PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Heparin-binding protein as a biomarker for the diagnosis of sepsis in the intensive care unit: a retrospective cross-sectional study in China
AUTHORS	Zuo, Lingyun; Li, Xiaoyun; Wang, Luhao; Yuan, Hao; Liao, Zihuai; Zhou, Si; Wu, JianFeng; Guan, XiangDong; Liu, YongJun

VERSION 1 – REVIEW

REVIEWER	Liu, Zhanguo Southern Medical University Second Clinical Medical College
REVIEW RETURNED	08-Nov-2023

GENERAL COMMENTS	Thank you for the opportunity to review this interesting manuscript. The authors conducted a retrospective study to assess the diagnostic utility of HBP as a biomarker in sepsis. The findings indicated that the diagnostic ability of HBP alone for sepsis is limited, whereas it exhibits a robust diagnostic ability when combined with other biomarkers. And a predictive tool for sepsis is generated based on the result of combined diagnostic model. This is an interesting study. But I have a few feedbacks as mentioned underneath. Major concerns： 1.Does the type of study meet the criteria for a cohort study appropriately need to be further verified. 2.The authors have mentioned that there were some supportive evidences on the value of HBP in sepsis diagnosis. And the authors highlighted the heterogeneity of previous studies as a primary factor contributing to inconsistent performance of HBP in sepsis diagnosis. However, the authors did not explicitly tell the distinguishing features of this study compared to previous works. It is my belief that a more robust rationale should be provided to demonstrate the necessity and uniqueness of this research. 3.The time window for sepsis diagnosis and data collection (except SOFA score and APACHE II score) should be provided. Without this information, it would be difficult to judge the temporal sequence between biomarker results and sepsis diagnosis, especially when the data is generated after determining sepsis, potentially leading to erroneous conclusions. 4.The one of inclusion criteria is including the patients with HBP test. Why new tests were conducted using frozen samples? 5.According to the baseline characteristics, the sofa score in infection group is 4.0 (2.3-7.0) and the mortality is similar between the infection group and the sepsis group. It is concerning that

	some patients in the infection group perhaps reach the sepsis-3 criterion in fact. Minor 1.The method of adjustment for multiple comparisons in the statistical analyses was not specified. 2.Statistical differences for comparisons of AUC value are not given 3.The result of decision curve analysis lacks of further explanation. What is the criterion for high clinical net benefit?

REVIEWER	Giamarellos-Bourboulis, Evangelos National and Kapodistrian University of Athens, 4th Department of Internal Medicine
REVIEW RETURNED	30-Nov-2023

GENERAL COMMENTS	In this submission, the authors run a retrospective analysis to investigate the diagnostic validity of heparin binding protein (HBP) for the diagnosis of sepsis. I have major concerns for this submission. • The authors need to explain if this a retrospective analysis in patients with already measured HBP or a retrospective measurement of HBP in prospectively collected samples. The first part of the Methods suggests the first design and the second part of the Methods suggests the second design. • The study title and the Introduction imply for a design which investigates early sepsis detection. The submission includes patients who are already at sepsis. • The derivation of the equation of the model and the positioning of HBP in the model is not defined. • The manuscript is not elegantly written. More precisely: A) The Introduction fails to explain the real study objective. B) Half of the Results section is unfocused in presenting the demographics of the patients which is not the endpoint. C) The Discussion is extremely wordy and requires shortening. D) Major recent publications for HBP are missing.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Major concerns：

[1] Does the type of study meet the criteria for a cohort study appropriately need to be further verified.

We thank the reviewer for reading our manuscript carefully and giving positive comments. Regarding the research design for this study, we have carefully considered and consulted with statistical experts. The purpose of this study was to investigate the diagnostic value of HBP in sepsis and establish a diagnostic model to predict sepsis or not. After discussions with the research team, the study has been identified as a retrospective cross-sectional study with an investigation time of 34 months. In

response to this determination, revisions have been made in the abstract and methods sections of the manuscript.

[2] The authors have mentioned that there were some supportive evidences on the value of HBP in sepsis diagnosis. And the authors highlighted the heterogeneity of previous studies as a primary factor contributing to inconsistent performance of HBP in sepsis diagnosis. However, the authors did not explicitly tell the distinguishing features of this study compared to previous works. It is my belief that a more robust rationale should be provided to demonstrate the necessity and uniqueness of this research.

