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Heparin-Binding Protein Improved Early Diagnosis of Sepsis in the Intensive Care Unit: A Retrospective Cohort Study

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Abstract

Objectives: This study aims to investigate the diagnostic value of heparin-binding protein (HBP) in sepsis and develop a sepsis diagnostic model incorporating HBP with key biomarkers and disease-related scores for an early, rapid, and accurate diagnosis of sepsis.

Design: Retrospective cohort study.

Setting: A comprehensive teaching tertiary hospital in China.

Participants: Adult patients (age⩾18years) who had tested HBP in intensive care unit (ICU).

Main outcome measures: HBP, C-reactive protein (CRP), procalcitonin (PCT), white blood cell count (WBC), interleukin-6 (IL-6), lactate (LAC), acute physiology and chronic health evaluation II (APACHE II) and sequential organ failure assessment (SOFA) score were recorded.

Results: From March 2019 and December 2021, 326 patients were enrolled in this study. The patients were categorized into the non-infection group (control group), infection group, sepsis group, and septic shock group as per the Sepsis-3 criteria. The levels of HBP in the sepsis group and septic shock group were 45.7 and 69.0 ng/mL, significantly higher than those in the control group and infection group, 18.0 and 24.0 ng/mL, respectively ($p < 0.001$). The AUC value of HBP for diagnosing sepsis was 0.733, which was lower than those corresponding to PCT, CRP, and SOFA, but higher than those of IL-6, LAC, and APACHE II. Multivariate binary logistic regression analysis identified HBP, PCT, CRP, IL-6, and SOFA as valuable indicators for

diagnosing sepsis. A sepsis diagnostic model was constructed based on these indicators, whose AUC was 0.901, with a sensitivity of 79.7% and specificity of 86.9%.

Conclusions: HBP could serve as a biomarker for early diagnosis of sepsis. Compared with single indicators, the sepsis diagnostic model constructed with HBP, PCT, CRP, IL-6, and SOFA further enhanced the diagnostic performance of sepsis.

Strengths and limitations of this study: This study included a highly heterogeneous population, making it highly applicable to sepsis patients in ICU. Moreover, most of the biomarkers included in this diagnostic model were widely used in clinical practice, making them easily obtainable, highly reproducible, and operationally feasible. HBP could serve as a biomarker for early diagnosis of sepsis, sepsis diagnostic model constructed with HBP and other biomarkers further enhanced the diagnostic performance of sepsis. This study was an ICU single-center retrospective research, the results might not be applicable to sepsis patients in the emergency department or general wards.

Keywords: HBP, Sepsis, Diagnostic model

Background

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection. Sepsis, when accompanied by severe circulatory impairment and cellular metabolic disorders, is referred to as septic shock, which is the leading cause of death in septic patients [1]. With the worsening of aging and various factors leading to an increasing number of immunocompromised hosts, the incidence of sepsis has been rising every year. The Global Burden of Sepsis study published in 2020 reported 48.9 million cases of sepsis worldwide in 2017, with 11 million deaths attributed to sepsis, accounting for 19.7% of global deaths [2]. Another domestic study showed that the incidence of sepsis in the intensive care unit (ICU) was 20.6%, with a 90-day mortality rate of 35.5%, and the mortality rate for septic shock was as high as 50% or more [3]. Kumar et al. demenstrated that the mortality rate of septic shock was correlated with hypotension and delayed use of antibiotics [4]. Another study indicated that early fluid resuscitation was closely related to the prognosis of patients with sepsis [5]. Therefore, early diagnosis of sepsis and timely appropriate treatment are crucial for sepsis management.

Early diagnosis and identification of sepsis require a comprehensive approach based on the patient's clinical symptoms, conventional cultures, biomarkers, and disease-specific scoring systems. However, clinical symptoms and signs of sepsis are often nonspecific, and conventional pathogen culture is relatively lagging behind [6]. Therefore, early diagnosis of sepsis in the ICU largely relies on biomarkers and disease-specific scoring systems. Currently, there are over 200 sepsis-related biomarkers

reported in the literature, among which heparin-binding protein (HBP) is a novel biomarker [7]. HBP is a serine protease-like protein secreted by neutrophils after infection and has functions such as altering endothelial cell permeability, antimicrobial activity, chemotaxis, and regulation of cell apoptosis [8]. It has been identified as an early diagnostic indicator for severe sepsis/septic shock in Chinese Guidelines for the Management of Severe Sepsis/Septic Shock (2014) [9] and Chinese Expert Consensus on Early Prevention and Interruption of Sepsis in Emergency Medicine (2020) [10]. In addition, an increasing number of studies have furnished evidence regarding the use of HBP for diagnosing sepsis in recent years. Studies have demonstrated that HBP can be used for sepsis diagnosis and monitoring the severity [8, 11, 12]. On the other hand, a few studies have indicated that elevated levels of HBP irrespective of infectious etiology and no correlation with severity and outcome [13]. Furthermore, differences and inconsistencies have been noted among various studies in regard to the diagnostic performance of HBP for sepsis [14]. Therefore, HBP has not been widely applied in clinical practice for sepsis diagnosis. The aim of this study was to explore the early diagnostic value of HBP in sepsis and to develop a sepsis diagnostic model combining HBP with multiple biomarkers and disease-specific scoring systems in order to facilitate early identification and diagnosis of sepsis.

Methods

Study Population

Data were collected retrospectively from patients admitted to the ICU of the First

Affiliated Hospital of Sun Yat-sen University, China, from March 2019 to December 2021. The inclusion criteria were as follows: (1) HBP had been tested, (2) The clinical data were complete, and (3) age over 18 years. The exclusion criteria were as follows: (1) Patients with neutropenia due to hematological malignancies, and (2) patients who underwent immunosuppressive therapy. Patients were classified into four groups, namely, the infection group, sepsis group, septic shock group, and control group in accordance with the Sepsis-3 criteria [15]. The protocols were approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University and conducted in accordance with the Declaration of Helsinki.

Measurement Indicators and Methods

Blood samples of enrolled patients were retrieved from the freezer. After gradual thawing, the samples were centrifuged at 1,000 rounds/min for 10 min, and 100 μL of supernatants were collected for plasma level of HBP determination using an immunofluorescence dry quantitative method (Jet-iStar3000, Hangzhou, Joinstar Biomedical Technology Co,.LTD). The procedure strictly followed the instructions provided with the reagent kit, and regular quality control was performed.

General information such as gender, age, underlying diseases, site of infection, and pathogens was recorded for each group of patients. General vital signs including body temperature, heart rate, blood pressure, respiratory rate, peripheral oxygen saturation (SpO_2), and urine output were collected. Infection biomarkers such as procalcitonin (PCT), white blood cell count (WBC), C-reactive protein (CRP),

interleukin-6 (IL-6), and blood lactate (LAC) were measured. Laboratory indicators such as blood biochemistry, liver enzymes, liver function, coagulation function, and platelet count were evaluated. Organ function indicators such as Glasgow Coma Scale (GCS) score, respiratory support measures, oxygenation index, and vasopressor use were documented. Medication use including albumin and heparin, as well as interventions such as continuous renal replacement therapy (CRRT) and extracorporeal membrane oxygenation (ECMO), were recorded. Acute Physiology and Chronic Health Evaluation II (APACHE II) score and Sequential Organ Failure Assessment (SOFA) score were calculated within 24 h of ICU admission. The length of ICU and survival outcomes (3-day improvement rate, 28-day mortality rate) were also recorded for each group of patients.

Statistical Methods

For baseline measurement data, median and interquartile range (IQR) were used to describe the data. If continuous variables followed a normal distribution, one-way ANOVA was used for intergroup comparisons; otherwise, the Kruskal–Wallis H test was used. Percentage calculations were performed for categorical data, and differences between groups were tested using the chi-square test or Fisher's exact test.

Receiver operating characteristic (ROC) curves were used to assess the diagnostic performance of HBP, PCT, WBC, CRP, IL-6, LAC, APACHE II score, and SOFA score for sepsis. The area under the ROC curve (AUC) was also estimated. The optimal cut-off values for diagnosing sepsis were determined based on the maximum Youden

index, and corresponding sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

To improve the diagnostic performance of sepsis, a multivariate binary logistic regression model was constructed. Random selection of 70% of all patients was used as the training set, while the remaining 30% served as the test set to assess the model's performance. AUC was calculated for both the training and test sets. The Hosmer–Lemeshow goodness-of-fit test and calibration curve were used to evaluate the model's goodness-of-fit for both datasets. Decision curves were also plotted to evaluate the clinical utility of the regression model. All hypothesis tests were two-tailed, and a significance level of $P < 0.050$ was set. Statistical analysis was performed using R 4.1.1 and SPSS 25.0.

Results

Characteristics of the patients

Table 1 encapsulates the baseline characteristics of the patients. A total of 326 patients were enrolled in this study, including 93 in the control group, 94 in the infection group, 53 in the sepsis group, and 86 in the septic shock group. The median ages of patients in the control group, infection group, sepsis group, and septic shock group were 56, 63, 58, and 64 years, respectively, with statistically significant differences among the groups ($p = 0.023$). No significant differences were noted among the groups in terms of gender, prevalence of hypertension, diabetes, heart disease, malignancy, liver disease, and other comorbidities.

In the control group, the patients were undergoing postoperative recovery. For patients in the infection group, the respiratory tract infection was the predominant source of infection (48.9%), followed by abdominal infection (33.0%) and skin and soft tissue infection (17.0%). In the sepsis group and septic shock group, the proportions of abdominal infections (56.6%, 73.3%) and bloodstream infections (15.1%, 18.6%) were significantly higher than those in the infection group (33.0%, 4.3%). The proportions of multiple-site infection of the sepsis group and septic shock group (28.3%, 30.2%) were significantly higher than those in the infection group (8.6%).

Among all enrolled patients, blood cultures were obtained from 206 patients, with 32 reporting positive results. Abdominal drainage cultures were obtained from 149 patients, with 76 reporting positive results. Sputum cultures were obtained from 122 patients, with 90 reporting positive results. Urine cultures and cerebrospinal fluid cultures were obtained from 98 patients, with 35 reporting positive results. In terms of pathogens, the positivity rates of *Escherichia coli*, *Enterococcus species*, *fungi*, *Klebsiella species*, and *Pseudomonas aeruginosa* were significantly higher in sepsis and septic shock patients compared with the infection group. Among them, septic shock patients had higher positivity rates, with 38 cases (44.1%) of *fungi*, 24 cases (27.9%) of *Escherichia coli*, 19 cases (22.1%) of *Enterococcus species*, and 14 cases (16.3%) of *Klebsiella species*.

The APACHE II and SOFA scores in the sepsis and septic shock groups were significantly higher than those in the control and infection groups. The median length of ICU stay in the control group, infection group, sepsis group, and septic shock group

were 2, 5, 6, and 8 days, respectively, with statistically significant differences ($p < 0.001$). In terms of survival analysis, the patients in the control group had the highest 3-day improvement rate and the lowest 28-day overall mortality rate, and the primary causes of death in three patients were hemorrhagic shock or cardiogenic shock. The patients in the septic shock group had the lowest 3-day improvement rate and the highest 28-day overall mortality rate, with all deaths attributed to septic shock. Among the 28 patients who succumbed to septic shock, 20 cases were due to abdominal infection.

Levels of HBP and other biomarkers in each group of patients

The median (IQR) levels of HBP in the control, infection, sepsis, and septic shock groups were 18.0 (9.9–32.1), 24.0 (14.1–56.4), 45.7 (24.8–107.9), and 69.0 (33.8–150.9) ng/mL, respectively ($p < 0.001$). HBP was capable of effectively distinguishing between patients with and without infection or sepsis, and its efficacy was superior to IL-6, LAC, and WBC. However, in distinguishing septic patients with or without shock, HBP was inferior to PCT, IL-6, and LAC. Additionally, there were no statistical differences were noted in WBC levels among the groups (Figure 1).

When comparing HBP levels among different infection sites in the infection, sepsis, and septic shock groups, statistical differences were observed among the subgroups except for multi-infection site (Supplementary Table 1). As the severity of infection increased, APACHE II and SOFA scores gradually increased, showing statistical differences. However, no statistical difference was observed when comparing

the infection group with the sepsis group (Figure 1).

Analysis of the diagnostic accuracy of different biomarkers for sepsis

HBP demonstrated promising diagnostic performance for early detection of sepsis, with an AUC of 0.733 (95% CI, 0.678–0.789), which was higher than AUCs corresponding to IL-6, LAC, and APACHE II scores (AUCs of 0.658, 0.632, and 0.688, respectively), but lower than PCT, CRP, and SOFA scores (AUCs of 0.812, 0.775, and 0.801, respectively). When the HBP cut-off value was set at 35.2 ng/mL, the sensitivity and specificity for diagnosing sepsis were 65.5% and 74.9%, respectively (Table 2, Supplementary Figure 1).

Relationship between HBP and other biomarkers

No significant correlation was observed between HBP levels and CRP, PCT, WBC, IL-6, LAC, APACHE II scores, and SOFA scores (Supplementary Figure 2).

Sepsis diagnostic model and test

Based on the training set, variables were selected through univariate regression analysis for patient demographics (such as gender, age, underlying diseases, infection sites, and pathogens), infection biomarkers (HBP, PCT, WBC, CRP, IL-6, and LAC), APACHE II scores, and SOFA scores. Variables with statistical significance were included in the multivariate regression model (Supplementary Table 2). Furthermore, insignificant variables were removed from the multivariate model to streamline the

predictive model. The final results of the regression model were shown in Figure 2.

To evaluate the predictive performance of the model, the remaining 30% of patients were used as a test set to validate the model. In the training set, the model achieved an AUC of 0.901 (95% CI, 0.863−0.940). When the Youden index was maximized, the cut-off value was determined to be 0.439, resulting in a sensitivity of 79.4% and a specificity of 86.5%. In the test set population, the model obtained an AUC of 0.913 (95% CI, 0.860−0.966). Applying the cut-off value obtained from the training set to the test set, the sensitivity and specificity were 80.5% and 87.7%, respectivly (Figure 3). Furthermore, to obtain a more accurate cut-off value, all patients were included in the diagnostic model, resulting in a cut-off value of 0.439. The sensitivity and specificity for diagnosing sepsis with this cut-off value were 79.7% and 86.9%, respectively.

The diagnostic model constructed using the training set exhibited a good predictive performance based on the Hosmer–Lemeshow goodness-of-fit test in both the training and test sets ($\chi^2 = 4.91$, $p = 0.767$; $\chi^2 = 5.12$, $p = 0.745$; Supplementary Figure 3) Additionally, the decision curve analysis (DCA) plot demonstrated a high clinical net benefit for the constructed sepsis diagnostic model (Supplementary Figure 4).

Discussion

Sepsis is a major cause of mortality in critically ill patients, with high morbidity and mortality. Approximately 20%–30% of severely infected patients do not exhibit

typical symptoms of organ dysfunction upon admission but rapidly progress to sepsis [6]. Therefore, early identification of sepsis is crucial for developing appropriate and effective treatment strategies and reducing mortality. Clinicians require more specific and sensitive biomarkers to identify the early diagnosis of sepsis. Currently, WBC, CRP, and PCT are proposed commonly in clinical practice as inflammatory biomarkers [7]. However, WBC and CRP are nonspecific markers of systemic inflammation and cannot effectively differentiate among bacterial, non-bacterial, and sterile inflammation. PCT has a higher specificity for bacterial infections but performs poorly in predicting sepsis-associated organ dysfunction [6, 16]. In recent years, numerous studies have proven that HBP has good predictive performance for infection, sepsis, or organ function assessment, superior to PCT, CRP, and other biomarkers [6, 8, 11, 12, 17, 18].

HBP, also known as heparin-binding protein or CAP37, is a protein molecule stored in the secretory granules of neutrophils and azurophilic granules. It contains a large number of positively charged amino acid residues, which are concentrated on one side of the protein [18]. A hydrophobic pocket structure formed by amino acid residues 20–44 exhibits a high affinity for endotoxins [6]. Therefore, HBP was initially discovered for its antimicrobial activity. Subsequent research confirmed that HBP was a multifunctional innate immune defense molecule that played a crucial role in the host's infection and inflammatory response [6, 18]. These characteristics made HBP a promising novel infection biomarker. Recent studies have reported that HBP could assist in the diagnosis of various diseases, such as respiratory and circulatory failure, sepsis, acute kidney injury, acute lung injury, meningitis, urinary tract infections, as

well as skin and soft tissue infections [6, 8, 11, 19, 20]. However, its clinical use has not yet been widely adopted, so further clinical research is required to validate its utility.

This study further confirmed that HBP was a promising biomarker in sepsis. In this study, the levels of HBP in infected patients (infection group, sepsis group, and septic shock group) were significantly higher than those of non-infected patients (control group). The HBP levels in sepsis patients (sepsis group and septic shock group) were significantly elevated compared with non-sepsis patients (infection group and control group). Therefore, HBP levels could effectively differentiate whether patients had an infection and whether infected patients had sepsis. Furthermore, its discriminative value was found to be superior to LAC, IL-6, WBC, SOFA, and APACHE II scores. Similar findings have been reported in previous studies [7, 11]. These results were likely related to the biological characteristics of HBP. It was stored in neutrophil secretory granules and azurophilic granules, and upon stimulation by pathogens, it could be rapidly and massively released into the bloodstream, inducing rearrangement of the endothelial cell cytoskeleton, leading to vascular leakage and edema formation. Additionally, HBP regulated the function of monocytes and macrophages, further amplifying the inflammatory response and enhancing the body's immune response to infection. Moreover, as neutrophils infiltrated into the tissues, HBP continued to be released, resulting in tissue damage and organ dysfunction [18, 21]. Therefore, HBP levels were significantly elevated in patients with infection and/or sepsis.

Regarding the diagnostic performance of HBP in sepsis, a study by Linder et al.

found that the AUC of HBP for predicting sepsis was 0.85, with a sensitivity of 87% and specificity of 95%, which were significantly higher than those of PCT, CRP, WBC, IL-6, and other biomarkers [7]. Furthermore, HBP had the ability to predict the occurrence of organ dysfunction and circulatory failure at an early stage, providing indications for timely interventions such as fluid resuscitation and antibiotic use, which were indispensable components of sepsis bundle therapy [7, 11, 22]. In addition, the favorable predictive value of HBP was validated in pediatric patients with severe sepsis [23]. The emergence of this phenomenon was considered to be related to the pathological process in which HBP was involved in vascular leakage and organ dysfunction in septic patients, and its release occurred earlier than CRP, PCT, and other markers [17, 18, 21]. In this study, the AUC for HBP in predicting sepsis was 0.733, which was not superior to PCT, CRP, and SOFA. Previous studies reported varying diagnostic accuracy of HBP for sepsis at different time points [17]. Meta-analyses also revealed that HBP often performs better in diagnosing sepsis in emergency department patients compared with ICU patients [13, 14, 17]. Based on the above analysis, it was considered that a correlation between the more severe condition of ICU patients and the complexity of intervention measures may be the reasons. First, most ICU patients had multiple influencing factors such as surgery, trauma, procedures, and infections. Second, patients received broad-spectrum antibiotics, fluid resuscitation, and other sepsis-related treatments in emergency departments or general wards prior to being transferred to the ICU, indicating a relatively advanced stage of the disease. Lastly, ICU patients had complex medication regimens and multiple intervention measures, such as heparin, albumin, and

CRRT, among others [24-28]. All of these factors might potentially affect the plasma level of HBP. Furthermore, this phenomenon also reflected the limitations of a single biomarker, as it could not fully reflect the clinical reality and accurately diagnose sepsis.

The pathophysiological mechanisms of sepsis are complex. They involve different immune states, sites of infection, and pathogens. The immune response patterns vary, and so do the pathophysiological processes of various biomarkers. Additionally, the severity of organ dysfunction also varies. During its occurrence and progression, there are always dual factors that simultaneously lead to an exaggerated inflammatory response and immune dysfunction. Systemic inflammatory response and immune suppression do not generally exist as simple independent entities but rather co-exist. Therefore, a single biomarker cannot serve as a reliable diagnostic indicator for sepsis [7, 10]. In this study, we also observed that HBP showed almost no correlation with PCT, CRP, IL-6, LAC, APACHE II, and SOFA scores. This suggested that HBP, as a biomarker, could provide unique information for the diagnosis of sepsis that was independent of other biomarkers. We hypothesized that establishing a diagnostic model combining HBP with PCT, CRP, IL-6, LAC, APACHE II, SOFA scores, and other indicators could become a new approach for early diagnosis of sepsis. Currently, relevant studies have been conducted in this regard. Gibot et al. found that a biological scoring system combining soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), PCT, and CD64 had an AUC of 0.95 for diagnosing sepsis, which was higher than any single marker [29]. Furthermore, a prospective observational study suggested that CRP, PCT, and CD64 were good predictive markers for sepsis, and their

combination further improved the diagnostic accuracy of sepsis [30]. However, many of the biomarkers mentioned in the above studies have not been widely used in clinical practice, making them less practical. In this study, commonly used biomarkers in clinical settings were included. Based on the ROC analysis of various markers, a sepsis diagnostic model using binary logistic regression was constructed. Upon test, the sepsis diagnostic model exhibited an AUC above 0.90, indicating its high clinical applicability.

Some limitations of this study should be discussed. First, the study population consisted of patients from a comprehensive ICU, and the model might not be applicable to sepsis patients in the emergency department or general wards. Second, in many septic shock patients, the HBP levels exceeded the upper limit of measurement, which could potentially reduce the statistical differences. Lastly, as a single-center retrospective study, the sample size was relatively small, which affected the statistical power. Subsequent research can be conducted in the form of multi-center prospective studies, involving multiple specialties, and monitoring HBP dynamically to further evaluate its predictive value in sepsis patients.

Conclusion

This study confirmed the value of plasma HBP in the early diagnosis of sepsis in the ICU. It also constructed a sepsis early diagnostic model that includes HBP, PCT, CRP, IL-6, and SOFA scores. This model demonstrated high accuracy and clinical utility, further enhancing the early predictive role in sepsis. It had potential clinical diagnostic value in the early detection of sepsis.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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References

1. Evans, L., et al., *Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021.* Crit Care Med, 2021. **49**(11): p. e1063-e1143.
2. Rudd, K.E., et al., *Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study.* Lancet, 2020. **395**(10219): p. 200-211.
3. Xie, J., et al., *The Epidemiology of Sepsis in Chinese ICUs: A National Cross-Sectional*

Survey. Crit Care Med, 2020. **48**(3): p. e209-e218.

