## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Usefulness of the heparin-binding protein level to diagnose sepsis and septic shock according to Sepsis-3 compared to procalcitonin and C reactive protein: a prospective cohort in China |
| **AUTHORS** | Zhou, Yixuan; Liu, Zhen; Huang, Jun; Li, Guiling; Li, Fengying; Cheng, Yulan; Xie, Xinyou; Zhang, Jun |

## VERSION 1 - REVIEW

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| **REVIEWER** | Fernando Martinez-Sagasti<br>Intensive Care Unit Hospital Clinico San Carlos, Madrid (Spain) |
| **REVIEW RETURNED** | 10-Oct-2018 |

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| **GENERAL COMMENTS** | Dear authors, first of all thank you very much for considering BMJ Open to publish your work. Your study is interesting but many important changes should be made before publishing it. In fact, I consider that this version of your work is a good draft but a new review in depth of it is needed in order to be clearer and more consistent.<br>Some of my recommendations are the following:<br><br>1.- You must try to show the results clearer. You have studied 4 groups as shown in Table 1: healthy people, patients with local infections, septic patients and patients in septic shock. You can simplify “sepsis non-shock group” to “sepsis” because you are following the new sepsis definitions, so “sepsis” means infection with organ disfunction without shock.<br>It is also important to specify how long the patients were septic when the blood sample was taken, I mean: what was the time window to include a patient into the study?<br><br>2.- On the Table 1 “n” must be at the beginning (below “characteristics”). All the variables need to specify the units they are expressed in; and the units must be consistent. For instance, if age is expressed as mean the standard deviation must be shown as well, but if it is expressed as median then interquartile range must accompany it. In the case of SOFA score the units must be also properly shown and the point of calculation (at enrolment, I suppose). It should be also appropriate to define better “cardiovascular disease”, “respiratory disease”, “liver disease” and so on because they are very ambiguous. For instance, reporting the number of patients with NYHA III or IV instead of “cardiovascular disease”, Child score as a substitute of “liver disease”, the number of patients receiving hemodialysis as an alternative to “renal disease”. Of course, the units you have used |

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| | to express the results (mean□standard deviation / median and interquartile range) must be clarified.<br>3.- It would be very welcome a new Table with the four groups showing the values of the biomarkers: HBP, CRP, PCT, SOFA score and WBC and the p values. If you have additional data as lactate it would be very interesting to know it. Knowing the source of infection would be very welcome.<br><br>4.- Figure 1 is hardly seen. It should be split in 2 or 3 Figures so they can be bigger.<br><br>5.- When expressing the results on the text they must not be redundant with those on the Tables. I mean, you do not need to explain all the values and p values on the text when they are on the Tables, but if you decide to show some of them because you consider them particularly important the way you are giving them must be different in order to better understanding. For instance, the sentence between lines 170 to 175 can be simplified and must be more accurate. If you are expressing the median and interquartile range it must be changed to: “At enrollment, the median HBP level in septic shock group 139.75 ng/ml (range 112.90-166.61 ng/ml) was significantly higher than in the other groups”. You do not need to add the p values because they are on the Table.<br>You can follow with something like: “The HBP level of sepsis group (67.78 ng/ml, range 55.93-79.62 ng/ml) was significantly higher than that of normal local infection group (15.59ng/ml, range 9.67-21.52 ng/ml) and the healthy control group (4.72ng/ml, range 4.00-5.43 ng/m) (Figure 1A)”.<br>There are several sentences along the text that should be changed to this structure for better understanding, for example those between lines 176-181 and lines 225-228.<br><br>6.- You must review all the data shown as 95%CI because it is not clear what you mean.<br><br>7.- Regarding the 8 cases of non-septic shock you have studied I think that they do not give important information because they are very few to draw conclusions and your main objective was to study the diagnostic value of heparin-binding protein in sepsis and septic shock under the new diagnostic criteria, so I would remove the paragraph between lines 221 to 229. I encourage you to run a new study analyzing these biomarkers in a population of patients with non-septic shock.<br><br>8.- I am sure you can improve the discussion.<br>You have to focus your discussion on comparing your results in patients with infection, sepsis and septic shock with those previously published. I recommend you to read the recent study of Holub M et al (Selected Biomarkers Correlate with the Origin and Severity of Sepsis. Mediators Inflamm. 2018) because it can help you to present your results better and<br>You should clarified some sentences because they are theoretical reasons why HBP release might be influenced on but they would not have impacted in your patients. For example, between lines 281 to 283 you mention gly-pro-arg-pro, simvastatin, tizolsetan, heaparin and aprotinin as potential drugs influencing the plasma levels of HBP but I suppose that your patients did not receive these treatments, otherwise you must explain it. |