Thank you for your rigorous comment. The studies mentioned in our manuscript by Linder et al. included patients from the emergency department, who were in the relatively early stages of the disease, with less severity and organ damage, resulting in a less impact on HBP. Their studies results were more applicable to emergency patients. Further exploration was needed to assess the diagnostic value of HBP with critically ill patients in the ICU. It is well known that sepsis is a common condition in the ICU. Due to the overall complexity of patients' conditions and more severe organ dysfunction, the mortality rate remains high. Therefore, conducting this study was of great significance for the early diagnosis and timely treatment of sepsis. Compared to previous studies, first, the control group in this study comprised not only healthy individuals but mostly surgical postoperative recovery patients. Second, patients with infections (infection group, sepsis group, septic shock group) exhibited a more diverse range of infection sites and stages. The progression stages of the disease were relatively later, with more interventions such as drug or mechanical assistance. These factors might influence the HBP levels, reflect the real clinical scene of ICU patients, making the results more applicable to ICU patients. More importantly, in the ICU, it was possible to observe the evolution of patients' conditions over a longer period, establish the final diagnosis, and understand the prognosis. This allowed for a more in-depth exploration of the value of HBP in the clinical diagnosis and treatment process

[3] The time window for sepsis diagnosis and data collection (except SOFA score and APACHE II score) should be provided. Without this information, it would be difficult to judge the temporal sequence between biomarker results and sepsis diagnosis, especially when the data is generated after determining sepsis, potentially leading to erroneous conclusions.

We are very sorry for our incorrect writing and the confusion it resulted. Plasma biomarkers, including HBP, were collected upon the patient's admission to the ICU. The diagnosis of infection or sepsis was based on the final diagnosis at the time of discharge from ICU or clinical death, determined by the attending physicians. The above information has been revised in the Methods section of the manuscript.

[4] The one of inclusion criteria is including the patients with HBP test. Why new tests were conducted using frozen samples?

Regarding this issue, we apologize for any ambiguity in the manuscript's description. Based on practical considerations, the patients included in this study had HBP tested upon ICU admission, or had plasma collected upon ICU admission for subsequent HBP testing. The HBP levels for all patients were measured at the time of ICU admission. The decision on whether to test HBP or collect blood samples was made by their attending physicians. The above information has been revised in the inclusion criteria section of the manuscript.

[5] According to the baseline characteristics, the sofa score in infection group is 4.0 (2.3-7.0) and the mortality is similar between the infection group and the sepsis group. It is concerning that some patients in the infection group perhaps reach the sepsis-3 criterion in fact.

Thanks for your rigorous consideration. This issue was not clearly described in the manuscript. As a retrospective research, the grouping criteria for patients in this study were based on the final diagnosis at the time of ICU discharge or clinical death, determined by the attending physicians which was obtained from the electronic medical records. The SOFA scores recorded in the study were automatically been extracted by the electronic scoring system, representing the absolute values of SOFA scores, which were not delta values. Some practical issues might affect the scores. For example, many patients in the control and infection groups were postoperative recovery with invasive mechanical ventilation, the electronic scoring system might assign a respiratory system score of 3-4 points. Additionally, it was quite common with an increased score of coagulation system, due to intraoperative bleeding leading to a decrease in platelets. These situations might be the primary reasons for higher SOFA scores (>2) in the infection group. To address this issue, we have added annotations to Table 1 and made revisions in the limitations section of the study.

Table 1. Characteristics of the patients.

	Control (n = 93)	Infection (n = 94)	Sepsis (n = 53)	Septic shock (n = 86)	*P*
Age, years, median (IQR)	56 (45.0–69.0)	63 (51.0–73.8)	58 (49.0–70.0)	64 (53.0–70.0)	0.023
Sex, male, n (%)	50 (53.8)	64 (68.1)	34 (64.2)	53 (61.6)	0.237
Comorbidity, n (%)					
Hypertension	30 (32.3)	38 (40.4)	15 (28.3)	29 (33.7)	0.459
Diabetes	15 (16.1)	25 (26.6)	10 (18.9)	15 (17.4)	0.281
Cardiovascular	21 (22.6)	24 (25.5)	5 (9.4)	15 (17.4)	0.100
Liver disease	3 (3.2)	3 (3.2)	3 (5.7)	5 (5.8)	0.739
Malignant tumor	34 (36.6)	36 (38.3)	18 (34.0)	42 (48.8)	0.243
Others	26 (28.0)	47 (50.0)	15 (28.3)	37 (43.0)	0.005