4. Kumar, A., et al., *Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock.* Crit Care Med, 2006. **34**(6): p. 1589-96.

5. Kuttab, H.I., et al., Evaluation and Predictors of Fluid Resuscitation in Patients With Severe Sepsis and Septic Shock. Crit Care Med, 2019. 47(11): p. 1582-1590.

6. Yang, Y., et al., *A Promising Candidate: Heparin-Binding Protein Steps onto the Stage of Sepsis Prediction.* J Immunol Res, 2019. **2019**: p. 7515346.

7. Pierrakos, C., et al., *Biomarkers of sepsis: time for a reappraisal.* Crit Care, 2020. **24**(1): p. 287.

8. Linder, A., et al., *Heparin-binding protein: an early marker of circulatory failure in sepsis.* Clin Infect Dis, 2009. **49**(7): p. 1044-50.

9. Cai, G., J. Yan, and H. Qiu, *[The standardization of diagnosis and treatment of severe sepsis/septic shock and its practice].* Zhonghua Nei Ke Za Zhi, 2015. **54**(6): p. 484-5.

10. Shock, et al., *[Chinese expert consensus on diagnosis and management of immunosuppression in sepsis].* Zhonghua Wei Zhong Bing Ji Jiu Yi Xue, 2020. **32**(11): p. 1281-1289.

11. Linder, A., et al., *Heparin-Binding Protein Measurement Improves the Prediction of Severe Infection With Organ Dysfunction in the Emergency Department.* Crit Care Med, 2015. **43**(11): p. 2378-86.

12. Linder, A., et al., *Elevated plasma levels of heparin-binding protein in intensive care unit patients with severe sepsis and septic shock.* Crit Care, 2012. **16**(3): p. R90.

13. Chew, M.S., et al., *Increased plasma levels of heparin-binding protein in patients with shock: a prospective, cohort study.* Inflamm Res, 2012. **61**(4): p. 375-9.

14. Llewelyn, M.J., et al., *Sepsis biomarkers in unselected patients on admission to intensive or high-dependency care.* Crit Care, 2013. **17**(2): p. R60.

15. Singer, M., et al., *The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).* JAMA, 2016. **315**(8): p. 801-10.

16. Tang, B.M., et al., *Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis.* Lancet Infect Dis, 2007. **7**(3): p. 210-7.

17. Wu, Y.L., et al., *Accuracy of Heparin-Binding Protein in Diagnosing Sepsis: A Systematic Review and Meta-Analysis.* Crit Care Med, 2021. **49**(1): p. e80-e90.

18. Fisher, J. and A. Linder, *Heparin-binding protein: a key player in the pathophysiology of organ dysfunction in sepsis.* J Intern Med, 2017. **281**(6): p. 562-574.

19. Kjolvmark, C., P. Akesson, and A. Linder, *Elevated urine levels of heparin-binding protein in children with urinary tract infection.* Pediatr Nephrol, 2012. **27**(8): p. 1301-8.

20. Linder, A., et al., *Heparin-binding protein: a diagnostic marker of acute bacterial meningitis.* Crit Care Med, 2011. **39**(4): p. 812-7.

21. Linder, A., O. Soehnlein, and P. Akesson, *Roles of heparin-binding protein in bacterial infections.* J Innate Immun, 2010. **2**(5): p. 431-8.

22. Kahn, F., et al., *Heparin-Binding Protein as a Prognostic Biomarker of Sepsis and Disease Severity at the Emergency Department.* Shock, 2019. **52**(6): p. e135-e145.

23. Liu, P., et al., *Heparin-binding protein as a biomarker of severe sepsis in the pediatric intensive care unit: A multicenter, prospective study.* Clin Chim Acta, 2023. **539**: p. 26-33.

24. Fisher, J., et al., *Is Heparin-Binding Protein Inhibition a Mechanism of Albumin's Efficacy in Human Septic Shock?* Crit Care Med, 2018. **46**(5): p. e364-e374.

25. Samuelsson, L., et al., *Renal clearance of heparin-binding protein and elimination during renal replacement therapy: Studies in ICU patients and healthy volunteers.* PLoS One, 2019. **14**(8): p. e0221813.

26. Honore, P.M., S. Redant, and D. De Bels, *Reliability of biomarkers of sepsis during extracorporeal therapies: the clinician needs to know what is eliminated and what is not.* Crit Care, 2020. **24**(1): p. 553.

27. Xing, L., et al., *Activation of M1 macrophages in sepsis-induced acute kidney injury in response to heparin-binding protein.* PLoS One, 2018. **13**(5): p. e0196423.

28. Fisher, J., et al., *Heparin-Binding Protein (HBP): A Causative Marker and Potential Target for Heparin Treatment of Human Sepsis-Induced Acute Kidney Injury.* Shock, 2017. **48**(3): p. 313-320.

29. Gibot, S., et al., *Combination biomarkers to diagnose sepsis in the critically ill patient.* Am J Respir Crit Care Med, 2012. **186**(1): p. 65-71.

30. Bauer, P.R., et al., *Diagnostic accuracy and clinical relevance of an inflammatory biomarker panel for sepsis in adult critically ill patients.* Diagn Microbiol Infect Dis, 2016. **84**(2): p. 175-80.

Tables

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, median (IQR)	9.0 (7.0–12.0)	12.0 (9.0–16.0)	13.0 (9.00–18.0)	16.5 (12.0–21.0)	<0.001
SOFA score, median (IQR)	2.0 (1.0–5.0)	4.0 (2.3–7.0)	5.0 (3.0–7.0)	10.0 (7.0–13.0)	<0.001
Length of ICU stay, days median (IQR)	2.0 (1.0–4.0)	5.0 (3.0–7.8)	6.0 (3.0–10.0)	8.0 (4.0–13.0)	<0.001
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.

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

Variable	AUC (95% CI)	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
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
WBC	0.541 (0.474–0.607)	21.0	20.1	95.7	77.8	61.7
PCT	0.812 (0.766–0.857)	0.9	85.6	59.9	61.1	84.2
CRP	0.775 (0.724–0.827)	107.7	66.9	77.0	68.4	75.8
LAC	0.632 (0.571–0.694)	1.9	53.2	72.2	58.7	67.5
APACHE II	0.688 (0.630–0.747)	12.5	65.5	63.6	64.3	64.8
SOFA	0.801 (0.755–0.848)	4.5	83.5	62.0	68.7	79.0

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: procalcitonin, SOFA: sequential organ failure assessment, WBC: white blood cell count.

Figure legends

Figure 1. 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: procalcitonin, SOFA: sequential organ failure assessment, WBC: white blood cell count. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Figure 2. A nomogram predicting the risk of spesis for patients. The value of each of variable was given a score on the point scale axis. A total score could be easily calculated by adding each single score and by projecting the total score to the lower total point scale. We were able to estimate the probability of Spesis. CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin, IL-6: procalcitonin, SOFA: sequential organ failure assessment.

Figure 3. ROC curve analysis of the sepsis training model and test model.

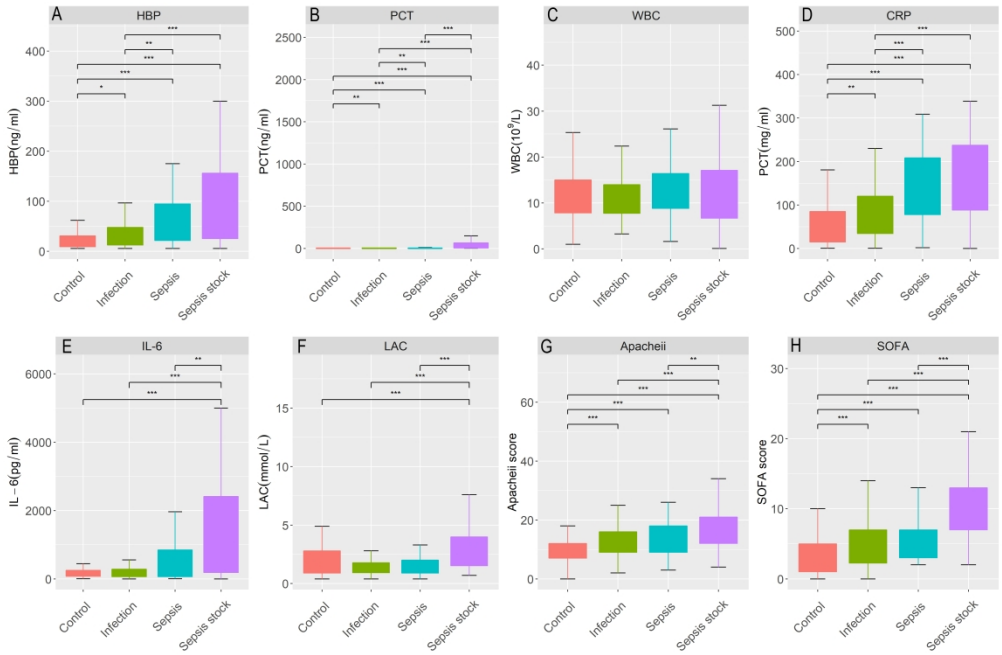

Figure 1. Comparison of plasma levels of biomarkers among different groups. A: HBP, B: PCT, C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: procalcitonin, SOFA: sequential organ failure assessment, WBC: white blood cell count. *: P < 0.05; **: P < 0.01; ***: P < 0.001.

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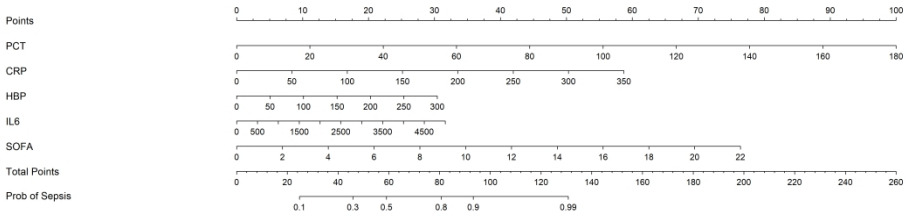

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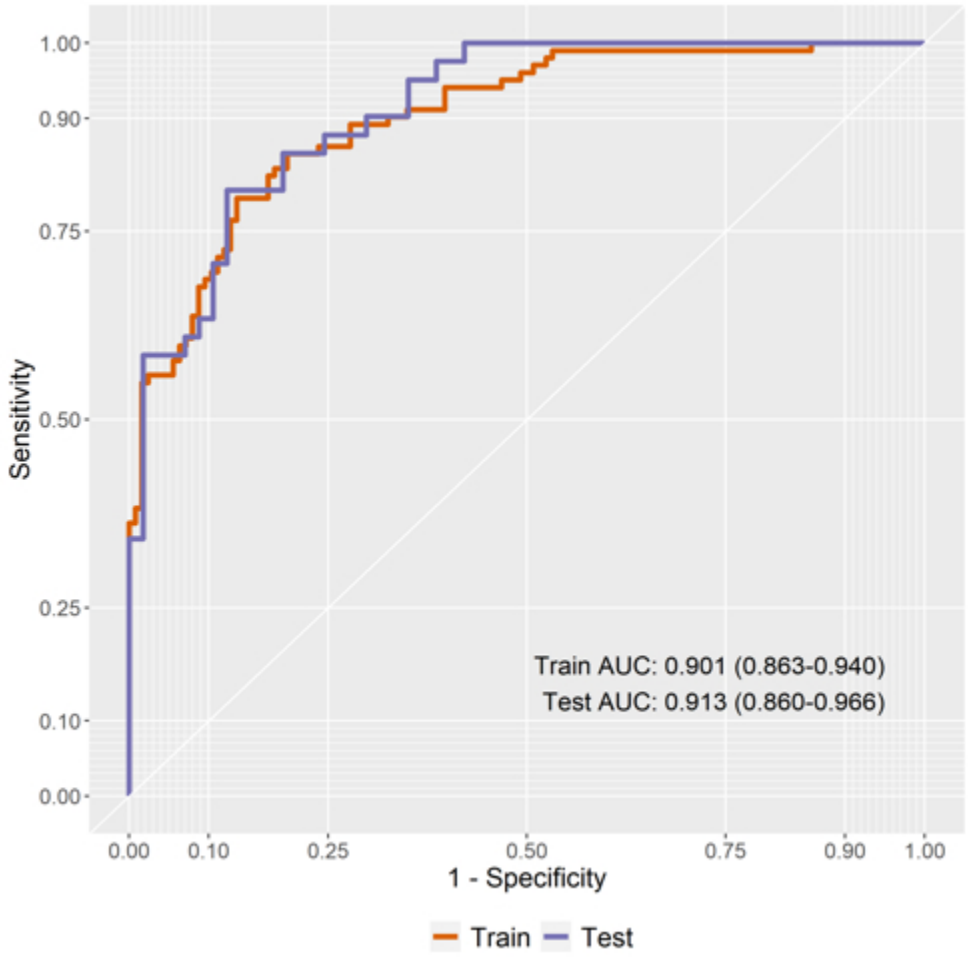


Figure 3. ROC curve analysis of the sepsis training model and test model.

82x82mm (150 x 150 DPI)

Supplementary Data

Supplementary Table 1. The comparison of HBP among different sites.

	Infection	Sepsis	Septic shock	*P*
Abdomen, median (IQR)	24.8 (14.0–74.5)	44.7 (25.9–108.0)	78.0 (38.6–156.3.0)	< 0.001
Respiratory median (IQR)	23.2 (10.8–55.3)	55.2 (37.8–73.9)	55.7 (14.1–300)	< 0.001
Blood median (IQR)	9.5*	80.4 (45.1–115.6)	207.6 (176.6–238.6)	< 0.001
Skin and soft tissues median (IQR)	25.5 (19.1–37.3)	27.3 (14.6–41.4)	61.8 (36.2–136)	0.027
Other median (IQR)	18.3 (14.5–22.5)	45.6 (27.0–64.3)	22.6 (19.5–86.7)	0.007
Multi-infection site median (IQR)	22.7 (20.9–32.8)	37.7 (18.0–110.6)	39.0 (23.7–134.6)	0.333

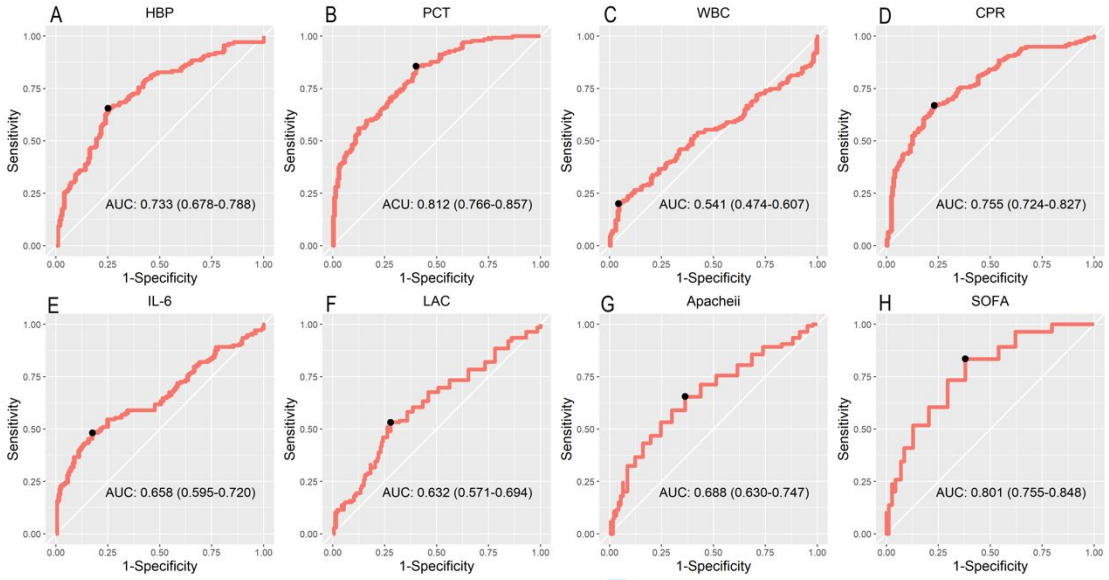
* Only one patient with bloodstream infection in the infection group, IQR: interquartile range.

Supplementary Table 2. The logistic regression model for sepsis diagnosis.

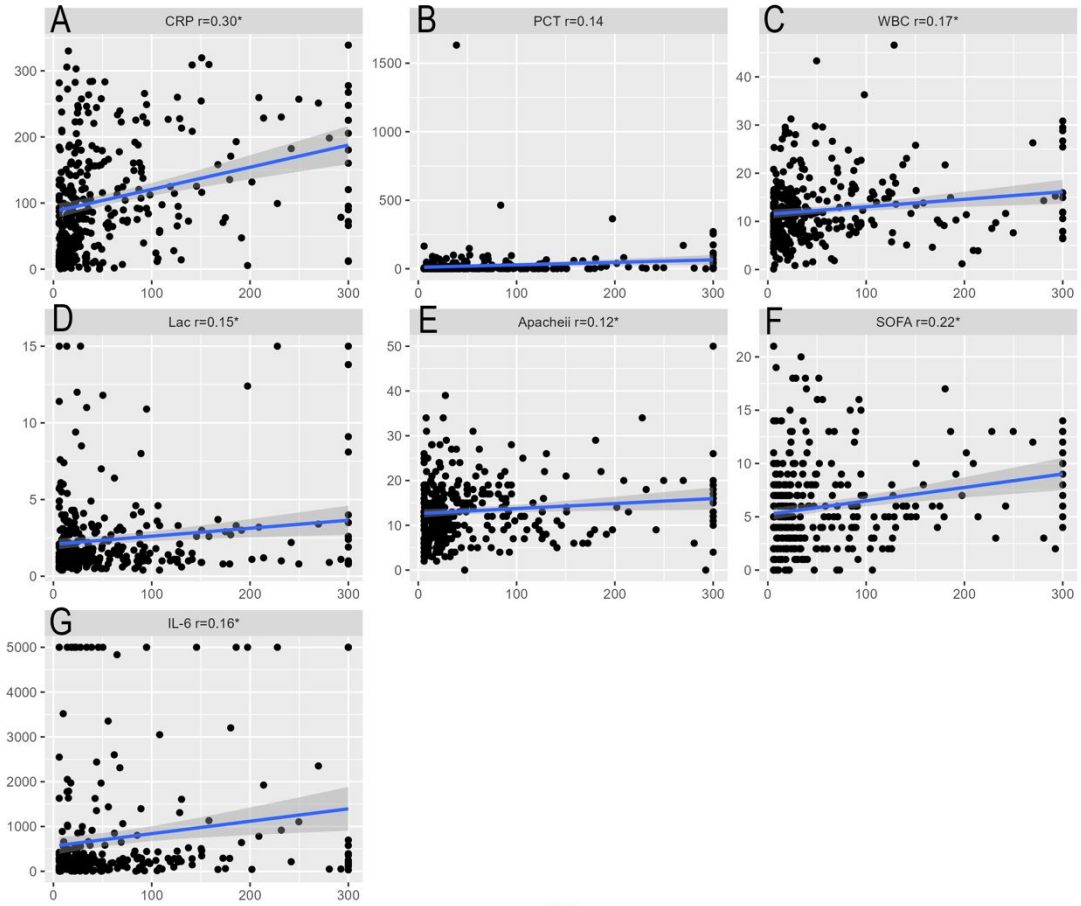
Variable	β	Z	P	OR (95%CI)
Intercept	−3.833	−7.29	<0.001	0.022 (0.008, 0.061)
PCT	0.034	2.63	0.009	1.034 (1.009, 1.060)
CRP	0.011	4.13	<0.001	1.011 (1.006, 1.016)
HBP	0.006	2.04	0.041	1.006 (1.000, 1.012)

IL-6	0.001	2.49	0.013	1.001 (1.000, 1.001)
SOFA	0.225	3.67	<0.001	1.252 (1.110, 1.412)

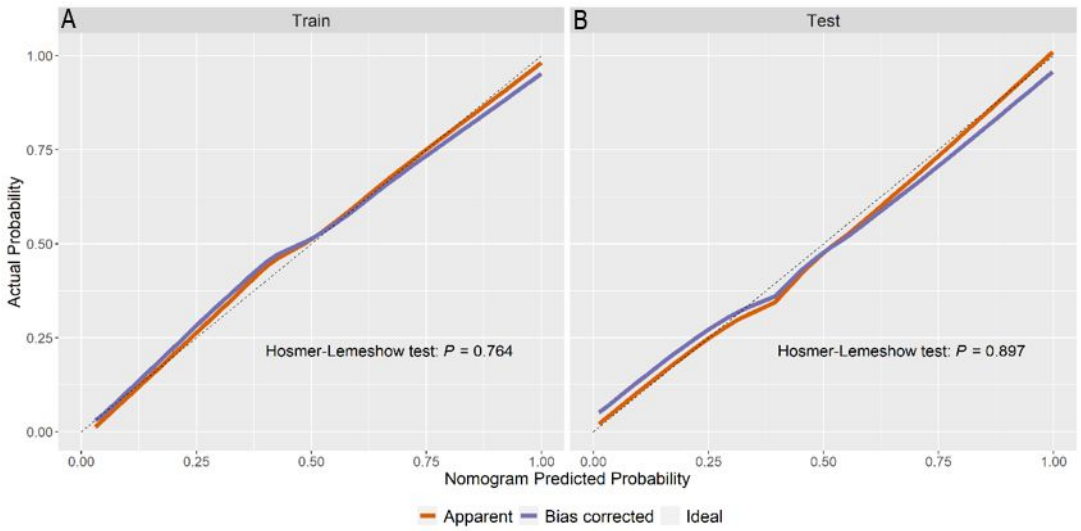
CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin, IL-6: procalcitonin, SOFA: sequential organ failure assessment.