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| | 9.- Regarding the conclusion<br>According to your results I agree that HBP would be a good marker for sepsis and septic shock but it is very speculative to say that early treatment should be carried out to improve the prognosis in patients found to have high HBP levels because this late statement cannot be drawn from your study.<br><br>10.- Concerning the references, all of them must be checked to follow the recommendations of the BMJ reference style (https://authors.bmj.com/writing-and-formatting/formatting-your-paper/). Particularly, list the names and initials of all authors if there are 3 or fewer; otherwise list the first 3 and add 'et al.' Use one space only between words up to the year and then no spaces. The journal title should be in italic and abbreviated according to the style of Medline. If the journal is not listed in Medline then it should be written out in full.<br><br>11. It is even more important to review that the references you are using really support your statements in the discussion. For instance, in line 240 you affirm that HBP was significantly increased in acute bacterial meningitis citing reference number 19: "Carina Mari Aparici, Aung Zaw Win. Acute Calculous Cholecystitis Missed on Computed Tomography and Ultrasound but Diagnosed with Fluorodeoxyglucose-Positron Emission Tomography/Computed Tomography. J Clin Imaging Sci 2016; 6:31". As you can observe this reference has nothing to do with bacterial meningitis.<br>In summary, your study is interesting but you must review it in depth. If you decide to write a new version presenting the results clearer, with more Tables describing your patients better, improving the quality of Figure 1, focusing the discussion on patients with infection, sepsis and septic shock, updating the references according to BMJ style and being more accurate on your conclusions I will be more than happy to review it again. |

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| **REVIEWER** | Adam Linder<br>Dept for Infection medicine, Lund University, Lund Sweden |
| **REVIEW RETURNED** | 17-Oct-2018 |

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| **GENERAL COMMENTS** | The authors present a study evaluating the neutrophil-derived Heparin-binding protein (HBP) as a prognostic biomarker for sepsis and septic shock using the new sepsis-3 definitions.<br>The study is well conducted and clearly presented and show that HBP is higher in sepsis vs milder infections and higher in septic shock vs non-septic shock.<br><br>However I have som concern that should be adressed.<br><br>The written language is overall poor and must be improved in order to merit publication.<br>The inclusion of the patients is unclear and must be clarified.<br>1. Where the patients included in the ED or wards or at admittance to the ICU?<br>2. Did the patients receive fluid, antibiotics, heparin before enrollment?<br>The authors should discuss the "outliers" i.e. patients with high HBP and no sepsis or septic shock - what were their diagnosis |

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| | and so on and also patients with sepsis/septic shock with low HBP. What where their characteristics compared to the other regarding, diagnosis, treatment type and time and so on.<br><br>Minor suggestions:<br>It´s unclear whether the median or mean are presented at page 12.<br>The references must be checked so they are in concordance<br>Maybe using a log2 scale would improve the visual impression of figure 1?<br>Figure 2 should be cut at 1.0<br>The title could be changed to ...shock using the Sepsis-3 criteria |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer: 1

1.- You must try to show the results clearer. You have studied 4 groups as shown in Table 1: healthy people, patients with local infections, septic patients and patients in septic shock. You can simplify “sepsis non-shock group” to “sepsis” because you are following the new sepsis definitions, so “sepsis” means infection with organ disfunction without shock.

It is also important to specify how long the patients were septic when the blood sample was taken, I mean: what was the time window to include a patient into the study?

Response: Thank you for the referee’s suggestion. Septic shock is defined as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone in the new definitions. For the sake of brevity, we simplified “sepsis nonshock group” to “sepsis group” and true “sepsis” to “sepsis (shock or not)”.