Source of infection, n (%)					
Abdomen	-	31 (33.0)	30 (56.6)	63 (73.3)	<0.001
Respiratory	-	46 (48.9)	17 (32.1)	23 (26.7)	0.006
Blood	-	4 (4.3)	8 (15.1)	16 (18.6)	0.009
Skin and soft tissues	-	16 (17.0)	5 (9.4)	8 (9.3)	0.220
Others	-	6 (6.4)	8 (15.1)	5 (5.8)	0.109
Pathogens, n (%)					
Escherichia coli	3 (3.2)	9 (9.6)	9 (17.0)	24 (27.9)	<0.001
Klebsiella genus	1 (1.1)	8 (8.5)	8 (15.1)	14 (16.3)	0.003
Other Enterobacteriaceae	2 (2.2)	2 (2.1)	4 (7.6)	9 (10.5)	0.030
Pseudomonas aeruginosa	1 (1.1)	5 (5.3)	7 (13.2)	9 (10.5)	0.015
Acinetobacter baumannii	1 (1.1)	7 (7.5)	4 (7.6)	4 (4.7)	0.112
Stenotrophomonas maltophilia	1 (1.1)	2 (2.1)	1 (1.9)	11 (12.8)	0.001
Enterococcus	1 (1.1)	8 (8.5)	9 (17.0)	19 (22.1)	<0.001
Other Gram-negative bacteria	1 (1.1)	0 (0.0)	2 (3.8)	9 (10.5)	0.001
Staphylococcus	1 (1.1)	12 (12.8)	5 (9.4)	7 (8.1)	0.024
Streptococcus	2 (2.2)	1 (1.1)	1 (1.9)	3 (3.5)	0.752
Anaerobic bacteria	1 (1.1)	1 (1.1)	1 (1.9)	4 (4.7)	0.377
Fungi	3 (3.2)	17 (18.1)	14 (26.4)	38 (44.1)	<0.001
APACHE II score,	9.0	12.0	13.0	16.5	<0.001
median (IQR)	(7.0–12.0)	(9.0–16.0)	(9.00–18.0)	(12.0–21.0)	
SOFA score*,	2.0	4.0	5.0	10.0	<0.001
median (IQR)	(1.0–5.0)	(2.3–7.0)	(3.0–7.0)	(7.0–13.0)	
Length of ICU stay, days	2.0	5.0	6.0	8.0	<0.001
median (IQR)	(1.0–4.0)	(3.0–7.8)	(3.0–10.0)	(4.0–13.0)	
3-day improvement, n (%)	88 (94.6)	83 (88.3)	47 (88.7)	64 (74.4)	0.001
28-day overall mortality, n (%)	3 (3.2)	9 (9.6)	6 (11.3)	28 (32.6)	<0.001

APACHE II score: acute physiology and chronic health evaluation II score, ICU: intensive care unit, IQR: interquartile range, SOFA score: sequential organ failure assessment score. * the absolute values of SOFA scores.

Minor

1.The method of adjustment for multiple comparisons in the statistical analyses was not specified.

This study was an exploratory study. Thus, no multiplicity correction was used due to the exploratory character of the comparisons.

2.Statistical differences for comparisons of AUC value are not given

We compared the differences of the AUCs between HBP and other indicators using the Delong test, as detailed in Table 2.

Table 2. Performance of biomarkers to discriminate sepsis from non-sepsis.

Variable	AUC (95% CI)	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*P*
HBP	0.733 (0.678–0.789)	35.2	65.5	74.9	65.9	74.5	
IL-6	0.658 (0.595–0.72)	328.9	48.2	82.4	67.0	68.1	0.060
WBC	0.541 (0.474–0.607)	21.0	20.1	95.7	77.8	61.7	＜0.001
PCT	0.812 (0.766–0.857)	0.9	85.6	59.9	61.1	84.2	0.021
CRP	0.775 (0.724–0.827)	107.7	66.9	77.0	68.4	75.8	0.237
LAC	0.632 (0.571–0.694)	1.9	53.2	72.2	58.7	67.5	0.185
APACHE II	0.688 (0.630–0.747)	12.5	65.5	63.6	64.3	64.8	0.128
SOFA	0.801 (0.755–0.848)	4.5	83.5	62.0	68.7	79.0	0.064