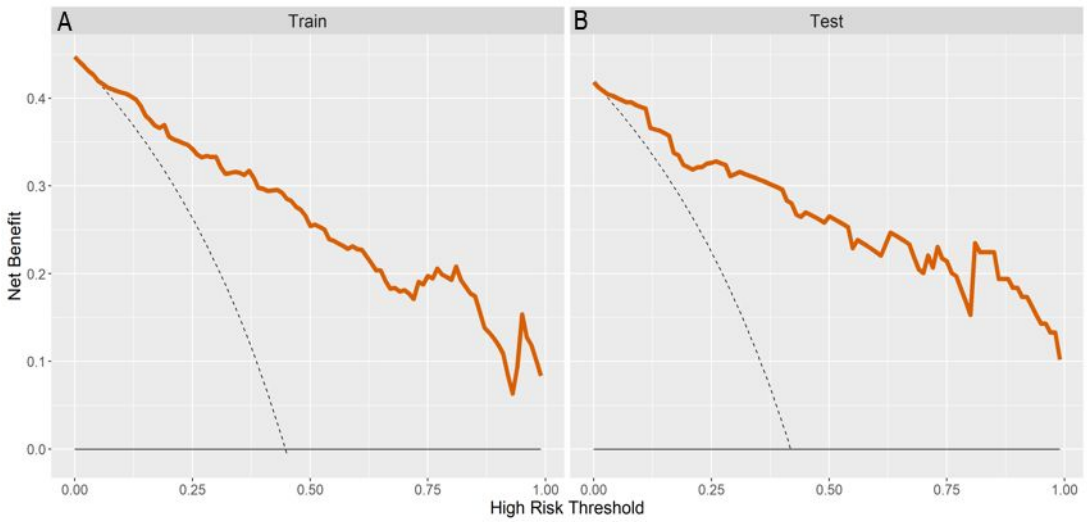
Supplementary Figure 1. ROC curves for biomarkers in distinguishing sepsis from non-sepsis. A: HBP, B: PCT, C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: procalcitonin, SOFA: sequential organ failure assessment, WBC: white blood cell count.



Supplementary Figure 2. The correlations of HBP with CRP (A) , PCT (B), WBC (C), LAC (D), APACHE II (E), SOFA (F), and IL-6(G). APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: procalcitonin, SOFA: sequential organ failure assessment, WBC: white blood cell count.



Supplementary Figure 3. Calibration test of the sepsis diagnostic model. A: training set, B: test set.



Supplementary Figure 4. Decision curve analysis (DCA) curve of the sepsis diagnostic model. A: training set, B: test set.

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Heparin-binding protein as a biomarker for early diagnosis of sepsis in the intensive care unit: a retrospective cross-sectional study in China

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Heparin-binding protein as a biomarker for early diagnosis of sepsis in the intensive care unit: a retrospective cross-sectional study in China

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Abstract

Objectives: This study aims to investigate the diagnostic value of heparin-binding protein (HBP) in sepsis and develop a sepsis diagnostic model incorporating HBP with key biomarkers and disease-related scores for an early, rapid, and accurate diagnosis of sepsis in the intensive care unit (ICU).

Design: Clinical retrospective cross-sectional study.

Setting: A comprehensive teaching tertiary hospital in China.

Participants: Adult patients (age≥18years) who had tested HBP or whose blood samples had been collected when admitted to ICU.

Main outcome measures: HBP, C-reactive protein (CRP), procalcitonin (PCT), white blood cell count (WBC), interleukin-6 (IL-6), lactate (LAC), acute physiology and chronic health evaluation II (APACHE II) and sequential organ failure assessment (SOFA) score were recorded.

Results: From March 2019 and December 2021, 326 patients were enrolled in this study. The patients were categorized into the non-infection group (control group), infection group, sepsis group, and septic shock group based on final diagnosis. The levels of HBP in the sepsis group and septic shock group were 45.7 and 69.0 ng/mL, significantly higher than those in the control group and infection group, 18.0 and 24.0 ng/mL, respectively ($p < 0.001$). The AUC value of HBP for diagnosing sepsis was 0.733, which was lower than those corresponding to PCT, CRP, and SOFA, but higher than those of IL-6, LAC, and APACHE II. Multivariate logistic regression analysis identified HBP, PCT, CRP, IL-6, and SOFA as valuable indicators for diagnosing

sepsis. A sepsis diagnostic model was constructed based on these indicators, whose AUC was 0.901, with a sensitivity of 79.7% and specificity of 86.9%.

Conclusions: HBP could serve as a biomarker for early diagnosis of sepsis in the ICU. Compared with single indicators, the sepsis diagnostic model constructed with HBP, PCT, CRP, IL-6, and SOFA further enhanced the diagnostic performance of sepsis.

Strengths and limitations of this study

- This study included a highly heterogeneous population, making it highly applicable to sepsis patients in ICU.
- Moreover, most of the biomarkers included in this diagnostic model were widely used in clinical practice, making them easily obtainable, highly reproducible, and operationally feasible.
- This study was an ICU single-center retrospective research, the results might not be applicable to sepsis patients in other settings.
- The SOFA scores in the study were absolute values automatically obtained by the electronic scoring system, rather than the delta values.
- Its design did not allow for the determination of causal relationships.

Keywords: HBP, Sepsis, Diagnostic model

Background

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection. Sepsis, when accompanied by severe circulatory impairment and cellular metabolic disorders, is referred to as septic shock, which is the leading cause of death in septic patients. [1] With the aging population and increase in immunocompromised hosts, the incidence of sepsis has been rising recent year. The Global Burden of Sepsis study published in 2020 reported 48.9 million cases of sepsis worldwide in 2017, with 11 million deaths attributed to sepsis, accounting for 19.7% of global deaths. [2] Another domestic study showed that the incidence of sepsis in the intensive care unit (ICU) was 20.6%, with a 90-day mortality rate of 35.5%, and the mortality rate for septic shock was as high as 50% or more. [3] Im et al. demenstrated that the mortality rate of septic shock was correlated with hypotension and delayed use of antibiotics. [4] Another study indicated that early fluid resuscitation was closely related to the prognosis of patients with sepsis. [5] Therefore, early diagnosis of sepsis and timely appropriate treatment are crucial for sepsis management.

Early diagnosis and identification of sepsis require a comprehensive approach based on the patient's clinical symptoms, conventional cultures, biomarkers, and disease-specific scoring systems. However, clinical symptoms and signs of sepsis are often nonspecific, and conventional pathogen culture is relatively delayed. [6] Therefore, early diagnosis of sepsis in the ICU mainly relies on biomarkers and disease-specific scoring systems. Currently, there are over 200 sepsis-related biomarkers reported in the literature, among which heparin-binding protein (HBP) is a novel

biomarker. [7] HBP is a serine protease-like protein secreted by neutrophils after infection and has functions such as altering endothelial cell permeability, antimicrobial activity, chemotaxis, and regulation of cell apoptosis. [8] It has been identified as an early diagnostic indicator for severe sepsis/septic shock in Chinese Guidelines for the Management of Severe Sepsis/Septic Shock (2014) [9] and Chinese Expert Consensus on Early Prevention and Interruption of Sepsis in Emergency Medicine (2020). [10] In addition, an increasing number of studies had furnished evidence regarding the use of HBP for diagnosing sepsis in recent years. The results demonstrated that HBP could be used for sepsis diagnosis and monitoring the severity. [8, 11, 12] On the other hand, a few studies had indicated that elevated levels of HBP irrespective of infectious etiology and no correlation with severity and outcome. [13] Furthermore, differences and inconsistencies have been noted among various studies in regard to the diagnostic performance of HBP of sepsis. [14, 15] Therefore, it remains controversial to use HBP for the early diagnosis of sepsis. The aim of this study was to analysis the early diagnostic value of HBP in sepsis and to develop a sepsis diagnostic model combining HBP with multiple biomarkers and disease-specific scoring systems retrospectively, in order to facilitate early identification and diagnosis of sepsis in the ICU.

Methods

Study population

This study included 2080 patients who admitted to the ICU of the First Affiliated Hospital of Sun Yat-sen University, China, from March 2019 to December 2021. The

strict inclusion and exclusion criteria were adopted for all patients, with the inclusion criteria being: (1) patients who had undergone HBP detection or whose blood samples had been collected for HBP detection at the time of ICU admission, (2) the clinical data were integrity, and (3) aged 18 years or older. The exclusion criteria were: (1) patients with neutropenia due to hematological malignancies, and (2) patients who underwent immunosuppressive therapy. Patients were categorized into four groups, namely, the infection group, sepsis group, septic shock group, and control group, based on the final diagnosis at the time of discharge from ICU or death, determined by the attending physician. Figure 1 showed the flow diagram of the participants. The protocols were approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University and conducted in accordance with the Declaration of Helsinki.

Measurement of plasma HBP and clinical data collection

The blood samples collected previously were sent to the central laboratory for the detection of plasma HBP levels. In briefly, the blood samples were centrifuged at 1,000 rounds/min for 10 min, and 100 μL of supernatants were collected for plasma level of HBP determination using an immunofluorescence dry quantitative method (Jet-iStar3000, Hangzhou, Joinstar Biomedical Technology Co,.LTD). The procedure strictly followed the instructions provided with the reagent kit, and the quality control was performed well.

General informations such as gender, age, underlying diseases, site of infection, and pathogens were collected. Laboratory tests such as HBP, procalcitonin (PCT),

white blood cell count (WBC), C-reactive protein (CRP), interleukin-6 (IL-6), and blood lactate (LAC) were measured at the time of ICU admission. Acute Physiology and Chronic Health Evaluation II (APACHE II) score and Sequential Organ Failure Assessment (SOFA) score were calculated within 24 h of ICU admission. The length of ICU and survival outcomes (3-day improvement rate, 28-day mortality rate) were also recorded for each group of patients.

Statistical Methods

For baseline measurement data, median and interquartile range (IQR) were used to describe the data. If continuous variables followed a normal distribution, one-way ANOVA was used for intergroup comparisons; otherwise, the Kruskal–Wallis H test was used. Percentage calculations were performed for categorical data, and differences between groups were tested using the chi-square test or Fisher's exact test.

Receiver operating characteristic (ROC) curves were used to assess the diagnostic performance of HBP, PCT, WBC, CRP, IL-6, LAC, APACHE II score, and SOFA score for sepsis. The area under the ROC curve (AUC) was also estimated. The optimal cut-off values for diagnosing sepsis were determined based on the maximum Youden index, and corresponding sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

To improve the diagnostic performance of sepsis, a multivariate binary logistic regression model was constructed. Random selection of 70% of all patients was used as the training set, while the remaining 30% served as the test set to assess the model's

performance. AUC was calculated for both the training and test sets. The Hosmer–Lemeshow goodness-of-fit test and calibration curve were used to evaluate the model's goodness-of-fit for both datasets. Decision curves were also plotted to evaluate the clinical utility of the regression model. All hypothesis tests were two-tailed, and a significance level of $P < 0.050$ was set. Statistical analysis was performed using R 4.1.1 and SPSS 25.0.

Patient and public involvement

This was a retrospective study. No Patients or public representatives were involved in setting the research question, nor in the design, conduct, or interpretation of the study.

Results

Characteristics of the patients

A total of 326 patients were enrolled at last, including 93 in the control group, 94 in the infection group, 53 in the sepsis group, and 86 in the septic shock group (Figure 1). Table 1 summerized the baseline characteristics of the patients. The median ages of patients in the control group, infection group, sepsis group, and septic shock group were 56, 63, 58, and 64 years, respectively, with statistically significant differences among the groups ($p = 0.023$). No significant differences were noted among the groups in terms of gender, prevalence of hypertension, diabetes, heart disease, malignancy, liver disease, and other comorbidities.

The control group consisted of postoperative recovery patients from various

surgical procedures, including gastrointestinal, hepatic, vascular, among others. The infection patients (including the infection group, sepsis group, and septic shock group) predominantly presented with pulmonary infections (48.9%, 32.1%, and 26.7%, respectively) and abdominal infections (33.0%, 56.6%, and 73.3%, respectively). Among all enrolled patients, 32 had positive blood cultures, 76 had positive peritoneal drainage fluid cultures, and 90 had positive sputum cultures. All sepsis patients (including the sepsis group and septic shock group) mainly suffered from bacterial infections and received antibiotic treatment. The APACHE II and SOFA scores of the sepsis and septic shock groups were significantly higher than the control and infection groups, with statistically significant difference among the four groups ($p < 0.001$). In the prognosis analysis, the 28-day mortality rates for the sepsis group and septic shock group were 11.32% and 32.56%, respectively significantly higher than those for the control and infection groups (3.2% and 9.6%) (Table 1).

Levels of HBP and other biomarkers in each group of patients

The median (IQR) levels of HBP in the control, infection, sepsis, and septic shock groups were 18.0 (9.9–32.1), 24.0 (14.1–56.4), 45.7 (24.8–107.9), and 69.0 (33.8–150.9) ng/mL, respectively ($p < 0.001$). HBP was capable of effectively distinguishing between patients with and without infection or sepsis, and its efficacy was superior to IL-6, LAC, and WBC. However, in distinguishing septic patients with or without shock, HBP was inferior to PCT, IL-6, and LAC. Additionally, there were no statistical differences were noted in WBC levels among the groups (Figure 2).

When comparing HBP levels among different infection sites in the infection, sepsis, and septic shock groups, statistical differences were observed among the subgroups except for multi-infection site (Supplementary Table 1). As the severity of infection increased, APACHE II and SOFA scores gradually increased, showing statistical differences. However, no statistical difference was observed when comparing the infection group with the sepsis group (Figure 2).

Analysis of the diagnostic accuracy of different biomarkers for sepsis

HBP demonstrated promising diagnostic performance for early detection of sepsis, with an AUC of 0.733 (95% CI 0.678–0.789), which was significantly higher than WBC (AUC 0.541, 95% CI 0.474–0.607) and higher than the AUCs of IL-6, LAC, and APACHE II scores (0.658, 0.632, and 0.688, respectively) but not statistical significantly. The AUC of HBP was significantly lower than PCT (AUC 0.812, 95%CI 0.766–0.857). When the HBP cut-off value was set at 35.2 ng/mL, the sensitivity, specificity, PPV and NPV for diagnosing sepsis were 65.5%, 74.9%, 65.9% and 74.5%, respectively (Table 2, Supplementary Figure 1).

Relationship between HBP and other biomarkers

No significant correlation was observed between HBP levels and CRP, PCT, WBC, IL-6, LAC, APACHE II scores, and SOFA scores (Supplementary Figure 2).

Construction of a sepsis diagnostic model

Based on the training set, variables were selected through univariate logistic regression analysis for patient demographics (such as gender, age, underlying diseases, infection sites, and pathogens), infection biomarkers (HBP, PCT, WBC, CRP, IL-6, and LAC), APACHE II scores, and SOFA scores. Variables with statistical significance ($p<0.05$) were included in the multivariate logistic regression model (Supplementary Table 2). Among the statistically significant variables in the univariate analysis were HBP、PCT、CRP、IL-6、LAC、APACHE II、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.

Validation of the sepsis diagnostic model

To evaluate the predictive performance of the model, the remaining 30% of patients were used as a test set to validate the model. In the training set, the model achieved an AUC of 0.901 (95% CI 0.863−0.940). When the Youden index was maximized, the cut-off value was determined to be 0.439, resulting in a sensitivity of 79.4% and a specificity of 86.5%. In the test set population, the model obtained an AUC of 0.913 (95% CI 0.860−0.966). Applying the cut-off value obtained from the training set to the test set, the sensitivity and specificity were 80.5% and 87.7%, respectively

(Supplementary Figure 3). Furthermore, to obtain a more accurate cut-off value, all patients were included in the diagnostic model, resulting in a cut-off value of 0.439. The sensitivity and specificity for diagnosing sepsis with this cut-off value were 79.7% and 86.9%, respectively.

The diagnostic model constructed using the training set exhibited a good predictive performance based on the Hosmer–Lemeshow goodness-of-fit test in both the training and test sets ($\chi^2 = 4.91$, $p = 0.767$; $\chi^2 = 5.12$, $p = 0.745$; Supplementary Figure 4) Additionally, the decision curve analysis (DCA) plot demonstrated a high clinical net benefit for the constructed sepsis diagnostic model that surpasses both Treat-all and Treat-no (Supplementary Figure 5).

Discussion

Sepsis is a major cause of mortality in critically ill patients, with high morbidity and mortality. Approximately 20%–30% of severely infected patients do not exhibit typical symptoms of organ dysfunction upon admission but rapidly progress to sepsis. [6] Therefore, early identification of sepsis is crucial for developing appropriate and effective treatment strategies and reducing mortality. Clinicians require more specific and sensitive biomarkers to identify the early diagnosis of sepsis. Currently, WBC, CRP, and PCT are proposed commonly in clinical practice as inflammatory biomarkers. [7] However, WBC and CRP are nonspecific markers of systemic inflammation and cannot effectively differentiate among bacterial, non-bacterial, and sterile inflammation. PCT has a higher specificity for bacterial infections but performs poorly in predicting sepsis-

associated organ dysfunction. [6, 16] In recent years, numerous studies have proven that HBP has good predictive performance for infection, sepsis, or organ function assessment, superior to PCT, CRP, and other biomarkers. [6, 8, 11, 12, 17, 18]

HBP, also known as heparin-binding protein or CAP37, is a protein molecule stored in the secretory granules of neutrophils and azurophilic granules. It contains a large number of positively charged amino acid residues, which are concentrated on one side of the protein. [18] A hydrophobic pocket structure formed by amino acid residues 20–44 exhibits a high affinity for endotoxins. [6] Therefore, HBP is initially discovered for its antimicrobial activity. Subsequent researches confirmed that HBP is a multifunctional innate immune defense molecule that played a crucial role in the host's infection and inflammatory response. [6, 18] These characteristics make HBP a promising novel infection biomarker. Recent studies have reported that HBP could assist in the diagnosis of various diseases, such as respiratory and circulatory failure, sepsis, acute kidney injury, acute lung injury, meningitis, urinary tract infections, as well as skin and soft tissue infections. [6, 8, 11, 19, 20] However, its clinical use has not yet been widely adopted, so further clinical research is required to validate its utility.

This study further confirmed that HBP was a promising biomarker in sepsis. In this study, HBP levels could effectively differentiate whether patients had an infection and whether infected patients had sepsis. Furthermore, its discriminative value was found to be superior to LAC, IL-6, WBC, SOFA, and APACHE II scores. Similar findings had been reported in previous studies. [7, 11] These results were likely related to the biological characteristics of HBP. It was stored in neutrophil secretory granules

and azurophilic granules, and upon stimulation by pathogens, it could be rapidly and massively released into the bloodstream, inducing rearrangement of the endothelial cell cytoskeleton, leading to vascular leakage and edema formation. Additionally, HBP regulated the function of monocytes and macrophages, further amplifying the inflammatory response and enhancing the body's immune response to infection. Moreover, as neutrophils infiltrated into the tissues, HBP continued to be released, resulting in tissue damage and organ dysfunction. [18, 21] Therefore, HBP levels were significantly elevated in patients with infection and/or sepsis.

Regarding the diagnostic performance of HBP in sepsis, a study by Linder et al. found that the AUC of HBP for predicting sepsis was 0.85, with a sensitivity of 87% and specificity of 95%, which were significantly higher than those of PCT, CRP, WBC, IL-6, and other biomarkers. [8] Furthermore, HBP had the ability to predict the occurrence of organ dysfunction and circulatory failure at an early stage, providing indications for timely interventions such as fluid resuscitation and antibiotic use, which were indispensable components of sepsis bundle therapy. [8, 11, 22] In addition, the favorable predictive value of HBP was validated in pediatric patients with severe sepsis. [23] The emergence of this phenomenon was considered to be related to the pathological process in which HBP was involved in vascular leakage and organ dysfunction in septic patients, and its release occurred earlier than CRP, PCT, and other markers. [17, 18, 21] In this study, the AUC of HBP in predicting sepsis was 0.733, which was not superior to PCT, CRP, and SOFA. Previous studies reported varying diagnostic accuracy of HBP for sepsis at different time points. [17] In this study, their

disease course was relatively later; although the detection of HBP or the collection of blood samples occurred upon admission to the ICU, the onset time was still later than emergency cases. Meta-analyses also revealed that HBP often performed better in diagnosing sepsis in emergency department patients compared with ICU patients. [13, 14, 17] Unlike previous studies, this research involved ICU patients rather than emergency patients. First, the control group in this study consisted not only of healthy individuals but mostly of surgical postoperative recovery patients. Additionally, ICU patients had more complex conditions, more severe organ damage, and require life support such as ventilators, vasopressors, continuous renal replacement therapy (CRRT), etc. Finally, patients had already received various treatments such as fluid resuscitation and antibiotics in the emergency room or ward. [24-28] In summary, these conditions might have some impact on HBP levels, but this study population was more representative of the actual situations of ICU patients. From another perspective, this phenomenon also reflected the limitations of a single biomarker, as it could not fully reflect the clinical reality and accurately diagnose sepsis in the ICU.

The pathophysiological mechanisms of sepsis are complex. They involve different immune states, sites of infection, and pathogens. The immune response patterns vary, and so do the pathophysiological processes of various biomarkers. During its occurrence and progression, there are always dual factors that simultaneously lead to an exaggerated inflammatory response and immune dysfunction. Systemic inflammatory response and immune suppression do not generally exist as simple independent entities but rather co-exist. Therefore, a single biomarker cannot serve as

a reliable diagnostic indicator for sepsis. [7, 10] In this study, we also observed that HBP showed almost no correlation with PCT, CRP, IL-6, LAC, APACHE II, and SOFA scores. This suggested that HBP, as a biomarker, could provide unique information for the diagnosis of sepsis that was independent of other biomarkers. We hypothesized that establishing a diagnostic model combining HBP with PCT, CRP, IL-6, LAC, APACHE II, SOFA scores, and other indicators could become a new approach for early diagnosis of sepsis. Currently, relevant studies had been conducted in this regard, [29, 30] but many of the biomarkers mentioned in the above studies have not been widely used in clinical practice, making them less practical. In this study, commonly used biomarkers in clinical settings were included. Based on the ROC analysis of various markers, a sepsis diagnostic model using multivariable logistic regression was constructed. Upon test, the sepsis diagnostic model exhibited an AUC above 0.90, indicating its high clinical applicability.

Conclusion

This study confirmed the value of plasma HBP in the early diagnosis of sepsis in the ICU. It also constructed a sepsis early diagnostic model that includes HBP, PCT, CRP, IL-6, and SOFA scores. This model demonstrated high accuracy and clinical utility, further enhancing the early predictive role in sepsis. It had potential clinical diagnostic value in the early detection of sepsis.