We have documented the exact time that the patients were septic when the blood sample was taken. “At the time of enrollment, 120 patients were diagnosed with sepsis, among whom 27 were diagnosed within 12 hours, 35 were diagnosed between 12 and 24 hours, 31 were diagnosed within 1-3 days, 26 were diagnosed from 3 to 7 days, and 1 was confirmed with sepsis after 10 days of attendance. Six patients were diagnosed with sepsis 24 hours after blood sampling.” (line165-168).

2.- On the Table 1 “n” must be at the beginning (below “characteristics”). All the variables need to specify the units they are expressed in; and the units must be consistent. For instance, if age is expressed as mean the standard deviation must be shown as well, but if it is expressed as median then interquartile range must accompany it. In the case of SOFA score the units must be also properly shown and the point of calculation (at enrolment, I suppose). It should be also appropriate to define better “cardiovascular disease”, “respiratory disease”, “liver disease” and so on because they are very ambiguous. For instance, reporting the number of patients with NYHA III or IV instead of “cardiovascular disease”, Child score as a substitute of “liver disease”, the number of patients receiving hemodialysis as an alternative to “renal disease”. Of course, the units you have used to express the results (mean□standard deviation / median and interquartile range) must be clarified.

Response: We greatly appreciate your constructive comments that help to improve the manuscript. We have refined table 1, added “n” below “characteristics” and improved the expression of variables. Additionally, we emphasized that SOFA score assessed at enrollment. Continuous variables were presented as medians (interquartile range).

We also refined the classification of patients. For example, “Cardiovascular disease” was subdivided into “NYHA I/II” and “NYHA III/IV”; “Liver disease” was subdivided into “Child A” “Child B” and “Child C”; “Renal disease” was subdivided into “Need hemodialysis” and “No hemodialysis”; “Abdominal disease” was divided into “Operation” and “No operation”.

3.- It would be very welcome a new Table with the four groups showing the values of the biomarkers: HBP, CRP, PCT, SOFA score and WBC and the p values. If you have additional data as lactate it would be very interesting to know it. Knowing the source of infection would be very welcome.

Response: This comment was highly appreciated. We created a new table (Table 2) to show the values of the biomarkers: HBP, CRP, PCT, SOFA score, WBC and the P values. We also added data on arterial lactic acid in patients with sepsis (shock or not). Unfortunately, we lacked complete data on lactate in healthy and local infection subjects over the same period. By comparing the lactate and sepsis 28-d survival, we found that lactate was significantly increased in the 28-d nonsurvivor group.

4.- Figure 1 is hardly seen. It should be split in 2 or 3 Figures so they can be bigger.

Response: Thank you very much for the suggestion. We split Figure 1 into two figures: Figure 1 displays “Plasma levels of HBP, PCT, CRP, WBC, and neutrophils, as well as the SOFA score, at enrollment”, indicating the distribution of values of different indicators in the four groups. Figure 2 displays “Plasma levels of HBP, PCT, CRP, WBC, and lactate, as well as the SOFA score, and the 28-d survival”, clarifying the values of different indicators in the survival group and death group.

5.- When expressing the results on the text they must not be redundant with those on the Tables. I mean, you do not need to explain all the values and p values on the text when they are on the Tables, but if you decide to show some of them because you consider them particularly important the way you are giving them must be different in order to better understanding. For instance, the sentence between lines 170 to 175 can be simplified and must be more accurate. If you are expressing the median and interquartile range it must be changed to: “At enrollment, the median HBP level in septic shock group 139.75 ng/ml (range 112.90-166.61 ng/ml) was significantly higher than in the other groups”. You do not need to add the p values because they are on the Table.

You can follow with something like: “The HBP level of sepsis group (67.78 ng/ml, range 55.93-79.62 ng/ml) was significantly higher than that of normal local infection group (15.59ng/ml, range 9.67-21.52 ng/ml) and the healthy control group (4.72ng/ml, range 4.00-5.43 ng/m) (Figure 1A)”.

There are several sentences along the text that should be changed to this structure for better understanding, for example those between lines 176-181 and lines 225-228.