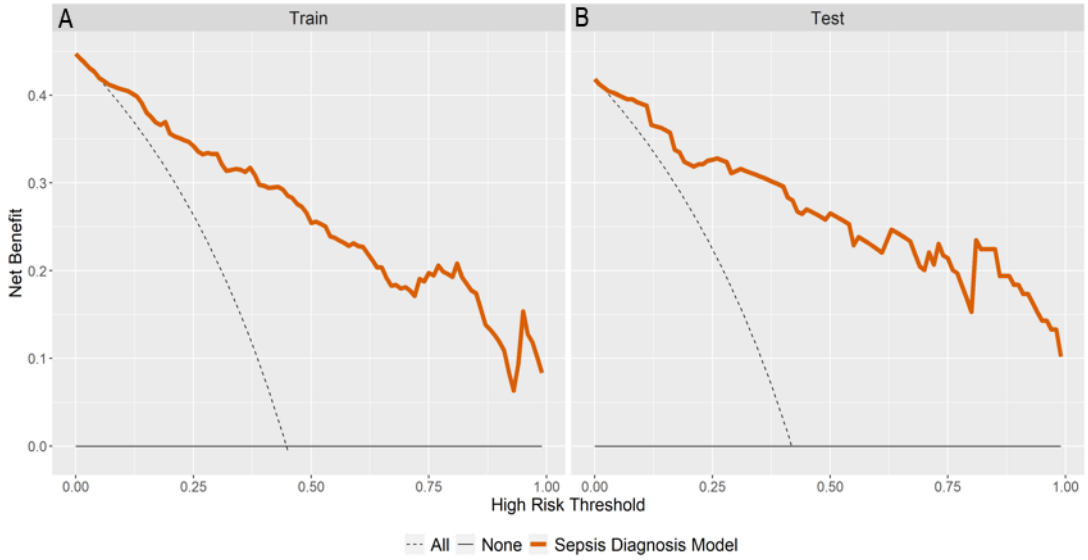
APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count. The P values between AUCs compared to HBP.

3.The result of decision curve analysis lacks of further explanation. What is the criterion for high clinical net benefit?

Thank you for your rigorous comment. Decision curve analyses were shown in Supplementary Figure 5. The black solid line was the net benefit of treating no patients(None), the black dashed line was the net benefit of treating all patients(All), the orange solid line was the net benefit of treating patients according to the sepsis diagnostic model. Throughout the entire threshold range(x-axis), the sepsis diagnostic model surpassed both Treat-all and Treat-no. Therefore, the decision curve analysis (DCA)

plot demonstrated a high clinical net benefit for the constructed sepsis diagnostic model. We have added annotations in Supplementary Figure 5.

For predictive models, net benefit was a composite metric that considers both true positives and false positives, namely the net true positive count. In this study, true positives referred to the correct identification of individuals with sepsis (eligible for appropriate treatment), while false positives were the incorrect identification of individuals as having sepsis (leading to unnecessary treatment). For instance, at a risk cut-off value of 43.9% (the maximum sum of sensitivity and specificity) and the corresponding benefit-harm ratio = 0.783 (odds [43.9%] = 0.439:0.561). After screening with this model, there were 110 true positive cases and 20 false positive cases. The net positive count was then calculated as 110 - 20 * 0.783 = 90. The net benefit was the ratio of the net positive count to the total study population, i.e., 0.276. This could be interpreted as, when using the current diagnostic model, approximately 27.6 individuals out of every 100 would be correctly diagnosed with sepsis.



Supplementary Figure 5. Decision curve analysis (DCA) curve of the sepsis diagnostic model. A: training set, B: test set. The black solid line is the net benefit of treating no patients, the black dashed line is the net benefit of treating all patients, the orange solid line is the net benefit of treating patients according to the sepsis diagnostic model. Throughout the entire threshold range(x-axis), the sepsis diagnostic model surpasses both Treat-all and Treat-no.

Reviewer: 2

[1] The authors need to explain if this a retrospective analysis in patients with already measured HBP or a retrospective measurement of HBP in prospectively collected samples. The first part of the Methods suggests the first design and the second

part of the Methods suggests the second design.

Thanks so much for your careful reading and checking. Regarding this issue, we apologize for any ambiguity in the manuscript's description. As a retrospective study, based on practical considerations, the patients included in this study were divided into two groups: one group had HBP tested upon ICU admission, while the other group had plasma collected upon ICU admission for subsequent HBP testing. Therefore, the HBP levels for all patients were measured at the time of ICU admission. The

above information has been revised in the inclusion criteria section and the participant flow diagram (Figure 1) in the manuscript.