Notes

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Author contributions. Study concept and design: Yongjun Liu, and Lingyun Zuo. Definition of the diagnostic algorithm: Yongjun Liu, Jianfeng Wu and Xiangdong Guan. Acquisition and analysis of data: Lingyun Zuo, Xiaoyun Li, Zihuai Liao, and Si Zhou. Interpretation of data: Luhao Wang and Hao Yuan. Drafting of manuscript: Lingyun Zuo, Xiaoyun Li, Luhao Wang, Hao Yuan and Yongjun Liu. Revision of manuscript: all authors.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Patient and public involvement. Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Ethics approval. This was a retrospective study that did not create any additional risks. Therefore, we did not obtain informed consent from the participants. Regarding the collection of blood samples for HBP testing during the holiday, the participants in our study had previously provided informed consent for the collection of biological samples.

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References

1. Evans, L., et al., *Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021.* Crit Care Med, 2021. **49**(11): p. e1063-e1143.
2. Rudd, K.E., et al., *Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study.* Lancet, 2020. **395**(10219): p. 200-211.
3. Xie, J., et al., *The Epidemiology of Sepsis in Chinese ICUs: A National Cross-Sectional Survey.* Crit Care Med, 2020. **48**(3): p. e209-e218.
4. Im, Y., et al., *Time-to-antibiotics and clinical outcomes in patients with sepsis and septic shock: a prospective nationwide multicenter cohort study.* Crit Care, 2022. **26**(1): p. 19.
5. Kuttab, H.I., et al., *Evaluation and Predictors of Fluid Resuscitation in Patients With Severe Sepsis and Septic Shock.* Crit Care Med, 2019. 47(11): p. 1582-1590.
6. Yang, Y., et al., *A Promising Candidate: Heparin-Binding Protein Steps onto the Stage of Sepsis Prediction.* J Immunol Res, 2019. **2019**: p. 7515346.
7. Pierrakos, C., et al., *Biomarkers of sepsis: time for a reappraisal.* Crit Care, 2020. **24**(1): p. 287.
8. Linder, A., et al., *Heparin-binding protein: an early marker of circulatory failure in sepsis.* Clin Infect Dis, 2009. **49**(7): p. 1044-50.
9. Cai, G., J. Yan, and H. Qiu, *[The standardization of diagnosis and treatment of severe sepsis/septic shock and its practice].* Zhonghua Nei Ke Za Zhi, 2015. **54**(6): p. 484-5.

10. Shock, et al., *[Chinese expert consensus on diagnosis and management of immunosuppression in sepsis]*. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue, 2020. **32**(11): p. 1281-1289.

11. Linder, A., et al., *Heparin-Binding Protein Measurement Improves the Prediction of Severe Infection With Organ Dysfunction in the Emergency Department.* Crit Care Med, 2015. **43**(11): p. 2378-86.

12. Zhou, Y., et al., *Usefulness of the heparin-binding protein level to diagnose sepsis and septic shock according to Sepsis-3 compared with procalcitonin and C reactive protein: a prospective cohort study in China.* BMJ Open, 2019. **9**(4): p. e026527.

13. Chew, M.S., et al., *Increased plasma levels of heparin-binding protein in patients with shock: a prospective, cohort study.* Inflamm Res, 2012. **61**(4): p. 375-9.

14. Llewelyn, M.J., et al., *Sepsis biomarkers in unselected patients on admission to intensive or high-dependency care.* Crit Care, 2013. **17**(2): p. R60.

15. Katsaros, K., et al., *Heparin Binding Protein for the Early Diagnosis and Prognosis of Sepsis in the Emergency Department: The Prompt Multicenter Study.* Shock, 2022. **57**(4): p. 518-525.

16. Jekarl, D.W., et al., *Procalcitonin as a prognostic marker for sepsis based on SEPSIS-3.* J Clin Lab Anal, 2019. **33**(9): p. e22996.

17. Wu, Y.L., et al., *Accuracy of Heparin-Binding Protein in Diagnosing Sepsis: A Systematic Review and Meta-Analysis.* Crit Care Med, 2021. **49**(1): p. e80-e90.

18. Fisher, J. and A. Linder, *Heparin-binding protein: a key player in the pathophysiology of organ dysfunction in sepsis.* J Intern Med, 2017. **281**(6): p. 562-574.

19. Kjolvmark, C., et al., *Heparin-binding protein: a diagnostic biomarker of urinary tract infection in adults.* Open Forum Infect Dis, 2014. **1**(1): p. ofu004.

20. Linder, A., et al., *Heparin-binding protein: a diagnostic marker of acute bacterial meningitis.* Crit Care Med, 2011. **39**(4): p. 812-7.

21. Linder, A., O. Soehnlein, and P. Akesson, *Roles of heparin-binding protein in bacterial infections.* J Innate Immun, 2010. **2**(5): p. 431-8.

22. Kahn, F., et al., *Heparin-Binding Protein as a Prognostic Biomarker of Sepsis and Disease Severity at the Emergency Department.* Shock, 2019. **52**(6): p. e135-e145.

23. Liu, P., et al., *Heparin-binding protein as a biomarker of severe sepsis in the pediatric intensive care unit: A multicenter, prospective study.* Clin Chim Acta, 2023. **539**: p. 26-33.

24. Fisher, J., et al., *Is Heparin-Binding Protein Inhibition a Mechanism of Albumin's Efficacy in Human Septic Shock?* Crit Care Med, 2018. **46**(5): p. e364-e374.

25. Samuelsson, L., et al., *Renal clearance of heparin-binding protein and elimination during renal replacement therapy: Studies in ICU patients and healthy volunteers.* PLoS One, 2019. **14**(8): p. e0221813.

26. Honore, P.M., S. Redant, and D. De Bels, *Reliability of biomarkers of sepsis during extracorporeal therapies: the clinician needs to know what is eliminated and what is not.* Crit Care, 2020. **24**(1): p. 553.

27. Xing, L., et al., *Activation of M1 macrophages in sepsis-induced acute kidney injury in response to heparin-binding protein.* PLoS One, 2018. **13**(5): p. e0196423.

28. Fisher, J., et al., *Heparin-Binding Protein (HBP): A Causative Marker and Potential Target for Heparin Treatment of Human Sepsis-Induced Acute Kidney Injury.* Shock, 2017. **48**(3):

p. 313-320.

29. Gibot, S., et al., *Combination biomarkers to diagnose sepsis in the critically ill patient.* Am J Respir Crit Care Med, 2012. **186**(1): p. 65-71.

30. Bauer, P.R., et al., *Diagnostic accuracy and clinical relevance of an inflammatory biomarker panel for sepsis in adult critically ill patients.* Diagn Microbiol Infect Dis, 2016. **84**(2): p. 175-80.

463 **Tables**

464 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, median (IQR)	9.0 (7.0–12.0)	12.0 (9.0–16.0)	13.0 (9.00–18.0)	16.5 (12.0–21.0)	<0.001
SOFA score*, median (IQR)	2.0 (1.0–5.0)	4.0 (2.3–7.0)	5.0 (3.0–7.0)	10.0 (7.0–13.0)	<0.001

Length of ICU stay, days median (IQR)	2.0 (1.0–4.0)	5.0 (3.0–7.8)	6.0 (3.0–10.0)	8.0 (4.0–13.0)	<0.001
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.

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

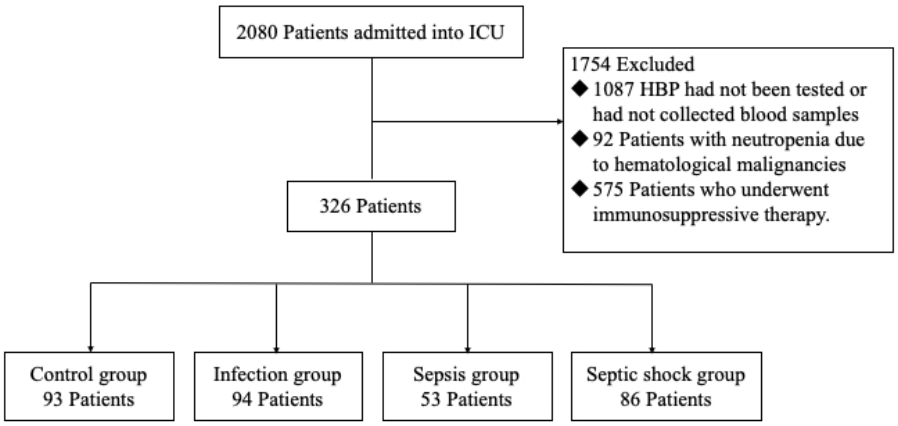
APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count. The *P* values between AUCs compared to HBP.

Figure legends

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

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. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Figure 3. A nomogram predicting the risk of sepsis for patients. The value of each of variable was given a score on the point scale axis. A total score could be easily calculated by adding each single score and by projecting the total score to the lower total point scale. We were able to estimate the probability of sepsis. CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment.



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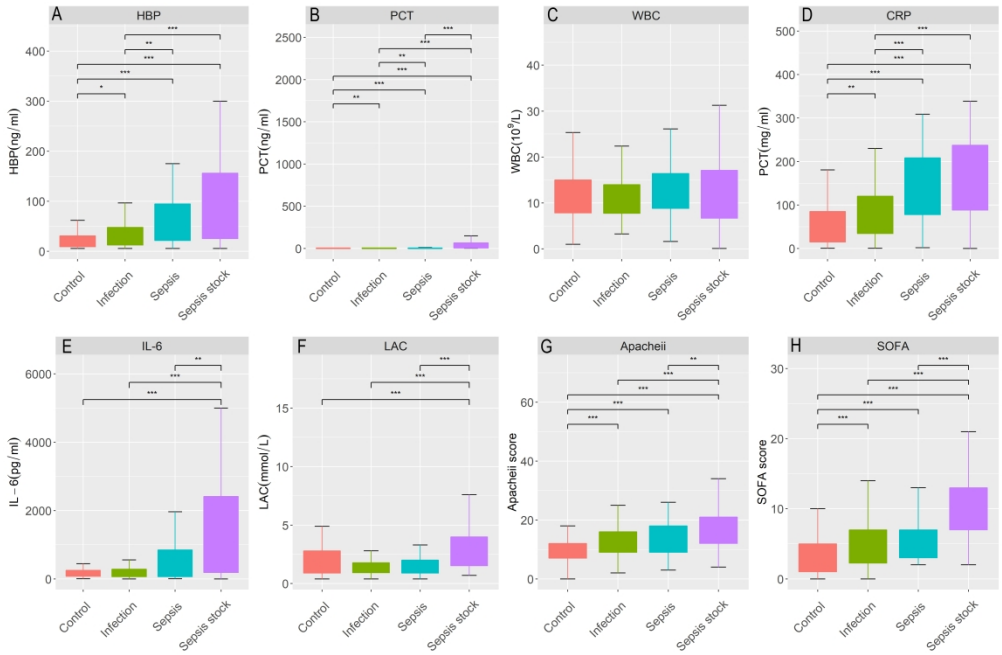

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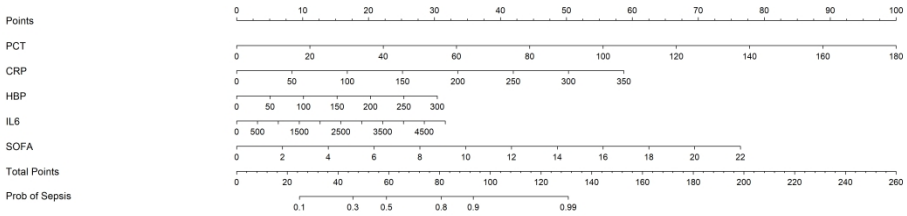

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Supplementary Data

Supplementary Table 1. The comparison of HBP among different sites.

	Infection	Sepsis	Septic shock	*P*
Abdomen, median (IQR)	24.8 (14.0–74.5)	44.7 (25.9–108.0)	78.0 (38.6–156.3.0)	＜0.001
Respiratory median (IQR)	23.2 (10.8–55.3)	55.2 (37.8–73.9)	55.7 (14.1–300)	＜0.001
Blood median (IQR)	9.5*	80.4 (45.1–115.6)	207.6 (176.6–238.6)	＜0.001
Skin and soft tissues median (IQR)	25.5 (19.1–37.3)	27.3 (14.6–41.4)	61.8 (36.2–136)	0.027
Other median (IQR)	18.3 (14.5–22.5)	45.6 (27.0–64.3)	22.6 (19.5–86.7)	0.007
Multi-infection site median (IQR)	22.7 (20.9–32.8)	37.7 (18.0–110.6)	39.0 (23.7–134.6)	0.333

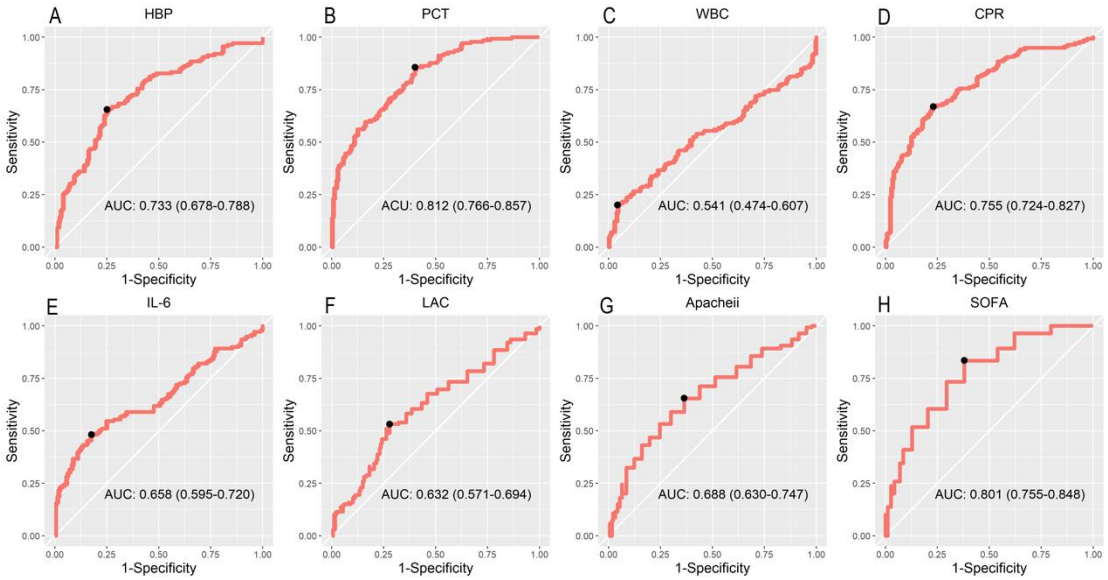
* Only one patient with bloodstream infection in the infection group, IQR: interquartile range.

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

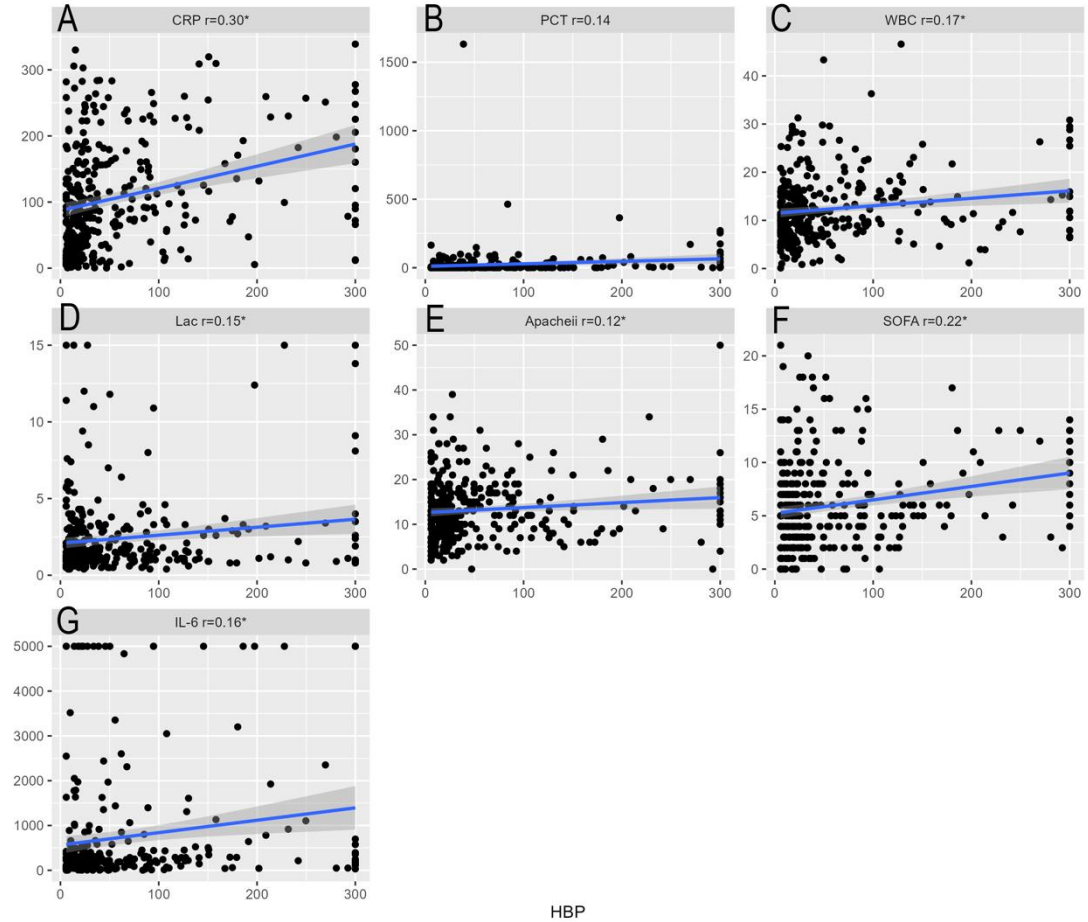
Variable	Univariate logistic regression analysis		Multivariate logistic regression analysis	
	OR (95%CI)	*P*	OR (95%CI)	*P*
Age	1.009 (0.993, 1.026)	0.276		
Sex	1.169 (0.683, 1.999)	0.569		
Hypertension	0.795 (0.450, 1.402)	0.427		
Diabetes	0.801 (0.418, 1.538)	0.505		
Cardiovascular	0.538 (0.288, 1.182)	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

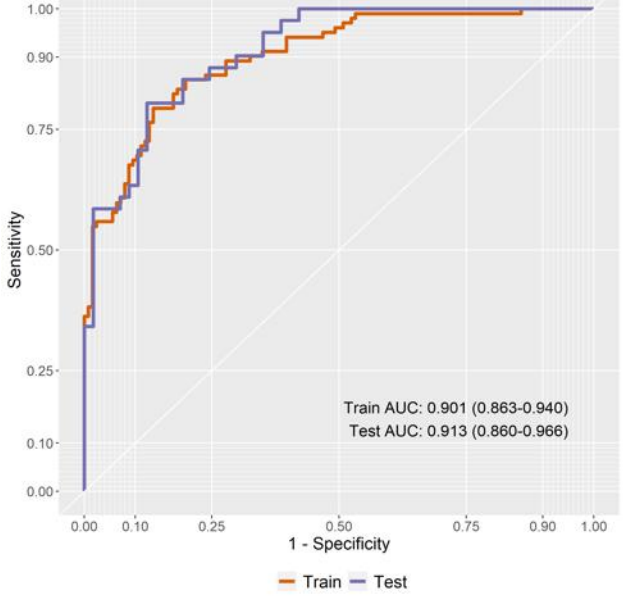
APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count.



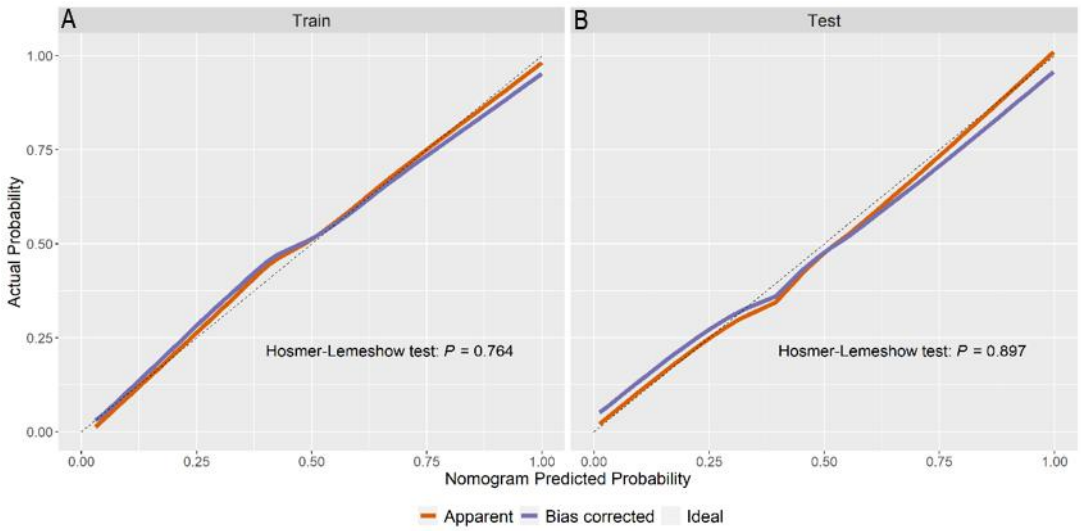
Supplementary Figure 1. ROC curves for biomarkers in distinguishing sepsis from non-sepsis. A: HBP, B: PCT, C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count.



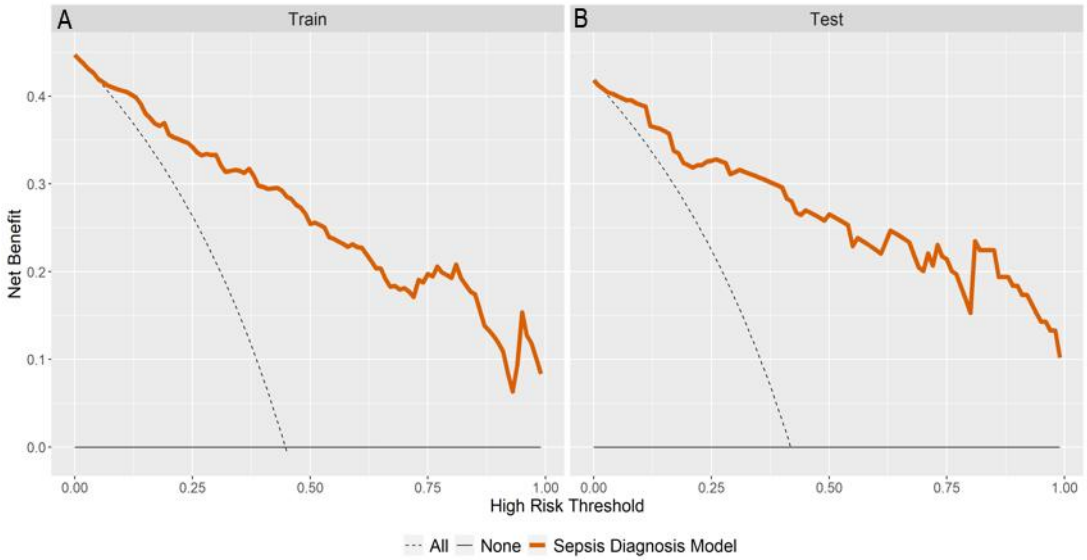
Supplementary Figure 2. The correlations of HBP with CRP (A), PCT (B), WBC (C), LAC (D), APACHE II (E), SOFA (F), and IL-6(G). APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count.