Response: We greatly appreciate the constructive comments. We improved the presentation of the results to make them concise and clear. Because of table 2, many specific values were not repeated. The sentence between lines 170 to 175 was changed to “At enrollment, the HBP level of the septic shock group was significantly higher than that of the other groups. The HBP level of the sepsis group was significantly higher than that of the normal local infection group. Compared with the healthy control group, the plasma level of HBP in the local infection group was also significantly increased (Figure 1A).” (line186-189). The sentence between lines 176-181 change into “Similar to the HBP level, the PCT level and SOFA score in septic shock patients were significantly higher than those in the other groups and were obviously higher in sepsis patients than in those with normal local infection”. (line189-191). The text of lines 225 to 228 have been deleted (please see the response to 7).

6.- You must review all the data shown as 95%CI because it is not clear what you mean.

Response: The suggested change was made. We improved the presentation of the results to make them concise and clear.

7.- Regarding the 8 cases of non-septic shock you have studied I think that they do not give important information because they are very few to draw conclusions and your main objective was to study the diagnostic value of heparin-binding protein in sepsis and septic shock under the new diagnostic criteria, so I would remove the paragraph between lines 221 to 229. I encourage you to run a new study analyzing these biomarkers in a population of patients with non-septic shock.

Response: Thank you for your valuable advice. We removed the text about different indicators in various shock statuses, lines 221 to 229. We will continue to enrich relevant research data to analyzing these biomarkers in a population of patients with nonseptic shock in a new study.

8.- I am sure you can improve the discussion.

You have to focus your discussion on comparing your results in patients with infection, sepsis and septic shock with those previously published. I recommend you to read the recent study of Holub M et al (Selected Biomarkers Correlate with the Origin and Severity of Sepsis. Mediators Inflamm. 2018) because it can help you to present your results better and You should clarified some sentences because they are theoretical reasons why HBP release might be influenced on but they would not have impacted in your patients. For example, between lines 281 to 283 you mention gly-pro-arg-pro, simvastatin, tizolsetan, heaparin and aprotinin as potential drugs influencing the plasma levels of HBP but I suppose that your patients did not receive these treatments, otherwise you must explain it.

Response: We greatly appreciate your constructive comments that help to improve the manuscript. We have carefully read the literature recommended, which is very helpful to our research. The discussion has been revised. The reasons for the similarities and differences between our research results and previous ones were analyzed. Additionally, the patient population characteristics of outliers were analyzed.

“Considering that heparin can inhibit the activity of HBP, the metabolism characteristics of heparin in the body also determine that the heparin elimination half-life varies with dose. The half-life of heparin after intravenous injection is 1 to 6 hours, with an average of 1.5 hours. We excluded individuals who were administered heparin within 3 days at the time of enrollment. The patients enrolled in this study have not been treated with Gyl-Pro-Arg-Pro, simvastatin, tezosentan or aprotinin.” (line 275-284).

9.- Regarding the conclusion

According to your results I agree that HBP would be a good marker for sepsis and septic shock but it is very speculative to say that early treatment should be carried out to improve the prognosis in patients found to have high HBP levels because this late statement cannot be drawn from your study.

Response: Thank you for your suggestion. We changed the Conclusions section to “We found that, as a potential diagnostic tool, an elevated level of HBP in the plasma is associated with sepsis and septic shock, and the HBP level was a potential diagnostic marker in patients with suspected sepsis.”

10.- Concerning the references, all of them must be checked to follow the recommendations of the BMJ reference style.

Response: Thank you for your kind advice. The suggested change was made.

11. It is even more important to review that the references you are using really support your statements in the discussion. For instance, in line 240 you affirm that HBP was significantly increased in acute bacterial meningitis citing reference number 19: “Carina Mari Aparici, Aung Zaw Win. Acute Calculous Cholecystitis Missed on Computed Tomography and Ultrasound but Diagnosed with Fluorodeoxyglucose-Positron Emission Tomography/Computed Tomography. J Clin Imaging Sci 2016; 6:31”. As you can observe this reference has nothing to do with bacterial meningitis.

Response: We sincerely apologize for the errors in the references. We carefully reviewed the references for accuracy and consistency.

Reviewer: 2

1- The written language is overall poor and must be improved in order to merit publication.

Response: We would like to express our sincerest gratitude for all the valuable and constructive comments we have received. This manuscript has been edited and proofread by American Journal Experts (Certificate Verification Key: 4274-A376-59C1-EF7B-1AA6).

