, and a paragraph (Page 14 to 15, line 278 to 289) to illustrate this issue.

Comment 4

How do you find the 2 components of PCA. You did not show the procedure in the article (the raw data?) (there were no figure S1 in my reviewing process)

Reply 4

We are sorry that the figure S1 was invisible to you due to some unknown reasons. We have actually uploaded it and embedded it in the supplement. We re-uploaded the supplement again and we wish it will be visible to you.

In figure S1, the coordinates in horizontal axis represents order of the components. The coordinates in vertical axis represents eigenvalue for each component. The components with eigenvalues larger than 0.9 are retained for subsequent analysis. This is the procedure how we find the two components.

The corresponding illustrations were described in method and result as follows:

In the method section:
"Principal components with eigenvalues above 0.9 were retained for subsequent analysis." (Page 9, line 162 to 163)
In the result section:
"In the principal component analysis, eigenvalues for each component were shown in scree plot (Fig. S1). The first two components returned an eigenvalue larger than 0.9, thus they were retained for subsequent analysis."

Comment 5
In analysis survival vs non-survival, why do you still use first and 2nd quadrant to compare their odds ratio for likelihood distribution. Did you try first quadrant only, and compared between survival and non-survival in culture positive and culture negative separately or all together?
Reply 5
Thank you for your question. In this study, we have used principal component analysis to study the relationship between infectious biomarkers and blood culture results, as well as patient prognosis. We found that positive blood cultures were more likely to be distributed in the first and second quadrants (where the component 2 has a positive value) as compared to negative blood cultures. To keep the consistency of our analysis, when we were analyzing the distribution characteristics of survivors and non-survivors using PCA method, we still focused on the area of first and second quadrant, instead of any other quadrant.

Comment 6
Although the odds ration could show the significant difference of distribution pattern in different type of blood culture and survival outcome, in clinically, we still more interesting in the prediction ability of PCA model of these four biomarkers which did not show in the article.
Reply 6
Thank you for your very constructive suggestion. We agree with the reviewer that the prediction ability (such as ROC curve) is very important for model assessment. Followed your suggestion, we have evaluated the diagnostic performance of the components and infection biomarkers in the discrimination of blood culture results and patient prognosis using ROC curves (see figure 9). Also, the corresponding diagnostic parameters were given in Table 3. Component 2 yield the largest AUCs in the discrimination of blood culture results and patient prognosis.

Comment 7
Since your data showed that procalcitonin and CRP was more strongly associated with blood culture result, why do you not test procalcitonin and CRP only PCA and compared with the existing four-biomarkers model or WBC/N% only model?
Reply 7

Thank you for your very constructive suggestion. Followed your suggestion, we have added the analysis that compared the prediction ability of the two components and the four biomarkers in discrimination of blood culture results and patient prognosis (see figure 9 and Table 3).

We also revised the abstract and main text for illustration. The revised parts are as follows:

In the abstract section:

"Method:……The diagnostic performance of components and infection biomarkers in discrimination of blood culture results and patient prognosis were compared using receiver operating characteristic (ROC) curves."(Page 3, line 47-49)

"Results:……PCT and CRP derived component had the largest AUC in discrimination of blood culture results (0.81) and patient prognosis (0.69)."(Page 4, line 61 to 63)

In the method section of the main text:

"Statistical analysis:……Finally, the diagnostic performance of the components and infection biomarkers in the discrimination of blood culture results and patient prognosis were compared using ROC curves. For each ROC curve, Youden's index was calculated (13). The optimal cut-off value was the point on the ROC curve where the Youden's index reached the maximum value. The corresponding diagnostic parameters at the optimal cut-off points were also computed, including the sensitivity, specificity, correctly classified ratio, positive likelihood ratio, and negative likelihood ratio." (Page 10, line 180 to 187)

In the results section of the main text:

*"**Diagnostic performance of the components and infection biomarkers for blood culture results and patient prognosis***

The ROC curves of the components and infection biomarkers for discrimination between positive and negative blood cultures and survivors and non-survivors were shown in Fig. 9A and Fig. 9B. The AUCs, the optimal cut-off values, and the corresponding diagnostic parameters for the individual components and infection biomarkers were summarized in Table 3. Component 2 yielded the highest AUC (0.81) in the discrimination of blood culture results, followed by PCT (0.77), CRP (0.65), N% (0.63), component 1 (0.54), and WBC (0.54) ($p < 0.001$). In addition, with regard to prognosis prediction, component 2 yielded a larger AUC (0.69) than the other indicators, including PCT (0.67), component 1 (0.63), N% (0.63), WBC (0.57), and CRP (0.56) ($p = 0.003$)." (Page 14 to 15, line 278 to 289)

In the discussion section of the main text:

"……This was further confirmed by the finding that the ROC curves of component 2 yielded the largest AUCs in the discrimination of blood culture results and patient prognosis……" (Page16, line 309 to 311)