Supplementary Figure 3. ROC curve analysis of the sepsis training model and test model.



Supplementary Figure 4. Calibration test of the sepsis diagnostic model. A: training set, B: test set.



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.

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Heparin-binding protein as a biomarker for early diagnosis of sepsis in the intensive care unit: a retrospective cross-sectional study in China

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Abstract

Objectives: This study aims to investigate the diagnostic value of heparin-binding protein (HBP) in sepsis and develop a sepsis diagnostic model incorporating HBP with key biomarkers and disease-related scores for early, rapid, and accurate diagnosis of sepsis in the intensive care unit (ICU).

Design: Clinical retrospective cross-sectional study.

Setting: A comprehensive teaching tertiary hospital in China.

Participants: Adult patients (age ≥18years) who underwent HBP testing or whose blood samples were collected when admitted to the ICU.

Main outcome measures: HBP, C-reactive protein (CRP), procalcitonin (PCT), white blood cell count (WBC), interleukin-6 (IL-6), lactate (LAC), acute physiology and chronic health evaluation II (APACHE II), and sequential organ failure assessment (SOFA) score were recorded.

Results: Between March 2019 and December 2021, 326 patients were enrolled in this study. The patients were categorized into a non-infection group (control group), infection group, sepsis group, and septic shock group based on the final diagnosis. The HBP levels in the sepsis group and septic shock group were 45.7 and 69.0 ng/mL, respectively, which were significantly higher than those in the control group (18.0 ng/mL) and infection group (24.0 ng/mL) ($p < 0.001$). The AUC value of HBP for diagnosing sepsis was 0.733, which was lower than those corresponding to PCT, CRP, and SOFA but higher than those of IL-6, LAC, and APACHE II. Multivariate logistic regression analysis identified HBP, PCT, CRP, IL-6, and SOFA as valuable indicators

for diagnosing sepsis. A sepsis diagnostic model was constructed based on these indicators, with an AUC of 0.901, a sensitivity of 79.7%, and a specificity of 86.9%.

Conclusions: HBP could serve as a biomarker for the early diagnosis of sepsis in the ICU. Compared with single indicators, the sepsis diagnostic model constructed using HBP, PCT, CRP, IL-6, and SOFA further enhanced the diagnostic performance of sepsis.

Strengths and limitations of this study

- This study included a highly heterogeneous population, making it highly applicable to patients with sepsis in the ICU.
- Moreover, most of the biomarkers included in this diagnostic model are widely used in clinical practice, making them easily obtainable, highly reproducible, and operationally feasible.
- This was an ICU single-center retrospective study, and the results might be inapplicable to sepsis patients in other settings.
- The SOFA scores in the study were absolute values automatically obtained by the electronic scoring system rather than the delta values.
- Its design dose not allow for the determination of causal relationships.

Keywords: HBP, Sepsis, Diagnostic model

Background

Sepsis is a life-threatening organ dysfunction caused by dysregulated host response to infection. Sepsis, when accompanied by severe circulatory impairment and cellular metabolic disorders, is referred to as septic shock and is the leading cause of death in patients with sepsis. [1] With the aging population and increase in immunocompromised hosts, the incidence of sepsis has recently been rising. The Global Burden of Sepsis study published in 2020 reported 48.9 million cases of sepsis worldwide in 2017, with 11 million deaths attributed to sepsis, accounting for 19.7% of the global deaths. [2] Another domestic study showed that the incidence of sepsis in the intensive care unit (ICU) was 20.6%, with a 90-day mortality rate of 35.5%, and the mortality rate for septic shock was as high as 50% or more. [3] Im et al. demonstrated that the mortality rate of septic shock is correlated with hypotension and the delayed use of antibiotics. [4] Another study indicated that early fluid resuscitation is closely linked to the prognosis of patients with sepsis. [5] Therefore, early diagnosis and timely and appropriate treatment are crucial for sepsis management.

Early diagnosis and identification of sepsis require a comprehensive approach based on the patient's clinical symptoms, conventional cultures, biomarkers, and disease-specific scoring systems. However, the clinical symptoms and signs of sepsis are often nonspecific, and conventional pathogen cultures are relatively delayed. [6] Therefore, the early diagnosis of sepsis in the ICU mainly relies on biomarkers and disease-specific scoring systems. Currently, there are over 200 sepsis-related biomarkers have been reported in the literature, among which heparin-binding protein

(HBP) is a novel biomarker. [7] HBP is a serine protease-like protein secreted by neutrophils after infection that has functions such as altering endothelial cell permeability, antimicrobial activity, chemotaxis, and regulation of cell apoptosis. [8] It has been identified as an early diagnostic indicator for severe sepsis/septic shock in Chinese Guidelines for the Management of Severe Sepsis/Septic Shock (2014) [9] and Chinese Expert Consensus on Early Prevention and Interruption of Sepsis in Emergency Medicine (2020). [10] In addition, an increasing number of studies have recently provided evidence regarding the use of HBP for diagnosing sepsis. The results demonstrate that HBP could be used for sepsis diagnosis and severity monitoring. [8, 11-14] On the other hand, a few studies have indicated that elevated levels of HBP irrespective of infectious etiology and no correlation with severity and outcome. [15] Furthermore, differences and inconsistencies have been noted among various studies regarding the diagnostic performance of HBP in sepsis. [16, 17] Therefore, it remains controversial to use HBP for the early diagnosis of sepsis. This study aimed to analyze the early diagnostic value of HBP in sepsis and develop a sepsis diagnostic model combining HBP with multiple biomarkers and disease-specific scoring systems retrospectively to facilitate early identification and diagnosis of sepsis in the ICU.

Methods

Study population

This study included 2080 patients who were admitted to the ICU of the First Affiliated Hospital of Sun Yat-sen University, China, from March 2019 to December

2021. Strict inclusion and exclusion criteria were adopted for all patients, with the following inclusion criteria: (1) patients who underwent HBP detection or whose blood samples were collected for HBP detection at the time of ICU admission, (2) Integrity of the clinical data, and (3) age 18 years or older. The exclusion criteria were as follows: (1) patients with neutropenia due to hematological malignancies, and (2) patients who underwent immunosuppressive therapy. Patients were categorized into four groups (infection, sepsis, septic shock, and control groups) based on the final diagnosis at the time of discharge from the ICU or death, determined by the attending physician. Figure 1 displays the flow diagram of the participants. The protocols were approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University and were conducted in accordance with the Declaration of Helsinki.

Measurement of plasma HBP and clinical data collection

The previously collected blood samples were sent to the central laboratory to detect plasma HBP levels. Briefly, the blood samples were centrifuged at 1,000 rounds/min for 10 min, and 100 μL of supernatants were collected for plasma level of HBP determination using an immunofluorescence dry quantitative method (Jet-iStar3000, Hangzhou, Joinstar Biomedical Technology Co., LTD). The procedure strictly followed the instructions provided with the reagent kit, and the quality control was performed well.

General information such as gender, age, underlying diseases, site of infection, and pathogens were collected. Laboratory tests, such as HBP, procalcitonin (PCT),

white blood cell count (WBC), C-reactive protein (CRP), interleukin-6 (IL-6), and blood lactate (LAC), were measured at the time of ICU admission. Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores were calculated within 24 h of ICU admission. The length of ICU and survival outcomes (3-day improvement rate and 28-day mortality rate) were also recorded for each group of patients.

Statistical Methods

For baseline measurement data, the median and interquartile range (IQR) were employed to describe the data. If continuous variables followed a normal distribution, one-way ANOVA was utilized for intergroup comparisons; otherwise, the Kruskal–Wallis H test was deployed. Percentage calculations were performed for categorical data, and differences between groups were tested using the chi-square test or Fisher's exact test.

Receiver operating characteristic (ROC) curves were used to assess the diagnostic performance of HBP, PCT, WBC, CRP, IL-6, LAC, APACHE II score, and SOFA score for sepsis. The area under the ROC curve (AUC) was calculated. The optimal cut-off values for diagnosing sepsis were determined based on the maximum Youden index, and the corresponding sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

To improve the diagnostic performance of sepsis, a multivariate binary logistic regression model was constructed. Random selection of 70% of all patients was used

as the training set, whereas the remaining 30% served as the test set to assess the model's performance. The AUC was calculated for both the training and test sets. The Hosmer–Lemeshow goodness-of-fit test and calibration curve were used to evaluate the model's goodness-of-fit for both datasets. Decision curves were plotted to evaluate the clinical utility of the regression model. All hypothesis tests were two-tailed, with a significance level of $P < 0.050$. Statistical analyses were performed using R 4.1.1 and SPSS 25.0.

Patient and public involvement

This was a retrospective study. No patients or public representatives were involved in setting the research question, nor in the study design, implementation, or interpretation.

Results

Characteristics of the patients

Finally, 326 patients were enrolled, including 93 in the control group, 94 in the infection group, 53 in the sepsis group, and 86 in the septic shock group (Figure 1). Table 1 summarizes the baseline characteristics of the patients. The median ages of patients in the control group, infection group, sepsis group, and septic shock group were 56, 63, 58, and 64 years, respectively, with statistically significant differences among the groups ($p = 0.023$). No significant differences were noted among the groups in terms of gender, prevalence of hypertension, diabetes, heart disease, malignancy, liver disease, or other comorbidities.

The control group consisted of patients who recovered postoperatively from various surgical procedures, including gastrointestinal, hepatic, vascular, among others. Patients with infection (including the infection, sepsis, and septic shock groups) predominantly presented with pulmonary infections (48.9%, 32.1%, and 26.7%, respectively) and abdominal infections (33.0%, 56.6%, and 73.3%, respectively). Among all enrolled patients, 32 had positive blood cultures, 76 had positive peritoneal drainage fluid cultures, and 90 had positive sputum cultures. All patients with sepsis (including the sepsis and septic shock groups) mainly suffered from bacterial infections and received antibiotic treatment. The APACHE II and SOFA scores of the sepsis and septic shock groups were significantly higher than those of the control and infection groups, with statistically significant differences among the four groups ($p < 0.001$). In the prognosis analysis, the 28-day mortality rates for the sepsis and septic shock groups were 11.32% and 32.56%, respectively, which were significantly higher than those for the control and infection groups (3.2% and 9.6%) (Table 1).

Levels of HBP and other biomarkers in each group of patients

The median (IQR) HBP levels in the control, infection, sepsis, and septic shock groups were 18.0 (9.9–32.1), 24.0 (14.1–56.4), 45.7 (24.8–107.9), and 69.0 (33.8–150.9) ng/mL, respectively ($p < 0.001$). HBP was capable of effectively distinguishing between patients with and without infection or sepsis, and its efficacy was superior to that of IL-6, LAC, and WBC. However, in distinguishing septic patients with or without shock, HBP was inferior to PCT, IL-6, and LAC. Additionally, no statistically

significant differences were noted in WBC counts among the groups (Figure 2).

When comparing HBP levels among different infection sites in the infection, sepsis, and septic shock groups, statistical differences were observed among the subgroups, except for the multi-infection site (Supplementary Table 1). As the severity of infection increased, the APACHE II and SOFA scores gradually increased, showing statistically significant differences. However, no statistical difference was observed between the infection and the sepsis groups (Figure 2).

Analysis of the diagnostic accuracy of different biomarkers for sepsis

HBP demonstrated promising diagnostic performance for the early detection of sepsis, with an AUC of 0.733 (95% CI 0.678–0.789), which was significantly higher than WBC (AUC 0.541, 95% CI 0.474–0.607) and higher than the AUCs of IL-6, LAC, and APACHE II scores (0.658, 0.632, and 0.688, respectively), but the difference was not statistically significant. The AUC for HBP was significantly lower than that for PCT (AUC 0.812, 95% CI 0.766–0.857). When the HBP cut-off value was set at 35.2 ng/mL, the sensitivity, specificity, PPV, and NPV for diagnosing sepsis were 65.5%, 74.9%, 65.9%, and 74.5%, respectively (Table 2, Supplementary Figure 1).

Relationship between HBP and other biomarkers

No significant correlation was observed between HBP levels and CRP, PCT, WBC, IL-6, LAC, APACHE II scores, and SOFA scores (Supplementary Figure 2).

Construction of a sepsis diagnostic model

Based on the training set, variables were selected using univariate logistic regression analysis for patient demographics (such as gender, age, underlying diseases, infection sites, and pathogens), infection biomarkers (HBP, PCT, WBC, CRP, IL-6, and LAC), APACHE II scores, and SOFA scores. Variables with statistical significance ($p < 0.05$) were included in the multivariate logistic regression model (Supplementary Table 2). Statistically significant variables in the univariate analysis were HBP, PCT, CRP, IL-6, LAC, APACHE II, and SOFA scores. 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, as illustrated in Figure 3.

Validation of the sepsis diagnostic model

To evaluate the predictive performance of the model, the remaining 30% of patients were used as a test set to validate the model. In the training set, the model achieved an AUC of 0.901 (95% CI 0.863–0.940). When the Youden index was maximized, the cut-off value was determined to be 0.439, resulting in a sensitivity of 79.4% and a specificity of 86.5%. In the test set population, the model obtained an AUC of 0.913 (95% CI 0.860–0.966). Applying the cut-off value obtained from the training set to the test set, the sensitivity and specificity were 80.5% and 87.7%, respectively

(Supplementary Figure 3). Furthermore, to obtain a more accurate cut-off value, all patients were included in the diagnostic model, resulting in a cut-off value of 0.439. The sensitivity and specificity for diagnosing sepsis with this cut-off value were 79.7% and 86.9%, respectively.

The diagnostic model constructed using the training set exhibited a good predictive performance based on the Hosmer–Lemeshow goodness-of-fit test in the training and test sets ($\chi^2 = 4.91$, $p = 0.767$; $\chi^2 = 5.12$, $p = 0.745$; Supplementary Figure 4). Additionally, the decision curve analysis (DCA) plot demonstrated a high clinical net benefit for the constructed sepsis diagnostic model that surpasses both Treat-all and Treat-no (Supplementary Figure 5).

Discussion

Sepsis is a major cause of mortality in critically ill patients and is associated with high morbidity and mortality rates. Approximately 20%–30% of severely infected patients do not exhibit typical symptoms of organ dysfunction upon admission but rapidly progress to sepsis. [6] Therefore, early identification of sepsis is crucial for developing appropriate and effective treatment strategies and reducing mortality. Clinicians require specific and sensitive biomarkers for the early diagnosis of sepsis. Currently, WBC, CRP, and PCT are commonly used as inflammatory biomarkers in clinical practice. [7] However, WBC and CRP are nonspecific markers of systemic inflammation and cannot effectively differentiate among bacterial, non-bacterial, and sterile inflammation. PCT has a higher specificity for bacterial infections but performs

poorly in predicting sepsis-associated organ dysfunction. [6, 18] In recent years, numerous studies have proven that HBP has good predictive performance for infection, sepsis, or organ function assessment, superior to PCT, CRP, and other biomarkers. [6, 8, 11, 12, 19, 20]

HBP, also known as heparin-binding protein (CAP37), is a protein that is stored in the secretory granules of neutrophils and azurophilic granules. It contains a large number of positively charged amino acid residues that are concentrated on one side of the protein. [20] A hydrophobic pocket structure formed by amino acid residues 20–44 exhibits a high affinity for endotoxins. [6] Therefore, HBP was initially discovered for its antimicrobial activity. Subsequent studies have confirmed that HBP is a multifunctional innate immune defense molecule that plays a crucial role in the host's infection and inflammatory responses. [6, 20] These characteristics make HBP a promising novel infection biomarker. Recent studies have reported that HBP could assist in diagnosing various diseases, such as respiratory and circulatory failure, sepsis, acute kidney injury, acute lung injury, meningitis, urinary tract infections, and skin and soft tissue infections. [6, 8, 11, 21-25] However, its clinical use has not yet been widely adopted; accordingly, further clinical research is required to validate its utility.

This study further confirms that HBP is a promising biomarker for sepsis. In this study, HBP levels could effectively differentiate whether patients had an infection and whether infected patients had sepsis. Furthermore, its discriminative value was found to be superior to that of the LAC, IL-6, WBC, SOFA, and APACHE II scores. Similar findings have been previously reported. [7, 11] These results were likely related to the

biological characteristics of HBP. It is stored in neutrophil secretory granules and azurophilic granules, and upon stimulation by pathogens, it can be rapidly and massively released into the bloodstream, inducing rearrangement of the endothelial cell cytoskeleton, leading to vascular leakage and edema formation. Additionally, HBP regulates the function of monocytes and macrophages, further amplifying the inflammatory response and enhancing the body's immune response to infection. Moreover, as neutrophils infiltrated into the tissues, HBP continued to be released, resulting in tissue damage and organ dysfunction. [20, 26] Consequently, HBP levels were significantly elevated in patients with infection and/or sepsis.

Regarding the diagnostic performance of HBP in sepsis, Linder et al. found that the AUC of HBP for predicting sepsis was 0.85, with a sensitivity of 87% and specificity of 95%, which were significantly higher than those of PCT, CRP, WBC, IL-6, and other biomarkers. [8] Furthermore, HBP can predict the occurrence of organ dysfunction and circulatory failure at an early stage, providing indications for timely interventions such as fluid resuscitation and antibiotic use, which are indispensable components of sepsis bundle therapy. [8, 11, 27] In addition, the favorable predictive value of HBP was validated in pediatric patients with severe sepsis. [28] The emergence of this phenomenon was considered to be linked to the pathological process in which HBP is involved in vascular leakage and organ dysfunction in septic patients, and its release occurred earlier than CRP, PCT, and other markers. [19, 20, 26] In this study, the AUC of HBP in predicting sepsis was 0.733, which was not superior to PCT, CRP, and SOFA. Previous studies have reported varying diagnostic accuracies of HBP for

sepsis at different time points. [19] In this study, the disease course was relatively later. Although detecting HBP or collecting blood samples occurred upon admission to the ICU, the onset time was still later than that in emergency cases. Meta-analyses also revealed that HBP often performed better in diagnosing sepsis in emergency department patients compared with ICU patients. [15, 16, 19] Unlike previous studies, this study involved ICU patients rather than emergency patients. First, the control group in this study consisted of surgical postoperative recovery patients without infection. Additionally, ICU patients have more complex conditions, have more severe organ damage, and require life support, such as ventilators, vasopressors, and continuous renal replacement therapy (CRRT). Finally, the patients already received various treatments, such as fluid resuscitation and antibiotics in the emergency room or ward. [29-33] In summary, these conditions might have some impact on HBP levels, but this study population was more representative of the actual situation of ICU patients. From another perspective, this phenomenon also reflects the limitations of a single biomarker, as it could not fully reflect the clinical reality and accurately diagnose sepsis in the ICU.

The pathophysiological mechanisms that underlie sepsis are complex. They are involved in different immune states, sites of infection, and pathogens. Immune response patterns vary, as do the pathophysiological processes of various biomarkers. During its occurrence and progression, dual factors that simultaneously lead to an exaggerated inflammatory response and immune dysfunction. Systemic inflammatory responses and immune suppression do not generally exist as simple independent entities but rather coexist. Therefore, a single biomarker cannot serve as a reliable diagnostic indicator for

sepsis. [7, 10] In this study, we also observed that HBP showed almost no correlation with PCT, CRP, IL-6, LAC, APACHE II, and SOFA scores. This suggests that HBP, as a biomarker, could provide unique information for diagnosing sepsis independent of other biomarkers. We hypothesized that establishing a diagnostic model combining HBP with PCT, CRP, IL-6, LAC, APACHE II, SOFA scores, and other indicators could be a new approach for the early diagnosis of sepsis. Currently, relevant studies have been conducted in this regard, [34, 35] however, many of the biomarkers mentioned in the above studies have not been widely used in clinical practice, making them less practical. In this study, biomarkers commonly used in clinical settings were included. Based on the ROC analysis of various markers, a sepsis diagnostic model was constructed using multivariable logistic regression. Upon testing, the sepsis diagnostic model exhibited an AUC of > 0.90, indicating its high clinical applicability.

Conclusion

This study confirmed the value of plasma HBP levels in the early diagnosis of sepsis in the ICU. It also constructed an early sepsis diagnostic model that includes HBP, PCT, CRP, IL-6, and SOFA scores. This model demonstrated a high accuracy and clinical utility, further enhancing its early predictive role in sepsis. It has potential clinical diagnostic value for the early detection of sepsis.

Notes

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Author contributions. Study concept and design: Yongjun Liu, and Lingyun Zuo. Definition of the diagnostic algorithm: Yongjun Liu, Jianfeng Wu, and Xiangdong Guan. Data acquisition and analysis: Lingyun Zuo, Xiaoyun Li, Zihuai Liao, and Si Zhou. Data interpretation: Luhao Wang and Hao Yuan. Manuscript drafting: Lingyun Zuo, Xiaoyun Li, Luhao Wang, Hao Yuan and Yongjun Liu. Manuscript revision: All authors.

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Ethics approval. This retrospective study did not introduce any additional risks. Therefore, informed consent was not obtained from all the participants. Regarding the collection of blood samples for HBP testing during holidays, the participants in our study were previously provided informed consent for collecting biological samples.