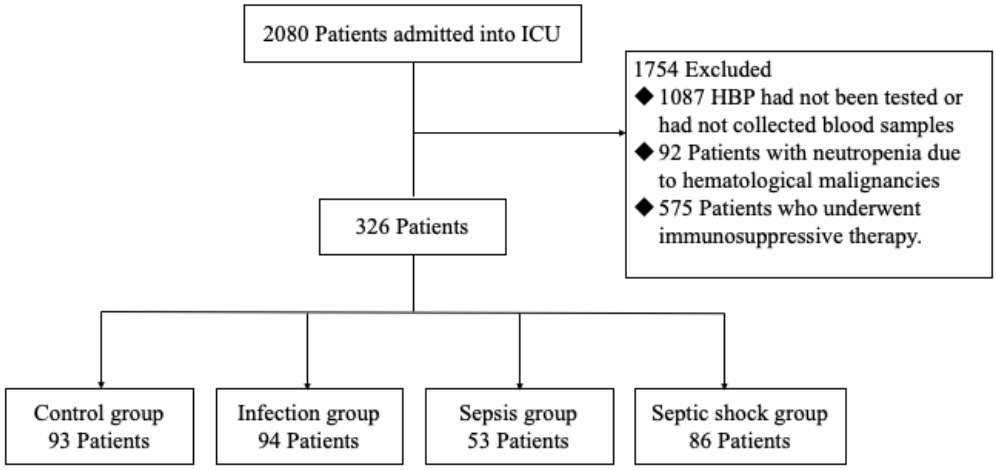


Figure 1. The flow diagram of participants. HBP: heparin-binding protein, ICU: intensive care unit.

[2] The study title and the Introduction imply for a design which investigates early sepsis detection. The submission includes patients who are already at sepsis.

We are very sorry for our incorrect writing and the confusion it resulted. This issue was not clearly described in the manuscript. The grouping criteria for patients in this study were based on the final diagnosis at the time of ICU discharge or clinical death, determined by the attending physicians. We retrospectively collected data after the patient's discharge or clinical death, so the final diagnosis was not influenced by any experimental factors. Due to the unstable vital signs of critically ill patients and the inability to undergo external examinations shortly after admission, coupled with the timeliness issues in some microbiological tests, clinical doctors might not obtain all examination results within a short time after admission, making it challenging to establish a definitive diagnosis. While it could not be ruled out that the initial diagnosis upon admission might be sepsis, a definitive diagnosis still required a subsequent comprehensive assessment based on the patient's clinical presentation, organ function, and examination results. Additionally, the timing of HBP testing or blood sample collection occurred at the time of ICU admission, preceding the final diagnosis. Therefore, the aim of our study was to explore the early diagnosis value of sepsis in the ICU using HBP. To address this issue, we have made relevant revisions in the Method section.

[3] The derivation of the equation of the model and the positioning of HBP in the model is not defined.

Thanks for your rigorous consideration. The derivation of the equation for this model was obtained through the following steps. Analysis of risk factors affecting sepsis diagnosis using univariate and multivariate logistic regression and construction of a sepsis diagnostic model. The variables for which univariate logistic regression obtained $P < 0.05$ were included in the multivariate logistic regression

model. Among the statistically significant variables in the univariate analysis were HBP, PCT, CRP, IL-6, LAC, APACHE II, and SOFA. The final multivariate logistic regression results showed that PCT (OR = 1.034, 95% CI 1.009-1.060, $p = 0.009$), CRP (OR = 1.011, 95% CI 1.006-1.016, $p < 0.001=$, HBP (OR = 1.006, 95% CI 1.000-1.012, $p = 0.041$), IL-6 (OR = 1.001 95% CI 1.000-1.001, $p = 0.013$), SOFA (OR = 1.252, 95% CI 1.110-1.412, $p < 0.001=$ were significantly associated with sepsis diagnosis. The sepsis diagnostic model was constructed based on the results of logistic regression that was shown in Figure 3. Regarding this issue, we have provided these details in the Results section of the manuscript, and supplemented the results of univariate logistic regression in Supplementary Table 2.

Supplementary Table 2. Univariate and multivariate logistic regression analysis of risk factors for sepsis diagnosis.

Variable	Univariate logistic regression analysis		Multivariate logistic regression analysis	
	OR (95%CI)	*P*	OR (95%CI)	*P*
Age	1.009 (0.993, 1.026)	0.276		
Sex	1.169 (0.683, 1.999)	0.569		
Hypertension	0.795 (0.450, 1.402)	0.427		
Diabetes	0.801 (0.418, 1.538)	0.505		
Cardiovascular	0.538 (0.288, 1.182)	0.135		
Liver disease	1.572 (0.411, 6.014)	0.509		
Malignant tumor	1.471 (0.861, 2.514)	0.158		
Other disease	0.998 (0.582, 1.712)	0.994		
PCT	1.068 (1.037, 1.101)	<0.001	1.034 (1.009, 1.060)	0.009
CRP	1.014 (1.009, 1.018)	<0.001	1.011 (1.006, 1.016)	<0.001
HBP	1.011 (1.006, 1.016)	<0.001	1.006 (1.000, 1.012)	0.041
IL-6	1.001 (1.000, 1.001)	<0.001	1.001 (1.000, 1.001)	0.013
LAC	1.198 (1.062, 1.352)	0.003		
WBC	1.034 (0.992, 1.076)	0.111		
APACHE II	1.108 (1.067, 1.152)	<0.001		
SOFA	1.383 (1.276, 1.501)	<0.001	1.252 (1.110, 1.412)	<0.001

APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count.