2-The inclusion of the patients is unclear and must be clarified.

1.Where the patients included in the ED or wards or at admittance to the ICU?

2.Did the patients receive fluid, antibiotics, heparin before enrollment?

Response: We greatly appreciate your constructive comments that help to improve the manuscript. The inclusion criteria of the patients were clarified. Patients diagnosed with sepsis (nonshock and shock) mainly came from the hospital intensive care unit (97/126), while the others come from the wards. All sepsis patients received antibiotics: 77% (97/126) were treated with steroids, and 80% (101/126) had been treated with vasopressors for more than 12 hours before the blood sample was taken. Three were administered heparin five days earlier, 3 a week earlier and 1 10 days earlier. (line156-157, 161-164).

3- The authors should discuss the "outliers" i.e. patients with high HBP and no sepsis or septic shock - what were their diagnosis and so on and also patients with sepsis/septic shock with low HBP. What where their characteristics compared to the other regarding, diagnosis, treatment type and time and so on.

Response: Thank you very much for the suggestion. We revised the Discussion section, including text about outliers: “In our study, among nonsepsis patients whose HBP level was greater than 24.5 ng/ml (6 cases), 3 (50%) cases were diagnosed as aortic dissection with pulmonary infection, which comprises arterial dissection, activation of the coagulation system, and elevated fibrinogen levels, causing leukocytes to release leukotriene B4, which binds to the BLT1 receptor on the surface of polymorphonuclear neutrophils and activates the intracellular phosphatidylinositol 3-kinase signaling pathway to release HBP. Interestingly, among sepsis patients without shock but with HBP > 118.7 (n = 9), 6 (67%) were diagnosed with acute pancreatitis (biliary acute pancreatitis/hyperlipidemic acute pancreatitis) complicated with abdominal cavity infection. All the patients were in the acute phase of the disease, during the strongest inflammatory response, when HBP acts as a chemoattractant to recruit more leukocytes to the site of local infection, enhancing the activation of monocytes, improving the phagocytosis of macrophages and increasing the pathogen-elimination effect.” (line 256-267).

4-It´s unclear whether the median or mean are presented at page 12.

Response: We greatly appreciate the constructive comments. We improved the presentation of the results to make them concise and clear.

5-The references must be checked so they are in concordance.

Response: Thank you for your kind advice. The suggested change was made.

6-Maybe using a log2 scale would improve the visual impression of figure 1?

Response: Thank you very much for the suggestion. We split Figure 1 into two figures.

7-Figure 2 should be cut at 1.0

Response: Thank you for your kind advice. The suggested change was made.

8-The title could be changed to ...shock using the Sepsis-3 criteria

Response: We change the title to “Diagnostic Value of the Heparin-Binding Protein Level in Sepsis and Septic Shock using the Third International Consensus Definitions”.

Thank you so much for your constructive comments. They have really helped in sharpening and clarifying the paper’s basic argument. We sincerely hope that you would like the changes we have made to the manuscript. Whatever the outcome, we would like to express our gratitude to you for helping us make this a much better paper.

**VERSION 2 – REVIEW**

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| **REVIEWER** | FERNANDO MARTINEZ-SAGASTI<br>Intensive Care Medicine Hospital Clinico San Carlos Madrid (Spain) |
| **REVIEW RETURNED** | 03-Jan-2019 |

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| **GENERAL COMMENTS** | Dear authors, first of all thank you very much for sending a new version of your work. It is clearer and more consistent.<br>Nevertheless I consider that some changes are necessary before publishing it.<br>I will refer my comments to the highlighted word file of your work.<br><br>Some of my recommendations are the following:<br><br>1.- Regarding the Title:<br><br>Your study not only analyzes the value of heparin-binding protein level to diagnose sepsis and septic shock but also you compare that biomarker with procalcitonin and C reactive protein so I suggest to change the title to something like:<br>“Usefulness of the heparin-binding protein level to diagnose sepsis and septic shock according to Sepsis-3 compared to Procalcitonin and C reactive protein”<br><br>2.-With regards to the Abstract:<br><br>It must be richer. For instance, on the paragraph “Participants” you should explain the four groups of your sample: the healthy group (n=56), local infection group (n=32), sepsis nonshock group (referred to as sepsis group) (n=75) and septic shock group (n=51) and the inclusion criteria.<br>On the paragraph “Main outcome measures” the biomarkers checked and the objectives must be reported. You should also briefly explain the statistical analysis you are going to do in order to study the usefulness of the biomarkers to identify the different clinical status of the septic patients. |