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References

1. Evans, L., et al., *Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021.* Crit Care Med, 2021. **49**(11): p. e1063-e1143.
2. Rudd, K.E., et al., *Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study.* Lancet, 2020. **395**(10219): p. 200-211.
3. Xie, J., et al., *The Epidemiology of Sepsis in Chinese ICUs: A National Cross-Sectional Survey.* Crit Care Med, 2020. **48**(3): p. e209-e218.
4. Im, Y., et al., *Time-to-antibiotics and clinical outcomes in patients with sepsis and septic shock: a prospective nationwide multicenter cohort study.* Crit Care, 2022. **26**(1): p. 19.
5. Kuttab, H.I., et al., *Evaluation and Predictors of Fluid Resuscitation in Patients with Severe Sepsis and Septic Shock.* Crit Care Med, 2019. 47(11): p. 1582-1590.
6. Yang, Y., et al., *A Promising Candidate: Heparin-Binding Protein Steps onto the Stage of Sepsis Prediction.* J Immunol Res, 2019. **2019**: p. 7515346.
7. Pierrakos, C., et al., *Biomarkers of sepsis: time for a reappraisal.* Crit Care, 2020. **24**(1): p. 287.
8. Linder, A., et al., *Heparin-binding protein: an early marker of circulatory failure in sepsis.* Clin Infect Dis, 2009. **49**(7): p. 1044-50.
9. Cai, G., J. Yan, and H. Qiu, *[The standardization of diagnosis and treatment of severe sepsis/septic shock and its practice].* Zhonghua Nei Ke Za Zhi, 2015. **54**(6): p. 484-5.

10. Shock, et al., *[Chinese expert consensus on diagnosis and management of immunosuppression in sepsis].* Zhonghua Wei Zhong Bing Ji Jiu Yi Xue, 2020. **32**(11): p. 1281-1289.

11. Linder, A., et al., *Heparin-Binding Protein Measurement Improves the Prediction of Severe Infection With Organ Dysfunction in the Emergency Department.* Crit Care Med, 2015. **43**(11): p. 2378-86.

12. Zhou, Y., et al., *Usefulness of the heparin-binding protein level to diagnose sepsis and septic shock according to Sepsis-3 compared with procalcitonin and C reactive protein: a prospective cohort study in China.* BMJ Open, 2019. **9**(4): p. e026527.

13. Tang J, Yuan H, Wu YL,et al. The Predictive Value of Heparin-Binding Protein and D-Dimer in Patients with Sepsis. Int J Gen Med. 2023 Jun 6; 16:2295-2303.

14. Li S, Xu Y, Wu Y,et al. Heparin-Binding Protein: A Prognostic Biomarker Associated with Severe or Complicated Community-Acquired Pneumonia in Children. J Inflamm Res. 2023 Jan 26; 16:321-331.

15. Chew, M.S., et al., *Increased plasma levels of heparin-binding protein in patients with shock: a prospective, cohort study.* Inflamm Res, 2012. **61**(4): p. 375-9.

16. Llewelyn, M.J., et al., *Sepsis biomarkers in unselected patients on admission to intensive or high-dependency care.* Crit Care, 2013. **17**(2): p. R60.

17. Katsaros, K., et al., *Heparin Binding Protein for the Early Diagnosis and Prognosis of Sepsis in the Emergency Department: The Prompt Multicenter Study.* Shock, 2022. **57**(4): p. 518-525.

18. Jekarl, D.W., et al., *Procalcitonin as a prognostic marker for sepsis based on SEPSIS-3.* J Clin Lab Anal, 2019. **33**(9): p. e22996.

19. Wu, Y.L., et al., *Accuracy of Heparin-Binding Protein in Diagnosing Sepsis: A Systematic Review and Meta-Analysis.* Crit Care Med, 2021. **49**(1): p. e80-e90.

20. Fisher, J. and A. Linder, *Heparin-binding protein: a key player in the pathophysiology of organ dysfunction in sepsis.* J Intern Med, 2017. **281**(6): p. 562-574.

21. Kjolvmark, C., et al., *Heparin-binding protein: a diagnostic biomarker of urinary tract infection in adults.* Open Forum Infect Dis, 2014. **1**(1): p. ofu004.

22. Linder, A., et al., *Heparin-binding protein: a diagnostic marker of acute bacterial meningitis.* Crit Care Med, 2011. **39**(4): p. 812-7.

23. Saridaki M, Metallidis S, Grigoropoulou S, et al. Integration of heparin-binding protein and interleukin-6 in the early prediction of respiratory failure and mortality in pneumonia by SARS-CoV-2 (COVID-19). Eur J Clin Microbiol Infect Dis. 2021 Jul;40(7):1405-1412.

24. Kong D, Lei Z, Wang Z, et al. A novel HCP (heparin-binding protein -C reactive protein-procalcitonin) inflammatory composite model can predict severe acute pancreatitis. Sci Rep. 2023 Jun 9;13(1):9440.

25. Kong Y, Ye Y, Ma J, et al. Accuracy of heparin-binding protein for the diagnosis of nosocomial meningitis and ventriculitis. Crit Care. 2022 Mar 8;26(1):56. （诊断脑炎）

26. Linder, A., O. Soehnlein, and P. Akesson, *Roles of heparin-binding protein in bacterial infections.* J Innate Immun, 2010. **2**(5): p. 431-8.

27. Kahn, F., et al., *Heparin-Binding Protein as a Prognostic Biomarker of Sepsis and Disease Severity at the Emergency Department.* Shock, 2019. **52**(6): p. e135-e145.

28. Liu, P., et al., *Heparin-binding protein as a biomarker of severe sepsis in the pediatric intensive care unit: A multicenter, prospective study.* Clin Chim Acta, 2023. **539**: p. 26-33.

29. Fisher, J., et al., *Is Heparin-Binding Protein Inhibition a Mechanism of Albumin's Efficacy in Human Septic Shock?* Crit Care Med, 2018. **46**(5): p. e364-e374.

30. Samuelsson, L., et al., *Renal clearance of heparin-binding protein and elimination during renal replacement therapy: Studies in ICU patients and healthy volunteers.* PLoS One, 2019. **14**(8): p. e0221813.

31. Honore, P.M., S. Redant, and D. De Bels, *Reliability of biomarkers of sepsis during extracorporeal therapies: the clinician needs to know what is eliminated and what is not.* Crit Care, 2020. **24**(1): p. 553.

32. Xing, L., et al., *Activation of M1 macrophages in sepsis-induced acute kidney injury in response to heparin-binding protein.* PLoS One, 2018. **13**(5): p. e0196423.

33. Fisher, J., et al., *Heparin-Binding Protein (HBP): A Causative Marker and Potential Target for Heparin Treatment of Human Sepsis-Induced Acute Kidney Injury.* Shock, 2017. **48**(3): p. 313-320.

34. Gibot, S., et al., *Combination biomarkers to diagnose sepsis in the critically ill patient.* Am J Respir Crit Care Med, 2012. **186**(1): p. 65-71.

35. Bauer, P.R., et al., *Diagnostic accuracy and clinical relevance of an inflammatory biomarker panel for sepsis in adult critically ill patients.* Diagn Microbiol Infect Dis, 2016. **84**(2): p. 175-80.

458

459 **Tables**

460 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, median (IQR)	9.0 (7.0 - 12.0)	12.0 (9.0 - 16.0)	13.0 (9.00 - 18.0)	16.5 (12.0 - 21.0)	<0.001
SOFA score*, median (IQR)	2.0 (1.0 - 5.0)	4.0 (2.3 - 7.0)	5.0 (3.0 - 7.0)	10.0 (7.0 - 13.0)	<0.001
Length of ICU stay, days median (IQR)	2.0 (1.0 - 4.0)	5.0 (3.0 - 7.8)	6.0 (3.0 - 10.0)	8.0 (4.0 - 13.0)	<0.001

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.

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.

Figure legends

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

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. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Figure 3. A nomogram predicting the risk of sepsis for patients. The value of each of variable was given a score on the point scale axis. A total score could be easily calculated by adding each single score and by projecting the total score to the lower total point scale. We were able to estimate the probability of sepsis. CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment.

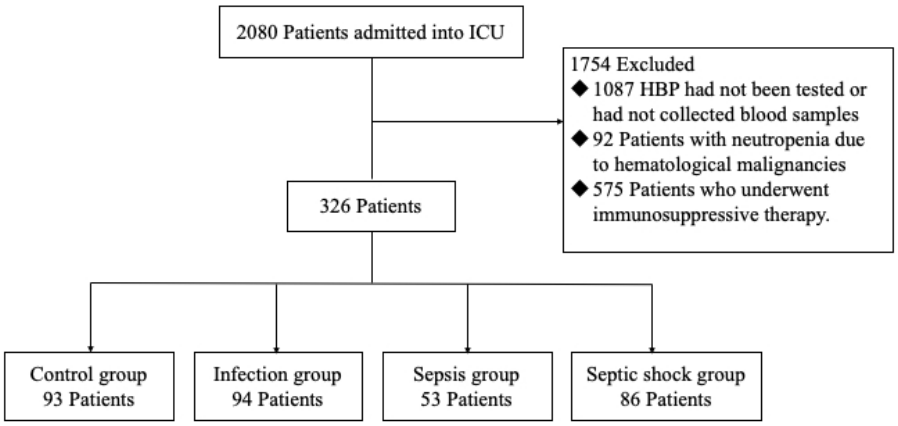

Figure 1. The flow diagram of participants. HBP: heparin-binding protein, ICU: intensive care unit.

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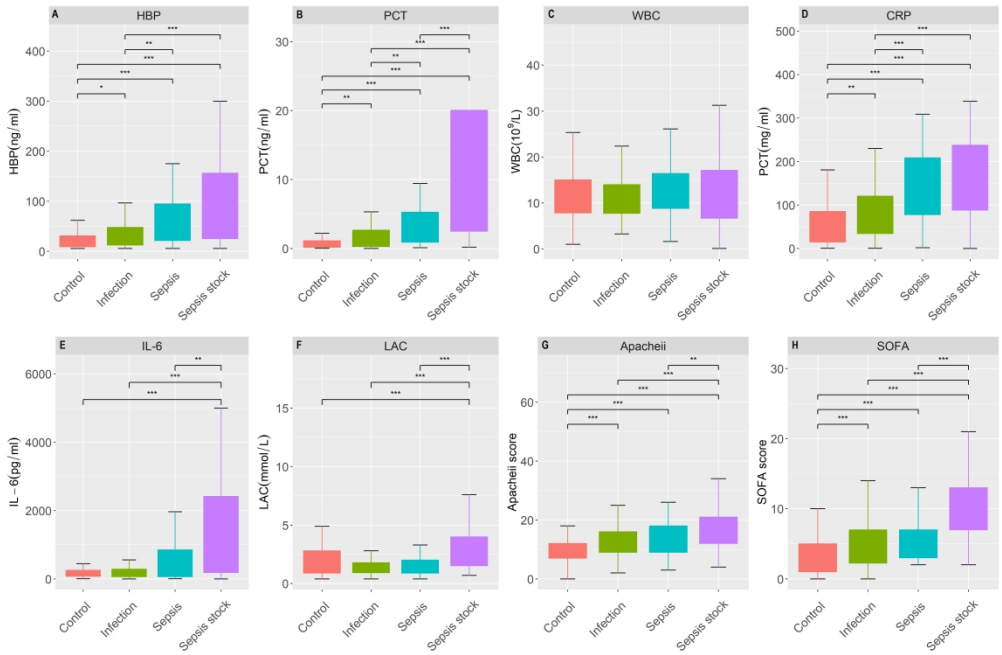

Comparison of plasma levels of biomarkers among different groups. A: HBP, B: PCT, C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: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

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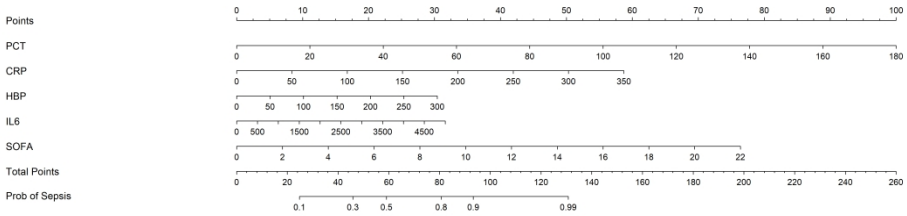

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423x127mm (300 x 300 DPI)

Supplementary Data

Supplementary Table 1. The comparison of HBP among different sites.

	Infection	Sepsis	Septic shock	*P*
Abdomen, median (IQR)	24.8 (14.0–74.5)	44.7 (25.9–108.0)	78.0 (38.6–156.3.0)	＜0.001
Respiratory median (IQR)	23.2 (10.8–55.3)	55.2 (37.8–73.9)	55.7 (14.1–300)	＜0.001
Blood median (IQR)	9.5*	80.4 (45.1–115.6)	207.6 (176.6–238.6)	＜0.001
Skin and soft tissues median (IQR)	25.5 (19.1–37.3)	27.3 (14.6–41.4)	61.8 (36.2–136)	0.027
Other median (IQR)	18.3 (14.5–22.5)	45.6 (27.0–64.3)	22.6 (19.5–86.7)	0.007
Multi-infection site median (IQR)	22.7 (20.9–32.8)	37.7 (18.0–110.6)	39.0 (23.7–134.6)	0.333

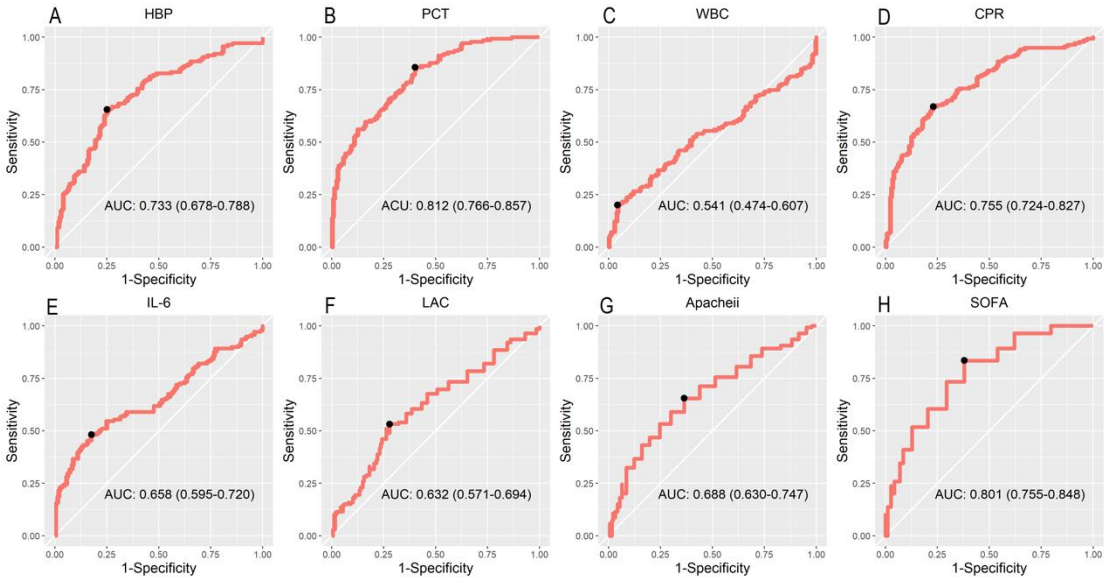
* Only one patient with bloodstream infection in the infection group, IQR: interquartile range.

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

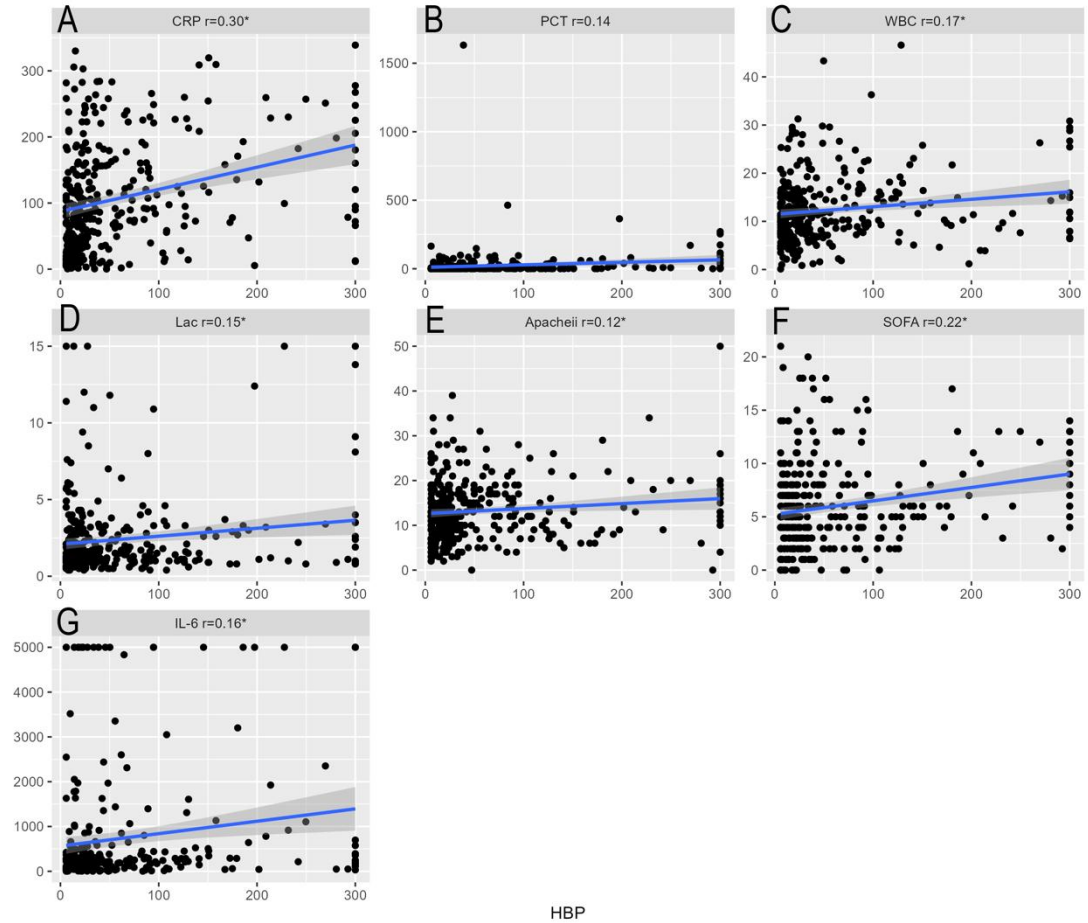
Variable	Univariate logistic regression analysis		Multivariate logistic regression analysis	
	OR (95%CI)	*P*	OR (95%CI)	*P*
Age	1.009 (0.993, 1.026)	0.276		
Sex	1.169 (0.683, 1.999)	0.569		
Hypertension	0.795 (0.450, 1.402)	0.427		
Diabetes	0.801 (0.418, 1.538)	0.505		
Cardiovascular	0.538 (0.288, 1.182)	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

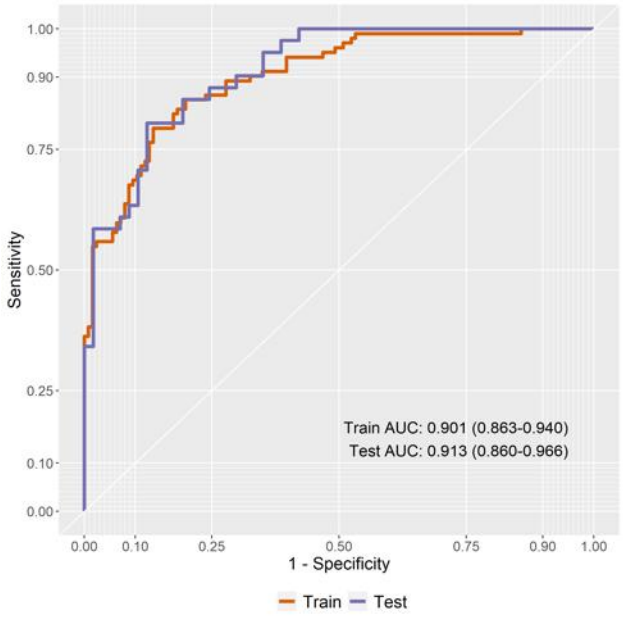
APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count.



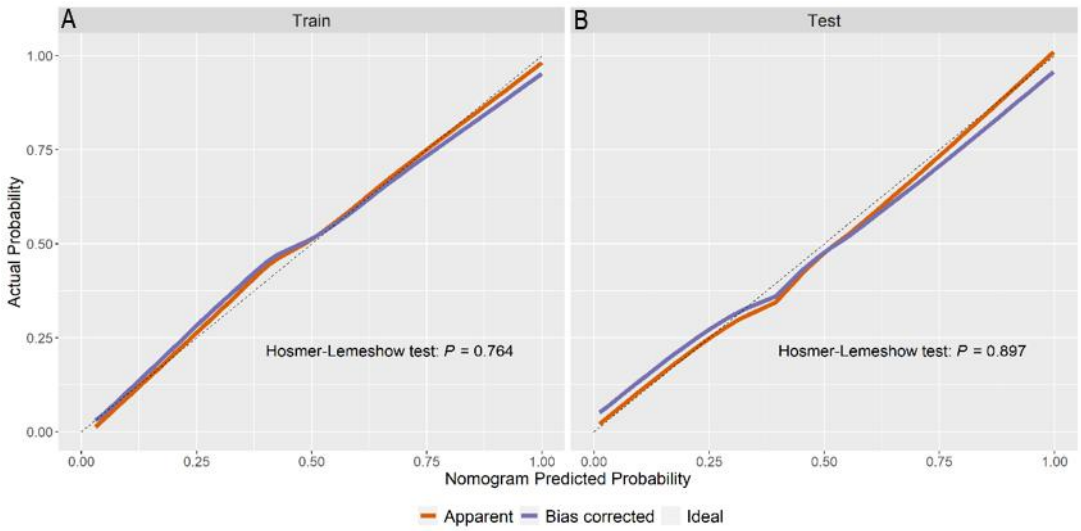
Supplementary Figure 1. ROC curves for biomarkers in distinguishing sepsis from non-sepsis. A: HBP, B: PCT, C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count.