[4] The manuscript is not elegantly written. More precisely: A) The Introduction fails to explain the real study objective. B) Half of the Results section is unfocused in presenting the demographics of the patients which is not the endpoint. C) The Discussion is extremely wordy and requires shortening. D) Major recent publications for HBP are missing.

Thanks so much for your careful reading and checking. Based on your suggestions, we have made the following modifications: A) In the background introduction section, we emphasized the real purpose of the study. B) In the results section, we deleted descriptions of demographic characteristics, that were not directly related to the study objectives. C) The Discussion section had been edited and reduced. D) The references section was supplemented with recent published literatures.

VERSION 2 – REVIEW

REVIEWER	Liu, Zhanguo Southern Medical University Second Clinical Medical College
REVIEW RETURNED	04-Feb-2024

GENERAL COMMENTS	The quality of the article has been greatly improved after revision, but there are still issues that need to be revised or clarified. 1.The study population was categorized into control, infection, sepsis, and septic shock groups by the authors. However, in the analysis of the diagnostic model for HBP, only the diagnostic performance of HBP in diagnosing sepsis was examined. It is recommended that further analysis be conducted on the diagnostic performance of HBP in septic shock and a cut-off value be provided. 2.The scale of the ordinate in Figure 2b should be adjusted to better represent the differences in PCT levels among the groups

REVIEWER	Giamarellos-Bourboulis, Evangelos National and Kapodistrian University of Athens, 4th Department of Internal Medicine
REVIEW RETURNED	16-Jan-2024

GENERAL COMMENTS	I acknowledge that the authors have done a great effort to revise and improve their submission. However, there are still major concerns remaining. • The authors fail to persuade that HBP is measured at an early time point to provide early sepsis detection. The way the manuscript is drafted follows the traditional approach of biomarker manuscripts comparing patients with already developed sepsis to comparators. The authors need to provide the time gap between sampling and sepsis onset so as to evidence if HBP can early predict sepsis progression. • There are still many grammar errors throughout the manuscript. • Major recent publications for HBP are still missing.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

1. The study population was categorized into control, infection, sepsis, and septic shock groups by the authors. However, in the analysis of the diagnostic model for HBP, only the diagnostic performance of HBP in diagnosing sepsis was examined. It is recommended that further analysis be conducted on the diagnostic performance of HBP in septic shock and a cut-off value be provided.

We appreciate the insightful feedback. Following your suggestion, we conducted an additional analysis to evaluate the diagnostic performance of HBP in diagnosing septic shock. Our findings indicated that HBP did not demonstrate excellent diagnostic efficiency for the early detection of septic shock across all patients with sepsis (including both sepsis and septic shock cohorts), with an AUC of 0.594 (95% CI 0.499–0.690), as shown in Figure 1. When the HBP cut-off value was set at 143.4 ng/mL, the sensitivity, specificity, PPV, and NPV for diagnosing septic shock were 29.1%, 88.7%, 80.6%, and 43.5%, respectively. It's worth noting that these results were not included in the manuscript due to the lack of statistical significance (P = 0.062).