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| | On “Results” you should report a bit more information of your findings and the sentence you have written on lines 48-49 “Thus, HBP was demonstrated to be the best indicator for the diagnosis of sepsis and septic shock” is more appropriate to move it to “Conclusions”<br><br>3.-Regarding the study:<br><br>On the “Introduction”, lines 85-87 must be better explained according to the four groups you have studied.<br>On the paragraph “Definitions” on the one hand you must explain the four groups of your sample and on the other hand you can delete the Box 1 with the definitions because they are well known and you give the reference.<br>On the “Results” you write on the paragraph “Plasma levels of HBP, PCT, and CRP, as well as the SOFA score, for sepsis and septic shock diagnosis”, lines 224-225: “The PCT level cannot identify sepsis shock (Table 4)”. You must change “sepsis shock” to “septic shock”. This same grammar mistake must be also checked on lines 251, 252, 253, 261 and 271.<br>Finally, the most important question and limitation of your study that should be discussed is the very large time window of the inclusion criteria. As you explain on lines 162 to 168 you have included patients between 12 hours to 7 days of diagnosing. This must be clarified and discussed because it might be a very important confounding factor because of the short half-life of the biomarkers you have analyzed, particularly HBP and PCT. I suggest to re-analyze the data excluding those cases in which the blood sample was taken later than 48 hours after diagnosing infection, sepsis or septic shock.<br>I will be happy to review the new version. |

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| **REVIEWER** | Adam Linder<br>Dept of Infection Medicine, Lund University Hospital, Lund Sweden |
| **REVIEW RETURNED** | 28-Dec-2018 |

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| **GENERAL COMMENTS** | I thank the authors for their revisions. I think the paper is much clearer now and show some very interesting results. However, I would appreciate if the number of blood culture positives could be added to the table. |

## VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

I thank the authors for their revisions. I think the paper is much clearer now and show some very interesting results. However, I would appreciate if the number of blood culture positives could be added to the table.

Response: We greatly appreciate your constructive comments that help to improve the manuscript. We have refined table 1, added the number of blood culture obtained and the number of blood culture

positives. “Most patients had blood cultures (83/93), but only a few had positive results (20/83).” (lines 164-165).

Reviewer: 1

1.- Regarding the Title:

Your study not only analyzes the value of heparin-binding protein level to diagnose sepsis and septic shock but also you compare that biomarker with procalcitonin and C reactive protein so I suggest to change the title to something like:

“Usefulness of the heparin-binding protein level to diagnose sepsis and septic shock according to Sepsis-3 compared to Procalcitonin and C reactive protein”

Response: We would like to express our sincerest gratitude for all the valuable and constructive comments we have received. We changed the title to “Usefulness of the heparin-binding protein level to diagnose sepsis and septic shock according to Sepsis-3 compared to procalcitonin and C reactive protein”, which could more appropriately express the setting and purpose of the research.

2.-With regards to the Abstract:

It must be richer. For instance, on the paragraph “Participants” you should explain the four groups of your sample: the healthy group (n=56), local infection group (n=32), sepsis nonshock group (referred to as sepsis group) (n=75) and septic shock group (n=51) and the inclusion criteria.

On the paragraph “Main outcome measures” the biomarkers checked and the objectives must be reported. You should also briefly explain the statistical analysis you are going to do in order to study the usefulness of the biomarkers to identify the different clinical status of the septic patients.

On “Results” you should report a bit more information of your findings and the sentence you have written on lines 48-49 “Thus, HBP was demonstrated to be the best indicator for the diagnosis of sepsis and septic shock” is more appropriate to move it to “Conclusions”

Response: We greatly appreciate your constructive comments that help to improve the manuscript. The abstract section has been revised in detail:

We explained the four groups and inclusion criteria on the paragraph “Participants”: “Adult infected patients with a suspected sepsis and person underwent physical examination was included. According to the health status and severity of illness, the research subjects were divided into healthy, local infection, sepsis nonshock and septic shock under Sepsis-3.” (lines 38-42).