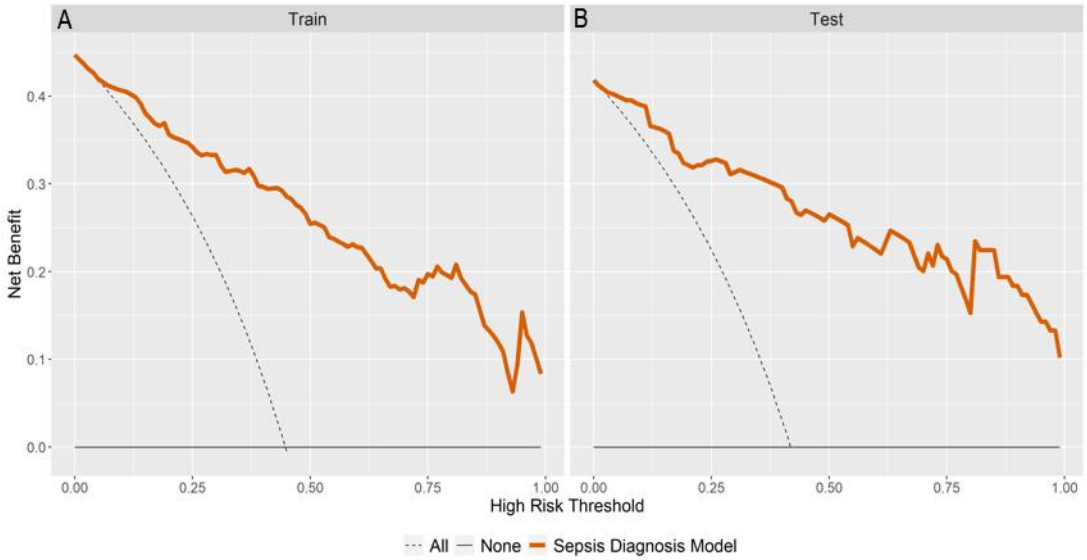
Supplementary Figure 2. The correlations of HBP with CRP (A), PCT (B), WBC (C), LAC (D), APACHE II (E), SOFA (F), and IL-6(G). APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count.



Supplementary Figure 3. ROC curve analysis of the sepsis training model and test model.



Supplementary Figure 4. Calibration test of the sepsis diagnostic model. A: training set, B: test set.



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.

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Heparin-binding protein as a biomarker for the diagnosis of sepsis in the intensive care unit: a retrospective cross-sectional study in China

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Abstract

Objectives: This study aims to investigate the diagnostic value of heparin-binding protein (HBP) in sepsis and develop a sepsis diagnostic model incorporating HBP with key biomarkers and disease-related scores for rapid, and accurate diagnosis of sepsis in the intensive care unit (ICU).

Design: Clinical retrospective cross-sectional study.

Setting: A comprehensive teaching tertiary hospital in China.

Participants: Adult patients (age ≥18years) who underwent HBP testing or whose blood samples were collected when admitted to the ICU.

Main outcome measures: HBP, C-reactive protein (CRP), procalcitonin (PCT), white blood cell count (WBC), interleukin-6 (IL-6), lactate (LAC), acute physiology and chronic health evaluation II (APACHE II), and sequential organ failure assessment (SOFA) score were recorded.

Results: Between March 2019 and December 2021, 326 patients were enrolled in this study. The patients were categorized into a non-infection group (control group), infection group, sepsis group, and septic shock group based on the final diagnosis. The HBP levels in the sepsis group and septic shock group were 45.7 and 69.0 ng/mL, respectively, which were significantly higher than those in the control group (18.0 ng/mL) and infection group (24.0 ng/mL) ($p < 0.001$). The AUC value of HBP for diagnosing sepsis was 0.733, which was lower than those corresponding to PCT, CRP, and SOFA but higher than those of IL-6, LAC, and APACHE II. Multivariate logistic regression analysis identified HBP, PCT, CRP, IL-6, and SOFA as valuable indicators

for diagnosing sepsis. A sepsis diagnostic model was constructed based on these indicators, with an AUC of 0.901, a sensitivity of 79.7%, and a specificity of 86.9%.

Conclusions: HBP could serve as a biomarker for the diagnosis of sepsis in the ICU. Compared with single indicators, the sepsis diagnostic model constructed using HBP, PCT, CRP, IL-6, and SOFA further enhanced the diagnostic performance of sepsis.

Strengths and limitations of this study

- This study included a highly heterogeneous population, making it highly applicable to patients with sepsis in the ICU.
- Moreover, most of the biomarkers included in this diagnostic model are widely used in clinical practice, making them easily obtainable, highly reproducible, and operationally feasible.
- This was an ICU single-center retrospective study, and the results might be inapplicable to sepsis patients in other settings.
- The SOFA scores in the study were absolute values automatically obtained by the electronic scoring system rather than the delta values.
- Its design dose not allow for the determination of causal relationships.

Keywords: HBP, Sepsis, Diagnostic model

Background

Sepsis is a life-threatening organ dysfunction caused by dysregulated host response to infection. Sepsis, when accompanied by severe circulatory impairment and cellular metabolic disorders, is referred to as septic shock and is the leading cause of death in patients with sepsis. [1] With the aging population and increase in immunocompromised hosts, the incidence of sepsis has recently been rising. The Global Burden of Sepsis study published in 2020 reported 48.9 million cases of sepsis worldwide in 2017, with 11 million deaths attributed to sepsis, accounting for 19.7% of the global deaths. [2] Another domestic study showed that the incidence of sepsis in the intensive care unit (ICU) was 20.6%, with a 90-day mortality rate of 35.5%, and the mortality rate for septic shock was as high as 50% or more. [3] Im et al. demonstrated that the mortality rate of septic shock is correlated with hypotension and the delayed use of antibiotics. [4] Another study indicated that early fluid resuscitation is closely linked to the prognosis of patients with sepsis. [5] Therefore, early diagnosis and timely and appropriate treatment are crucial for sepsis management.

Early diagnosis and identification of sepsis require a comprehensive approach based on the patient's clinical symptoms, conventional cultures, biomarkers, and disease-specific scoring systems. However, the clinical symptoms and signs of sepsis are often nonspecific, and conventional pathogen cultures are relatively delayed. [6] Therefore, the early diagnosis of sepsis in the ICU mainly relies on biomarkers and disease-specific scoring systems. Currently, there are over 200 sepsis-related biomarkers have been reported in the literature, among which heparin-binding protein (HBP) is a novel biomarker. [7] HBP is a serine protease-like protein secreted by

neutrophils after infection that has functions such as altering endothelial cell permeability, antimicrobial activity, chemotaxis, and regulation of cell apoptosis. [8] It has been identified as an early diagnostic indicator for severe sepsis/septic shock in Chinese Guidelines for the Management of Severe Sepsis/Septic Shock (2014) [9] and Chinese Expert Consensus on Early Prevention and Interruption of Sepsis in Emergency Medicine (2020). [10] In addition, an increasing number of studies have recently provided evidence regarding the use of HBP for diagnosing sepsis. The results demonstrate that HBP could be used for sepsis diagnosis and severity monitoring. [8, 11-14] On the other hand, a few studies have indicated that elevated levels of HBP irrespective of infectious etiology and no correlation with severity and outcome. [15] Furthermore, differences and inconsistencies have been noted among various studies regarding the diagnostic performance of HBP in sepsis. [16, 17] Therefore, it remains controversial to use HBP for the early diagnosis of sepsis. This study aimed to analyze the diagnostic value of HBP in sepsis and develop a sepsis diagnostic model combining HBP with multiple biomarkers and disease-specific scoring systems retrospectively to facilitate identification and diagnosis of sepsis in the ICU.

Methods

Study population

This study included 2080 patients who were admitted to the ICU of the First Affiliated Hospital of Sun Yat-sen University, China, from March 2019 to December 2021. Strict inclusion and exclusion criteria were adopted for all patients, with the

following inclusion criteria: (1) patients who underwent HBP detection or whose blood samples were collected for HBP detection at the time of ICU admission, (2) Integrity of the clinical data, and (3) age 18 years or older. The exclusion criteria were as follows: (1) patients with neutropenia due to hematological malignancies, and (2) patients who underwent immunosuppressive therapy. Patients were categorized into four groups (infection, sepsis, septic shock, and control groups) based on the final diagnosis at the time of discharge from the ICU or death, determined by the attending physician. Figure 1 displays the flow diagram of the participants. The protocols were approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University and were conducted in accordance with the Declaration of Helsinki.

Measurement of plasma HBP and clinical data collection

The previously collected blood samples were sent to the central laboratory to detect plasma HBP levels. Briefly, the blood samples were centrifuged at 1,000 rounds/min for 10 min, and 100 μL of supernatants were collected for plasma level of HBP determination using an immunofluorescence dry quantitative method (Jet-iStar3000, Hangzhou, Joinstar Biomedical Technology Co., LTD). The procedure strictly followed the instructions provided with the reagent kit, and the quality control was performed well.

General information such as gender, age, underlying diseases, site of infection, and pathogens were collected. Laboratory tests, such as HBP, procalcitonin (PCT), white blood cell count (WBC), C-reactive protein (CRP), interleukin-6 (IL-6), and

blood lactate (LAC), were measured at the time of ICU admission. Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores were calculated within 24 h of ICU admission. The length of ICU and survival outcomes (3-day improvement rate and 28-day mortality rate) were also recorded for each group of patients.

Statistical Methods

For baseline measurement data, the median and interquartile range (IQR) were employed to describe the data. If continuous variables followed a normal distribution, one-way ANOVA was utilized for intergroup comparisons; otherwise, the Kruskal–Wallis H test was deployed. Percentage calculations were performed for categorical data, and differences between groups were tested using the chi-square test or Fisher's exact test.

Receiver operating characteristic (ROC) curves were used to assess the diagnostic performance of HBP, PCT, WBC, CRP, IL-6, LAC, APACHE II score, and SOFA score for sepsis. The area under the ROC curve (AUC) was calculated. The optimal cut-off values for diagnosing sepsis were determined based on the maximum Youden index, and the corresponding sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

To improve the diagnostic performance of sepsis, a multivariate binary logistic regression model was constructed. Random selection of 70% of all patients was used as the training set, whereas the remaining 30% served as the test set to assess the

model's performance. The AUC was calculated for both the training and test sets. The Hosmer–Lemeshow goodness-of-fit test and calibration curve were used to evaluate the model's goodness-of-fit for both datasets. Decision curves were plotted to evaluate the clinical utility of the regression model. All hypothesis tests were two-tailed, with a significance level of $P < 0.050$. Statistical analyses were performed using R 4.1.1 and SPSS 25.0.

Patient and public involvement

This was a retrospective study. No patients or public representatives were involved in setting the research question, nor in the study design, implementation, or interpretation.

Results

Characteristics of the patients

Finally, 326 patients were enrolled, including 93 in the control group, 94 in the infection group, 53 in the sepsis group, and 86 in the septic shock group (Figure 1). Table 1 summarizes the baseline characteristics of the patients. The median ages of patients in the control group, infection group, sepsis group, and septic shock group were 56, 63, 58, and 64 years, respectively, with statistically significant differences among the groups ($p = 0.023$). No significant differences were noted among the groups in terms of gender, prevalence of hypertension, diabetes, heart disease, malignancy, liver disease, or other comorbidities.

The control group consisted of patients who recovered postoperatively from

various surgical procedures, including gastrointestinal, hepatic, vascular, among others. Patients with infection (including the infection, sepsis, and septic shock groups) predominantly presented with pulmonary infections (48.9%, 32.1%, and 26.7%, respectively) and abdominal infections (33.0%, 56.6%, and 73.3%, respectively). Among all enrolled patients, 32 had positive blood cultures, 76 had positive peritoneal drainage fluid cultures, and 90 had positive sputum cultures. All patients with sepsis (including the sepsis and septic shock groups) mainly suffered from bacterial infections and received antibiotic treatment. The APACHE II and SOFA scores of the sepsis and septic shock groups were significantly higher than those of the control and infection groups, with statistically significant differences among the four groups ($p < 0.001$). In the prognosis analysis, the 28-day mortality rates for the sepsis and septic shock groups were 11.32% and 32.56%, respectively, which were significantly higher than those for the control and infection groups (3.2% and 9.6%) (Table 1).

Levels of HBP and other biomarkers in each group of patients

The median (IQR) HBP levels in the control, infection, sepsis, and septic shock groups were 18.0 (9.9–32.1), 24.0 (14.1–56.4), 45.7 (24.8–107.9), and 69.0 (33.8–150.9) ng/mL, respectively ($p < 0.001$). HBP was capable of effectively distinguishing between patients with and without infection or sepsis, and its efficacy was superior to that of IL-6, LAC, and WBC. However, in distinguishing septic patients with or without shock, HBP was inferior to PCT, IL-6, and LAC. Additionally, no statistically significant differences were noted in WBC counts among the groups (Figure 2).

When comparing HBP levels among different infection sites in the infection, sepsis, and septic shock groups, statistical differences were observed among the subgroups, except for the multi-infection site (Supplementary Table 1). As the severity of infection increased, the APACHE II and SOFA scores gradually increased, showing statistically significant differences. However, no statistical difference was observed between the infection and the sepsis groups (Figure 2).

Analysis of the diagnostic accuracy of different biomarkers for sepsis

HBP demonstrated promising diagnostic performance for the detection of sepsis, with an AUC of 0.733 (95% CI 0.678–0.789), which was significantly higher than WBC (AUC 0.541, 95% CI 0.474–0.607) and higher than the AUCs of IL-6, LAC, and APACHE II scores (0.658, 0.632, and 0.688, respectively), but the difference was not statistically significant. The AUC for HBP was significantly lower than that for PCT (AUC 0.812, 95% CI 0.766–0.857). When the HBP cut-off value was set at 35.2 ng/mL, the sensitivity, specificity, PPV, and NPV for diagnosing sepsis were 65.5%, 74.9%, 65.9%, and 74.5%, respectively (Table 2, Supplementary Figure 1).

Relationship between HBP and other biomarkers

No significant correlation was observed between HBP levels and CRP, PCT, WBC, IL-6, LAC, APACHE II scores, and SOFA scores (Supplementary Figure 2).

Construction of a sepsis diagnostic model

Based on the training set, variables were selected using univariate logistic regression analysis for patient demographics (such as gender, age, underlying diseases, infection sites, and pathogens), infection biomarkers (HBP, PCT, WBC, CRP, IL-6, and LAC), APACHE II scores, and SOFA scores. Variables with statistical significance ($p < 0.05$) were included in the multivariate logistic regression model (Supplementary Table 2). Statistically significant variables in the univariate analysis were HBP, PCT, CRP, IL-6, LAC, APACHE II, and SOFA scores. 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, as illustrated in Figure 3.

Validation of the sepsis diagnostic model

To evaluate the predictive performance of the model, the remaining 30% of patients were used as a test set to validate the model. In the training set, the model achieved an AUC of 0.901 (95% CI 0.863–0.940). When the Youden index was maximized, the cut-off value was determined to be 0.439, resulting in a sensitivity of 79.4% and a specificity of 86.5%. In the test set population, the model obtained an AUC of 0.913 (95% CI 0.860–0.966). Applying the cut-off value obtained from the training set to the test set, the sensitivity and specificity were 80.5% and 87.7%, respectively

(Supplementary Figure 3). Furthermore, to obtain a more accurate cut-off value, all patients were included in the diagnostic model, resulting in a cut-off value of 0.439. The sensitivity and specificity for diagnosing sepsis with this cut-off value were 79.7% and 86.9%, respectively.

The diagnostic model constructed using the training set exhibited a good predictive performance based on the Hosmer–Lemeshow goodness-of-fit test in the training and test sets ($\chi^2 = 4.91$, $p = 0.767$; $\chi^2 = 5.12$, $p = 0.745$; Supplementary Figure 4). Additionally, the decision curve analysis (DCA) plot demonstrated a high clinical net benefit for the constructed sepsis diagnostic model that surpasses both Treat-all and Treat-no (Supplementary Figure 5).

Discussion

Sepsis is a major cause of mortality in critically ill patients and is associated with high morbidity and mortality rates. Approximately 20%–30% of severely infected patients do not exhibit typical symptoms of organ dysfunction upon admission but rapidly progress to sepsis. [6] Therefore, early identification of sepsis is crucial for developing appropriate and effective treatment strategies and reducing mortality. Clinicians require specific and sensitive biomarkers for the early diagnosis of sepsis. Currently, WBC, CRP, and PCT are commonly used as inflammatory biomarkers in clinical practice. [7] However, WBC and CRP are nonspecific markers of systemic inflammation and cannot effectively differentiate among bacterial, non-bacterial, and sterile inflammation. PCT has a higher specificity for bacterial infections but performs

poorly in predicting sepsis-associated organ dysfunction. [6, 18] In recent years, numerous studies have proven that HBP has good predictive performance for infection, sepsis, or organ function assessment, superior to PCT, CRP, and other biomarkers. [6, 8, 11, 12, 19, 20]

HBP, also known as heparin-binding protein (CAP37), is a protein that is stored in the secretory granules of neutrophils and azurophilic granules. It contains a large number of positively charged amino acid residues that are concentrated on one side of the protein. [20] A hydrophobic pocket structure formed by amino acid residues 20–44 exhibits a high affinity for endotoxins. [6] Therefore, HBP was initially discovered for its antimicrobial activity. Subsequent studies have confirmed that HBP is a multifunctional innate immune defense molecule that plays a crucial role in the host's infection and inflammatory responses. [6, 20] These characteristics make HBP a promising novel infection biomarker. Recent studies have reported that HBP could assist in diagnosing various diseases, such as respiratory and circulatory failure, sepsis, acute kidney injury, acute lung injury, meningitis, urinary tract infections, and skin and soft tissue infections. [6, 8, 11, 21-25] However, its clinical use has not yet been widely adopted; accordingly, further clinical research is required to validate its utility.

This study further confirms that HBP is a promising biomarker for sepsis. In this study, HBP levels could effectively differentiate whether patients had an infection and whether infected patients had sepsis. Furthermore, its discriminative value was found to be superior to that of the LAC, IL-6, WBC, SOFA, and APACHE II scores. Similar findings have been previously reported. [7, 11] These results were likely related to the

biological characteristics of HBP. It is stored in neutrophil secretory granules and azurophilic granules, and upon stimulation by pathogens, it can be rapidly and massively released into the bloodstream, inducing rearrangement of the endothelial cell cytoskeleton, leading to vascular leakage and edema formation. Additionally, HBP regulates the function of monocytes and macrophages, further amplifying the inflammatory response and enhancing the body's immune response to infection. Moreover, as neutrophils infiltrated into the tissues, HBP continued to be released, resulting in tissue damage and organ dysfunction. [20, 26] Consequently, HBP levels were significantly elevated in patients with infection and/or sepsis.

Regarding the diagnostic performance of HBP in sepsis, Linder et al. found that the AUC of HBP for predicting sepsis was 0.85, with a sensitivity of 87% and specificity of 95%, which were significantly higher than those of PCT, CRP, WBC, IL-6, and other biomarkers. [8] Furthermore, HBP can predict the occurrence of organ dysfunction and circulatory failure at an early stage, providing indications for timely interventions such as fluid resuscitation and antibiotic use, which are indispensable components of sepsis bundle therapy. [8, 11, 27] In addition, the favorable predictive value of HBP was validated in pediatric patients with severe sepsis. [28] The emergence of this phenomenon was considered to be linked to the pathological process in which HBP is involved in vascular leakage and organ dysfunction in septic patients, and its release occurred earlier than CRP, PCT, and other markers. [19, 20, 26] In this study, the AUC of HBP in predicting sepsis was 0.733, which was not superior to PCT, CRP, and SOFA. Previous studies have reported varying diagnostic accuracies of HBP for

sepsis at different time points. [19] In this study, patients underwent HBP testing upon ICU admission or had plasma collected at that time for subsequent HBP assessment. Consequently, HBP levels were measured for all patients at the time of ICU admission. Since a definitive diagnosis of sepsis required a comprehensive evaluation based on subsequent examinations, diagnoses were collected after patient discharge or death. Therefore, the timing of HBP testing or blood sample collection preceded the definitive diagnosis but might not represent the early stage of sepsis. Based on this, HBP did not demonstrate high diagnostic efficiency for the early detection of sepsis in this study. Meta-analyses also revealed that HBP often performed better in diagnosing sepsis in emergency department patients compared with ICU patients. [15, 16, 19] Unlike previous studies, this study involved ICU patients rather than emergency patients. First, the control group in this study consisted of surgical postoperative recovery patients without infection. Additionally, ICU patients have more complex conditions, have more severe organ damage, and require life support, such as ventilators, vasopressors, and continuous renal replacement therapy (CRRT). Finally, the patients already received various treatments, such as fluid resuscitation and antibiotics in the emergency room or ward. [29-33] In summary, these conditions might have some impact on HBP levels, but this study population was more representative of the actual situation of ICU patients. From another perspective, this phenomenon also reflects the limitations of a single biomarker, as it could not fully reflect the clinical reality and accurately diagnose sepsis in the ICU.

The pathophysiological mechanisms that underlie sepsis are complex. They are

involved in different immune states, sites of infection, and pathogens. Immune response patterns vary, as do the pathophysiological processes of various biomarkers. During its occurrence and progression, dual factors that simultaneously lead to an exaggerated inflammatory response and immune dysfunction. Systemic inflammatory responses and immune suppression do not generally exist as simple independent entities but rather coexist. Therefore, a single biomarker cannot serve as a reliable diagnostic indicator for sepsis. [7, 10] In this study, we also observed that HBP showed almost no correlation with PCT, CRP, IL-6, LAC, APACHE II, and SOFA scores. This suggests that HBP, as a biomarker, could provide unique information for diagnosing sepsis independent of other biomarkers. We hypothesized that establishing a diagnostic model combining HBP with PCT, CRP, IL-6, LAC, APACHE II, SOFA scores, and other indicators could be a new approach for the diagnosis of sepsis. Currently, relevant studies have been conducted in this regard, [34, 35] however, many of the biomarkers mentioned in the above studies have not been widely used in clinical practice, making them less practical. In this study, biomarkers commonly used in clinical settings were included. Based on the ROC analysis of various markers, a sepsis diagnostic model was constructed using multivariable logistic regression. Upon testing, the sepsis diagnostic model exhibited an AUC of > 0.90, indicating its high clinical applicability.

Conclusion

This study confirmed the value of plasma HBP levels in the diagnosis of sepsis in the ICU. It also constructed an sepsis diagnostic model that includes HBP, PCT, CRP, IL-6, and SOFA scores. This model demonstrated a high accuracy and clinical utility,

further enhancing its predictive role in sepsis. It has potential clinical diagnostic value for the detection of sepsis in the ICU.