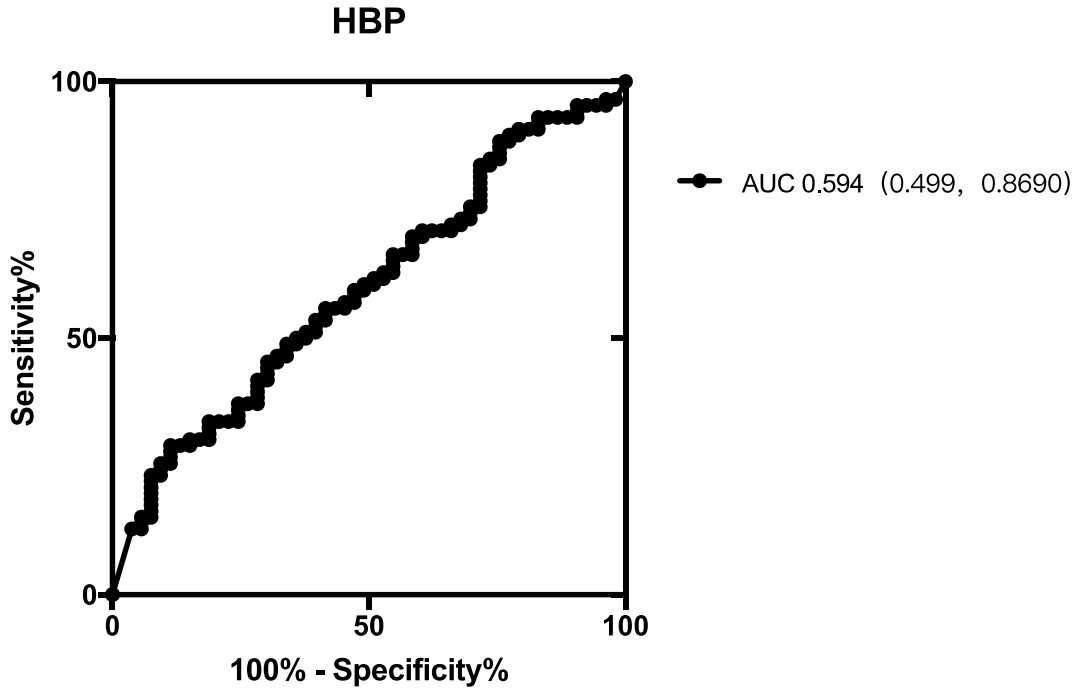


Figure 1. The AUC of HBP for diagnosing septic shock

2. The scale of the ordinate in Figure 2b should be adjusted to better represent the differences in PCT levels among the groups

Thank you for your attention to detail. We have adjusted the scale of the ordinate in Figure 2b to better represent the differences in PCT levels among the groups, ensuring improved clarity and accuracy in our presentation (Figure 2.).

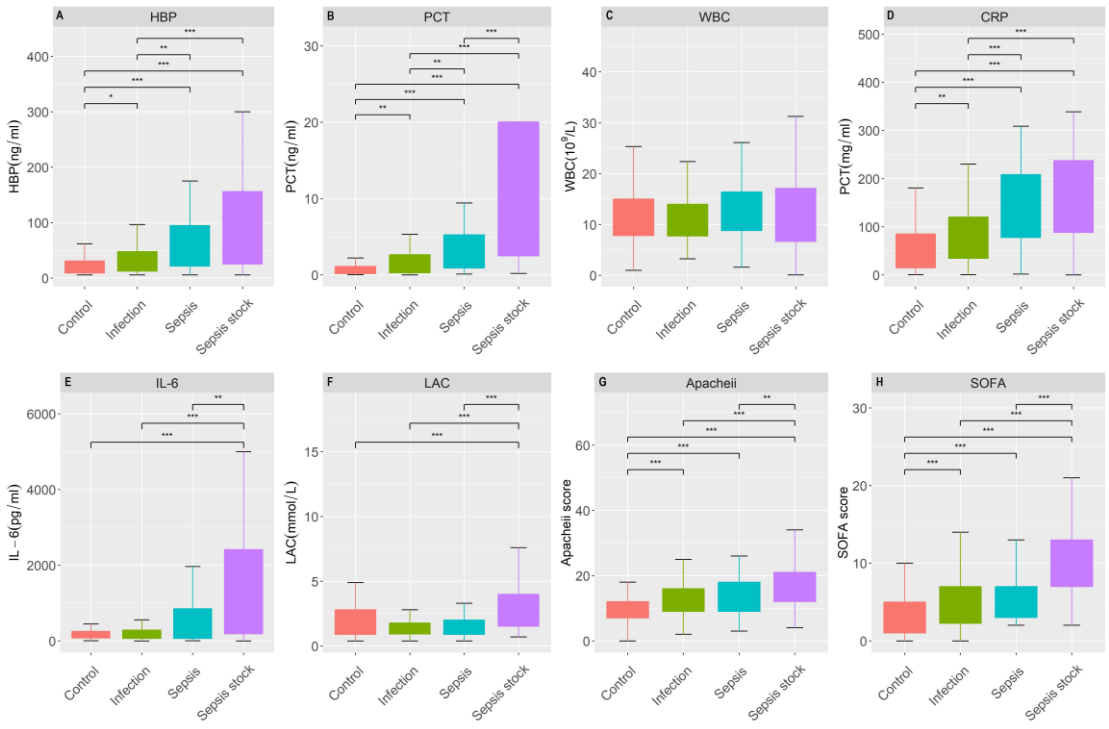


Figure 2. Comparison of plasma levels of biomarkers among different groups. A: HBP, B: PCT, C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Reviewer: 2

1. The authors fail to persuade that HBP is measured at an early time point to provide early sepsis detection. The way the manuscript is drafted follows the traditional approach of biomarker manuscripts comparing patients with already developed sepsis to comparators. The authors need to provide the time gap between sampling and sepsis onset so as to evidence if HBP can early predict sepsis progression.