We briefly showed the biomarkers test and statistical analysis on the paragraph “Main outcome measures”: “Plasma levels of HBP, procalcitonin (PCT), C-reactive protein (CRP), complete blood count were detected in all subjects. single-factor analysis of variance was used to compare the level of biomarkers of multiple groups. Receiver operating characteristic curve was used to assess the diagnostic capacity of each marker.” (lines 44-47).

We enriched the “Results” section: “HBP levels were significantly higher in patients with sepsis nonshock than in those with local infections (median 49.7 vs 11.8 ng/ml, $p < 0.01$) at enrollment. Moreover, HBP levels in septic shock patients were significantly higher than in patients with sepsis without shock (median 153.8 vs 49.7 ng/ml, $p < 0.01$). The areas under the receiver-operating characteristic (ROC) curve of HBP were 0.893 for sepsis which were higher than that of PCT (0.856) and CRP (0.699). Moreover, the areas under the ROC curve of HBP were 0.760 for septic shock which were higher than SOFA score (0.656). However, there was no significant difference between

28-d survivors (n=56) and 28-d nonsurvivors (n=37) with sepsis in terms of HBP value (p = 0.182)." (lines 49-56). The sentence "Thus, HBP was demonstrated to be the best indicator for the diagnosis of sepsis and septic shock." (Original lines 48-49) was removed.

3.-Regarding the study:

On the "Introduction", lines 85-87 must be better explained according to the four groups you have studied.

On the paragraph "Definitions" on the one hand you must explain the four groups of your sample and on the other hand you can delete the Box 1 with the definitions because they are well known and you give the reference.

On the "Results" you write on the paragraph "Plasma levels of HBP, PCT, and CRP, as well as the SOFA score, for sepsis and septic shock diagnosis", lines 224-225: "The PCT level cannot identify sepsis shock (Table 4)". You must change "sepsis shock" to "septic shock". This same grammar mistake must be also checked on lines 251, 252, 253, 261 and 271.

Finally, the most important question and limitation of your study that should be discussed is the very large time window of the inclusion criteria. As you explain on lines 162 to 168 you have included patients between 12 hours to 7 days of diagnosing. This must be clarified and discussed because it might be a very important confounding factor because of the short half-life of the biomarkers you have analyzed, particularly HBP and PCT. I suggest to re-analyze the data excluding those cases in which the blood sample was taken later than 48 hours after diagnosing infection, sepsis or septic shock.

Response: We greatly appreciate your constructive comments. On the "Introduction", we brief describe the four groups: "In this study, four groups (healthy, local infection, sepsis nonshock, septic shock) under the new diagnostic criteria were included. We analyzed the HBP and other biomarker level in different groups to assess their diagnostic value in infected patients with sepsis and nonsepsis, as well as in sepsis patients with septic shock and sepsis nonshock." (lines 91-95). Detailed grouping information was in the "Results" section. (lines 152-158).

The Box 1 was deleted. The grammar mistake were corrected: "The PCT level cannot identify sepsis shock (Table 4)." (original lines 224-225) modified to "The PCT level cannot identify septic shock (p= 0.195) (Table 4)." (lines225). "Linder A, et al. performed serial HBP measurements in sepsis patients before they developed septic shock and found that plasma HBP levels in shock sepsis patients were obviously higher than those in nonshock sepsis patients between 12 and 24 hours." (original lines 251-253) modified to "Linder A, et al. performed serial HBP measurements in sepsis patients before they developed septic shock and found that plasma HBP levels in septic shock patients were obviously higher than those in sepsis nonshock patients between 12 and 24 hours." (lines 251-253). And We carefully reviewed the article for similar grammatical problems.

Thank you very much for the advice of "time window", it's a very important confounding factor. We looked through the literature, and retrospective review of complete patient charts, laboratory and microbiological tests, so we excluded 33 patients who diagnosed with sepsis or septic shock for more than 48 hours. Since most patients with sepsis have a confirmed infection for more than 48 hours, the time window for infection is just limited to "infections exist". The data excluding those 33 cases in which the blood sample was taken later than 48 hours after diagnosing sepsis were re-analyze.