Thanks for your thorough comments. Sepsis represents a significant threat to critically ill patients, leading to elevated morbidity and mortality rates. Hence, there's a crucial need to explore highly specific and sensitive biomarkers for early sepsis detection within the ICU setting.

Considering practicalities, patients enrolled in this study underwent HBP testing upon admission to the ICU or had plasma collected at admission for subsequent HBP assessment. Consequently, HBP levels for all patients were measured at the time of ICU admission. Given the unstable vital signs of critically ill patients and constraints on external examinations shortly after admission, along with delays in obtaining microbiological results, clinicians might not promptly access all examination results after admission. This could impede the establishment of a definitive diagnosis.

While some patients might present with sepsis upon ICU admission, a definitive diagnosis of sepsis still necessitated comprehensive evaluation based on subsequent examinations. We collected diagnosis after the patient discharge or death. Therefore, the timing of HBP testing or blood sample collection preceded the time of definitive diagnosis.

Though this study reflected real-world scenarios, it had limitations. We intend to address these limitations by conducting a prospective multicenter study to dynamically monitor HBP for predicting sepsis, aiming to further elucidate the diagnostic value of HBP in sepsis.

2. There are still many grammar errors throughout the manuscript.

We have addressed the grammar errors in our manuscript, ensuring that it meets the highest standards of language proficiency.

3. Major recent publications for HBP are still missing.

Thank you for bringing this to our attention. We have updated the references section with major recent publications on HBP, enriching the literature cited in our manuscript.

In conclusion, we are grateful for the reviewers' insightful feedback, which has significantly contributed to the refinement of our manuscript. We are confident that the revisions we have made address all concerns raised and enhance the overall quality and clarity of our work. We eagerly await your decision regarding the publication of our revised manuscript.

VERSION 3 – REVIEW

REVIEWER	Giamarellos-Bourboulis, Evangelos National and Kapodistrian University of Athens, 4th Department of Internal Medicine
REVIEW RETURNED	28-Mar-2024

GENERAL COMMENTS	From the answer the authors have provided to my concerns, it is obvious that “early” is not the case in their study. Early should be removed from the title and the limitations provided in the letter should also be added in the Discussion.

VERSION 3 – AUTHOR RESPONSE

Response to Review Comments

We extend our gratitude for your attentive review, valuable comments, and constructive suggestions, which have significantly enhanced the quality of our manuscript.

We have meticulously considered the feedback provided by the reviewer 2 and made appropriate revisions to our manuscript. Below, we summarize our responses to reviewer's comments, addressing

the concerns comprehensively. We are confident that our revisions adequately address the point raised by the reviewer, and we hope our revised manuscript can be accepted for publication.

Reviewer: 2
1. From the answer the authors have provided to my concerns, it is obvious that “early” is not the case in their study. Early should be removed from the title and the limitations provided in the letter should also be added in the Discussion.

Thanks for your thorough comments. Following your suggestion, we removed the “early” from the title and full text, and added the limitations in the discussion (from line 313 to line 321 in untracked manuscript.

Sepsis represents a significant threat to critically ill patients, leading to elevated morbidity and mortality rates. Hence, there's a crucial need to explore highly specific and sensitive biomarkers for sepsis detection within the ICU setting.

Considering practicalities, patients enrolled in this study underwent HBP testing upon admission to the ICU or had plasma collected at admission for subsequent HBP assessment. Although detecting HBP or collecting blood samples occurred upon admission to the ICU, the onset time might not the early time point of sepsis course. Based on this, HBP could not demonstrate excellent diagnostic efficiency for the early detection of sepsis in this study.

Though this study reflected real-world scenarios, it had limitations. We intend to address these limitations by conducting a prospective multicenter study to dynamically monitor HBP for predicting sepsis, aiming to further elucidate the early diagnostic value of HBP in sepsis.

In conclusion, we are grateful for the reviewer' insightful feedback, which has significantly contributed to the refinement of our manuscript. We are confident that the revisions we have made address concerns raised and enhance the overall quality and clarity of our work. We eagerly await your decision regarding the publication of our revised manuscript.