## VERSION 3 - REVIEW

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| **REVIEWER** | Fernando Martinez Sagasti<br>Hospital Clinico San Carlos, Madrid (Spain) |

| REVIEW RETURNED | 16-Feb-2019 |
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| GENERAL COMMENTS | Dear authors, thank you for reviewing your manuscript that incorporates our previous recommendations. The article is much clearer at this time. I just want to draw your attention to some minor corrections that you must do before publishing it:<br><br>Abstract<br>On line 38 you state: “Adult infected patients with a suspected sepsis and person underwent physical examination was…”<br>Please change “person” to “people”<br>On line 40 you write: “into healthy, local infection, sepsis nonshock and septic shock under Sepsis-3”.<br>Please add “definitions” to the end of the sentence: “into healthy, local infection, sepsis nonshock and septic shock under Sepsis-3 definitions”.<br>On lines 50 to 53 you write: “The areas under the receiver-operating characteristic (ROC) curve of HBP were 0.893 for sepsis which were higher that of PCT (0.856) and CRP (0.699). Moreover, the areas under the ROC curve of HBP were 0.760 for septic shock which were higher than SOFA score (0.656)”.<br>I recommend to write it in singular, correcting some grammar mistake and give a bit more information. You can change it to something like:<br>“The area under the receiver-operating characteristic (ROC) curve of HBP (cut off ≥ 28.1 ng/mL) was 0.893 for sepsis which was higher than those of PCT (0.856) for a cut-off ≥ 2.05 ng/mL and of CRP (0.699) for a cut-off ≥ 151.9 mg/L. Moreover, the area under the ROC curve of HBP (cut-off ≥ 103.5 ng/mL) was 0.760 for septic shock which was higher than the ROC curve of SOFA score (0.656) for a cut-off ≥ 5.5”.<br>On lines 56-57 you state: “An elevated level of HBP in the plasma is associated with sepsis, which might be a serviceable diagnostic marker in patients with suspected sepsis”.<br>I recommend to change it to something like: “A high level of HBP in plasma is associated with sepsis, which might be a useful diagnostic marker in patients with suspected sepsis"<br>MATERIALS AND METHODS<br>Patient population<br>Between lines 100-102 you have written: “The inclusion criteria was possess etiological basis of infection with or without leukocyte elevation, and confirmed or clinically strongly suspected sepsis by the attending clinician”.<br>I suggest to simplify this sentence and to change it to something like: The inclusion criteria were confirmed or clinically strongly suspected sepsis by the attending clinician. |
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## VERSION 3 – AUTHOR RESPONSE

Reviewer: 1

Abstract

On line 38 you state: “Adult infected patients with a suspected sepsis and person underwent physical examination was…”

Please change “person” to “people”

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“The area under the receiver-operating characteristic (ROC) curve of HBP (cut off ≥ 28.1 ng/mL) was 0.893 for sepsis which was higher than those of PCT (0.856) for a cut-off ≥ 2.05 ng/mL and of CRP (0.699) for a cut-off ≥ 151.9 mg/L. Moreover, the area under the ROC curve of HBP (cut-off ≥ 103.5 ng/mL) was 0.760 for septic shock which was higher than the ROC curve of SOFA score (0.656) for a cut-off ≥ 5.5”.

On lines 56-57 you state: “An elevated level of HBP in the plasma is associated with sepsis, which might be a serviceable diagnostic marker in patients with suspected sepsis”.

I recommend to change it to something like: “A high level of HBP in plasma is associated with sepsis, which might be a useful diagnostic marker in patients with suspected sepsis"

MATERIALS AND METHODS

Patient population

Between lines 100-102 you have written: “The inclusion criteria was possess etiological basis of infection with or without leukocyte elevation, and confirmed or clinically strongly suspected sepsis by the attending clinician”.

I suggest to simplify this sentence and to change it to something like: The inclusion criteria were confirmed or clinically strongly suspected sepsis by the attending clinician.

Response: We greatly appreciate your constructive comments that help to improve the manuscript. We have carefully revised the above problems. We would like to express our sincerest thanks to your guidance in the revision.