Notes

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Ethics approval. This retrospective study did not introduce any additional risks. Therefore, informed consent was not obtained from all the participants. Regarding the

collection of blood samples for HBP testing during holidays, the participants in our study were previously provided informed consent for collecting biological samples.

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References

1. Evans, L., et al., *Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021.* Crit Care Med, 2021. **49**(11): p. e1063-e1143.
2. Rudd, K.E., et al., *Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study.* Lancet, 2020. **395**(10219): p. 200-211.
3. Xie, J., et al., *The Epidemiology of Sepsis in Chinese ICUs: A National Cross-Sectional Survey.* Crit Care Med, 2020. **48**(3): p. e209-e218.
4. Im, Y., et al., *Time-to-antibiotics and clinical outcomes in patients with sepsis and septic shock: a prospective nationwide multicenter cohort study.* Crit Care, 2022. **26**(1): p. 19.
5. Kuttab, H.I., et al., *Evaluation and Predictors of Fluid Resuscitation in Patients with Severe Sepsis and Septic Shock.* Crit Care Med, 2019. 47(11): p. 1582-1590.
6. Yang, Y., et al., *A Promising Candidate: Heparin-Binding Protein Steps onto the Stage of Sepsis Prediction.* J Immunol Res, 2019. **2019**: p. 7515346.
7. Pierrakos, C., et al., *Biomarkers of sepsis: time for a reappraisal.* Crit Care, 2020. **24**(1): p. 287.
8. Linder, A., et al., *Heparin-binding protein: an early marker of circulatory failure in sepsis.* Clin Infect Dis, 2009. **49**(7): p. 1044-50.

9. Cai, G., J. Yan, and H. Qiu, *[The standardization of diagnosis and treatment of severe sepsis/septic shock and its practice]*. Zhonghua Nei Ke Za Zhi, 2015. **54**(6): p. 484-5.

10. Shock, et al., *[Chinese expert consensus on diagnosis and management of immunosuppression in sepsis]*. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue, 2020. **32**(11): p. 1281-1289.

11. Linder, A., et al., *Heparin-Binding Protein Measurement Improves the Prediction of Severe Infection With Organ Dysfunction in the Emergency Department.* Crit Care Med, 2015. **43**(11): p. 2378-86.

12. Zhou, Y., et al., *Usefulness of the heparin-binding protein level to diagnose sepsis and septic shock according to Sepsis-3 compared with procalcitonin and C reactive protein: a prospective cohort study in China.* BMJ Open, 2019. **9**(4): p. e026527.

13. Tang J, Yuan H, Wu YL,et al. The Predictive Value of Heparin-Binding Protein and D-Dimer in Patients with Sepsis. Int J Gen Med. 2023 Jun 6; 16:2295-2303.

14. Li S, Xu Y, Wu Y,et al. Heparin-Binding Protein: A Prognostic Biomarker Associated with Severe or Complicated Community-Acquired Pneumonia in Children. J Inflamm Res. 2023 Jan 26; 16:321-331.

15. Chew, M.S., et al., *Increased plasma levels of heparin-binding protein in patients with shock: a prospective, cohort study.* Inflamm Res, 2012. **61**(4): p. 375-9.

16. Llewelyn, M.J., et al., *Sepsis biomarkers in unselected patients on admission to intensive or high-dependency care.* Crit Care, 2013. **17**(2): p. R60.

17. Katsaros, K., et al., *Heparin Binding Protein for the Early Diagnosis and Prognosis of Sepsis in the Emergency Department: The Prompt Multicenter Study.* Shock, 2022. **57**(4):

p. 518-525.

18. Jekarl, D.W., et al., *Procalcitonin as a prognostic marker for sepsis based on SEPSIS-3.* J Clin Lab Anal, 2019. **33**(9): p. e22996.

19. Wu, Y.L., et al., *Accuracy of Heparin-Binding Protein in Diagnosing Sepsis: A Systematic Review and Meta-Analysis.* Crit Care Med, 2021. **49**(1): p. e80-e90.

20. Fisher, J. and A. Linder, *Heparin-binding protein: a key player in the pathophysiology of organ dysfunction in sepsis.* J Intern Med, 2017. **281**(6): p. 562-574.

21. Kjolvmark, C., et al., *Heparin-binding protein: a diagnostic biomarker of urinary tract infection in adults.* Open Forum Infect Dis, 2014. **1**(1): p. ofu004.

22. Linder, A., et al., *Heparin-binding protein: a diagnostic marker of acute bacterial meningitis.* Crit Care Med, 2011. **39**(4): p. 812-7.

23. Saridaki M, Metallidis S, Grigoropoulou S, et al. Integration of heparin-binding protein and interleukin-6 in the early prediction of respiratory failure and mortality in pneumonia by SARS-CoV-2 (COVID-19). Eur J Clin Microbiol Infect Dis. 2021 Jul;40(7):1405-1412.

24. Kong D, Lei Z, Wang Z, et al. A novel HCP (heparin-binding protein -C reactive protein-procalcitonin) inflammatory composite model can predict severe acute pancreatitis. Sci Rep. 2023 Jun 9;13(1):9440.

25. Kong Y, Ye Y, Ma J, et al. Accuracy of heparin-binding protein for the diagnosis of nosocomial meningitis and ventriculitis. Crit Care. 2022 Mar 8;26(1):56.

26. Linder, A., O. Soehnlein, and P. Akesson, *Roles of heparin-binding protein in bacterial infections.* J Innate Immun, 2010. **2**(5): p. 431-8.

27. Kahn, F., et al., *Heparin-Binding Protein as a Prognostic Biomarker of Sepsis and Disease Severity at the Emergency Department.* Shock, 2019. **52**(6): p. e135-e145.

28. Liu, P., et al., *Heparin-binding protein as a biomarker of severe sepsis in the pediatric intensive care unit: A multicenter, prospective study.* Clin Chim Acta, 2023. **539**: p. 26-33.

29. Fisher, J., et al., *Is Heparin-Binding Protein Inhibition a Mechanism of Albumin's Efficacy in Human Septic Shock?* Crit Care Med, 2018. **46**(5): p. e364-e374.

30. Samuelsson, L., et al., *Renal clearance of heparin-binding protein and elimination during renal replacement therapy: Studies in ICU patients and healthy volunteers.* PLoS One, 2019. **14**(8): p. e0221813.

31. Honore, P.M., S. Redant, and D. De Bels, *Reliability of biomarkers of sepsis during extracorporeal therapies: the clinician needs to know what is eliminated and what is not.* Crit Care, 2020. **24**(1): p. 553.

32. Xing, L., et al., *Activation of M1 macrophages in sepsis-induced acute kidney injury in response to heparin-binding protein.* PLoS One, 2018. **13**(5): p. e0196423.

33. Fisher, J., et al., *Heparin-Binding Protein (HBP): A Causative Marker and Potential Target for Heparin Treatment of Human Sepsis-Induced Acute Kidney Injury.* Shock, 2017. **48**(3): p. 313-320.

34. Gibot, S., et al., *Combination biomarkers to diagnose sepsis in the critically ill patient.* Am J Respir Crit Care Med, 2012. **186**(1): p. 65-71.

35. Bauer, P.R., et al., *Diagnostic accuracy and clinical relevance of an inflammatory biomarker panel for sepsis in adult critically ill patients.* Diagn Microbiol Infect Dis, 2016. **84**(2): p. 175-80.

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Tables

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, median (IQR)	9.0 (7.0 - 12.0)	12.0 (9.0 - 16.0)	13.0 (9.00 - 18.0)	16.5 (12.0 - 21.0)	<0.001
SOFA score*, median (IQR)	2.0 (1.0 - 5.0)	4.0 (2.3 - 7.0)	5.0 (3.0 - 7.0)	10.0 (7.0 - 13.0)	<0.001
Length of ICU stay, days median (IQR)	2.0 (1.0 - 4.0)	5.0 (3.0 - 7.8)	6.0 (3.0 - 10.0)	8.0 (4.0 - 13.0)	<0.001

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.

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.

Figure legends

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

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. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Figure 3. A nomogram predicting the risk of sepsis for patients. The value of each of variable was given a score on the point scale axis. A total score could be easily calculated by adding each single score and by projecting the total score to the lower total point scale. We were able to estimate the probability of sepsis. CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment.

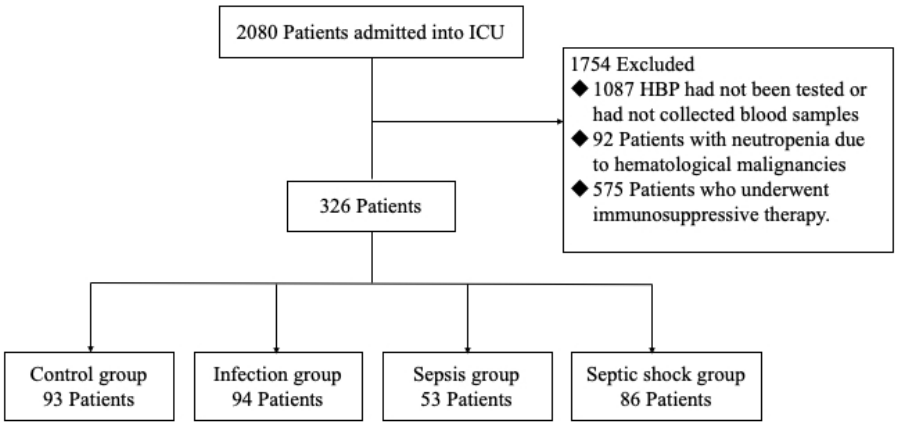

Figure 1. The flow diagram of participants. HBP: heparin-binding protein, ICU: intensive care unit.

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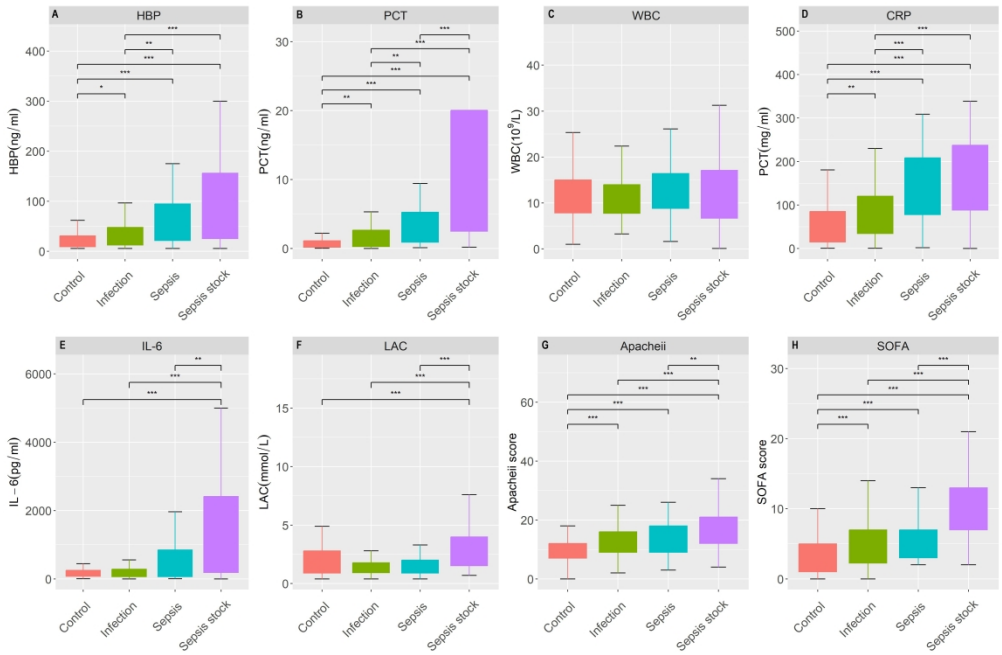

Comparison of plasma levels of biomarkers among different groups. A: HBP, B: PCT, C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: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

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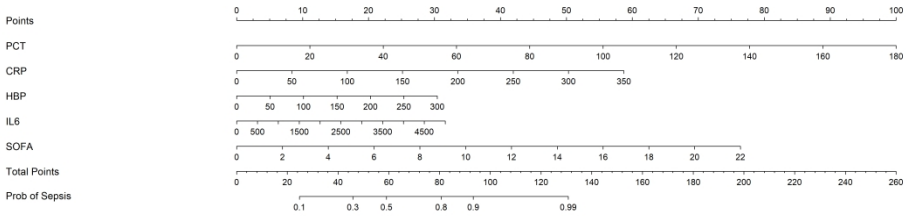

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Supplementary Data

Supplementary Table 1. The comparison of HBP among different sites.

	Infection	Sepsis	Septic shock	*P*
Abdomen, median (IQR)	24.8 (14.0–74.5)	44.7 (25.9–108.0)	78.0 (38.6–156.3.0)	＜0.001
Respiratory median (IQR)	23.2 (10.8–55.3)	55.2 (37.8–73.9)	55.7 (14.1–300)	＜0.001
Blood median (IQR)	9.5*	80.4 (45.1–115.6)	207.6 (176.6–238.6)	＜0.001
Skin and soft tissues median (IQR)	25.5 (19.1–37.3)	27.3 (14.6–41.4)	61.8 (36.2–136)	0.027
Other median (IQR)	18.3 (14.5–22.5)	45.6 (27.0–64.3)	22.6 (19.5–86.7)	0.007
Multi-infection site median (IQR)	22.7 (20.9–32.8)	37.7 (18.0–110.6)	39.0 (23.7–134.6)	0.333

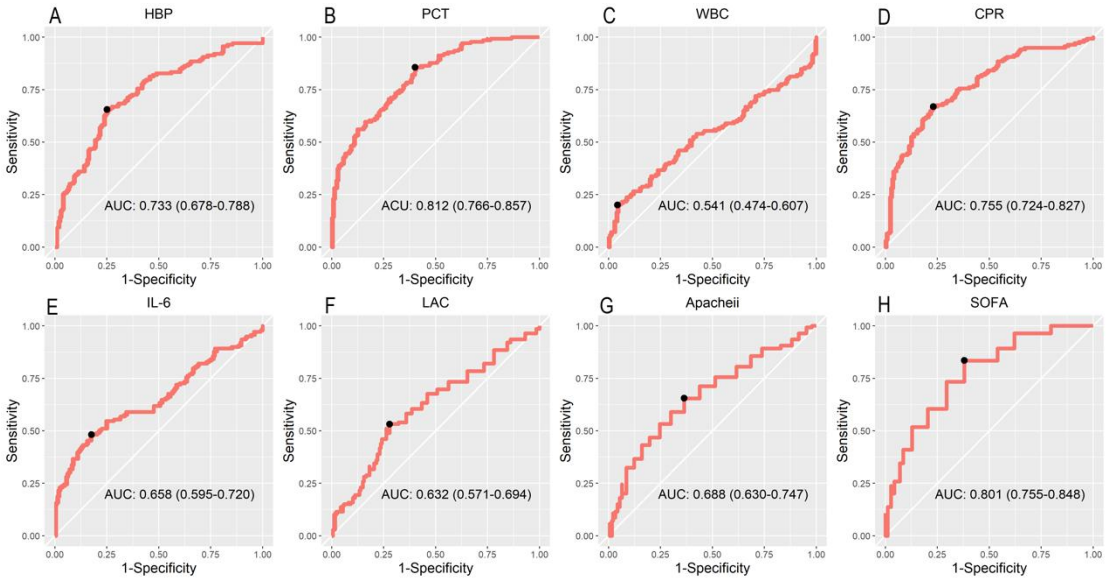
* Only one patient with bloodstream infection in the infection group, IQR: interquartile range.

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

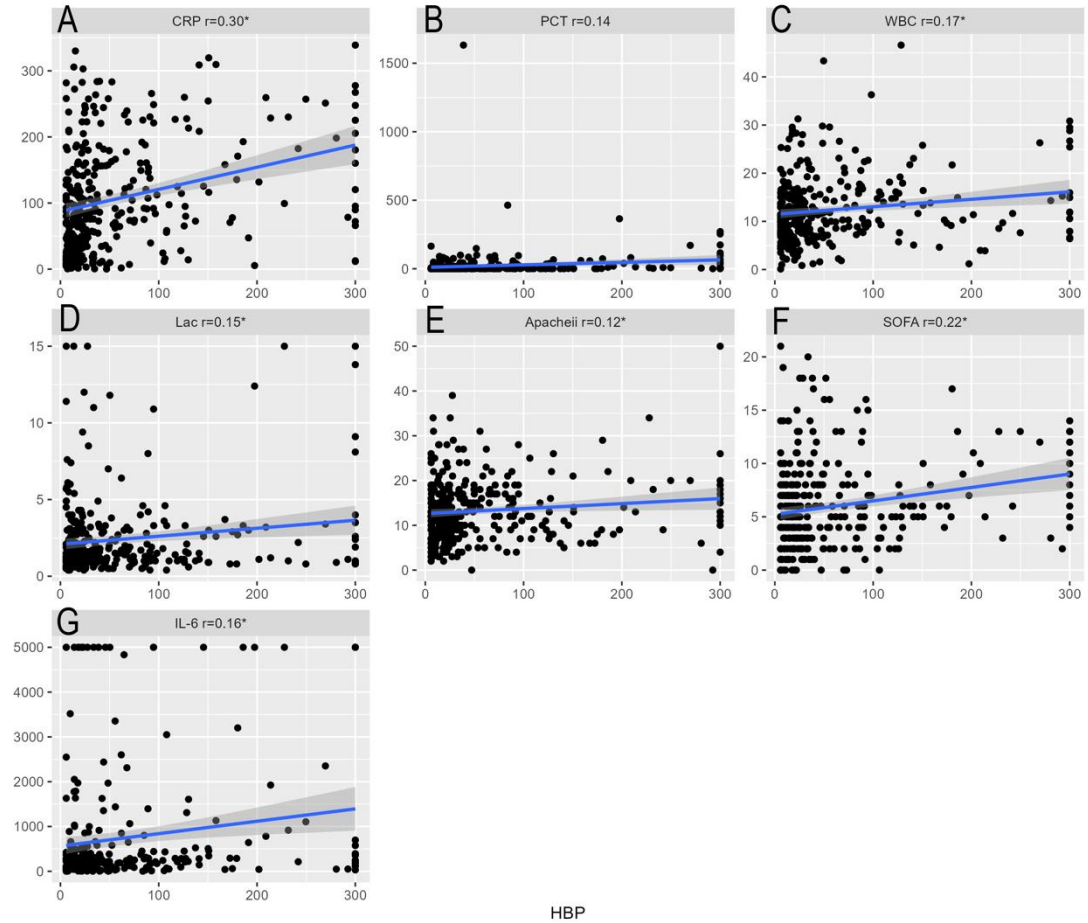
Variable	Univariate logistic regression analysis		Multivariate logistic regression analysis	
	OR (95%CI)	*P*	OR (95%CI)	*P*
Age	1.009 (0.993, 1.026)	0.276		
Sex	1.169 (0.683, 1.999)	0.569		
Hypertension	0.795 (0.450, 1.402)	0.427		
Diabetes	0.801 (0.418, 1.538)	0.505		
Cardiovascular	0.538 (0.288, 1.182)	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

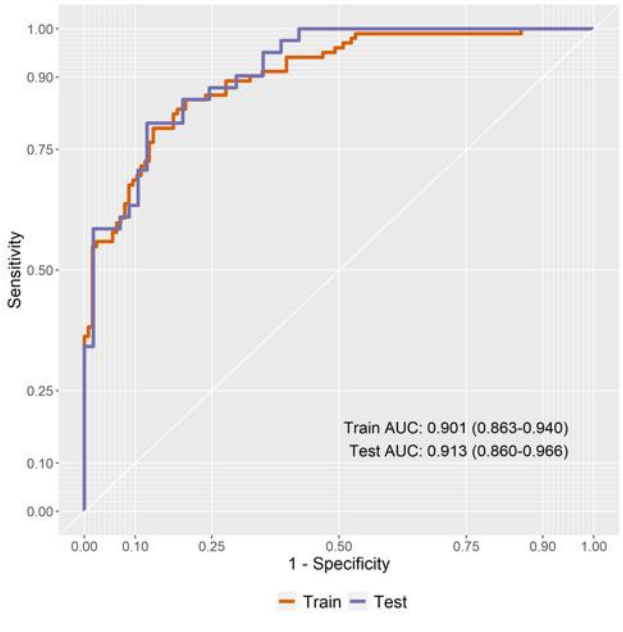
APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count.



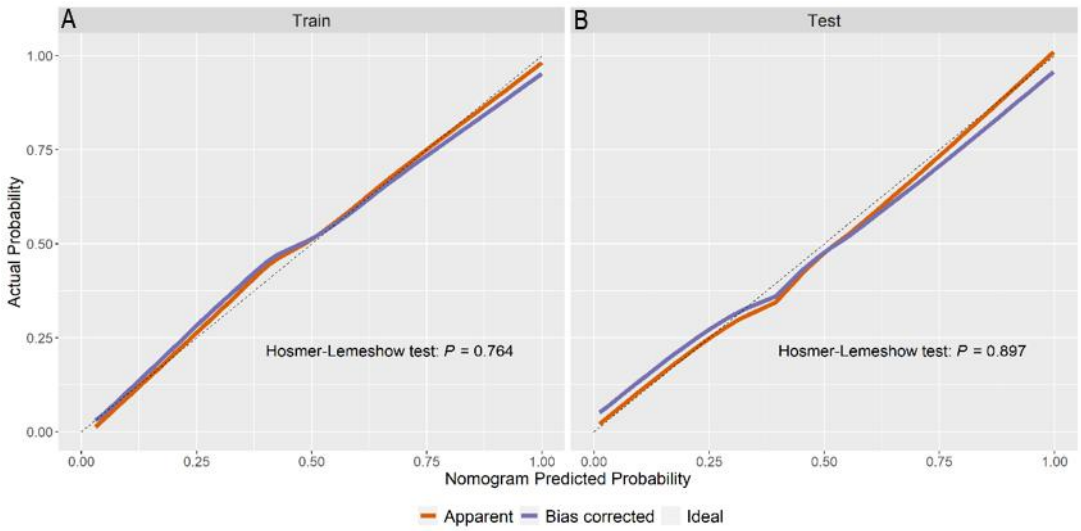
Supplementary Figure 1. ROC curves for biomarkers in distinguishing sepsis from non-sepsis. A: HBP, B: PCT, C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count.



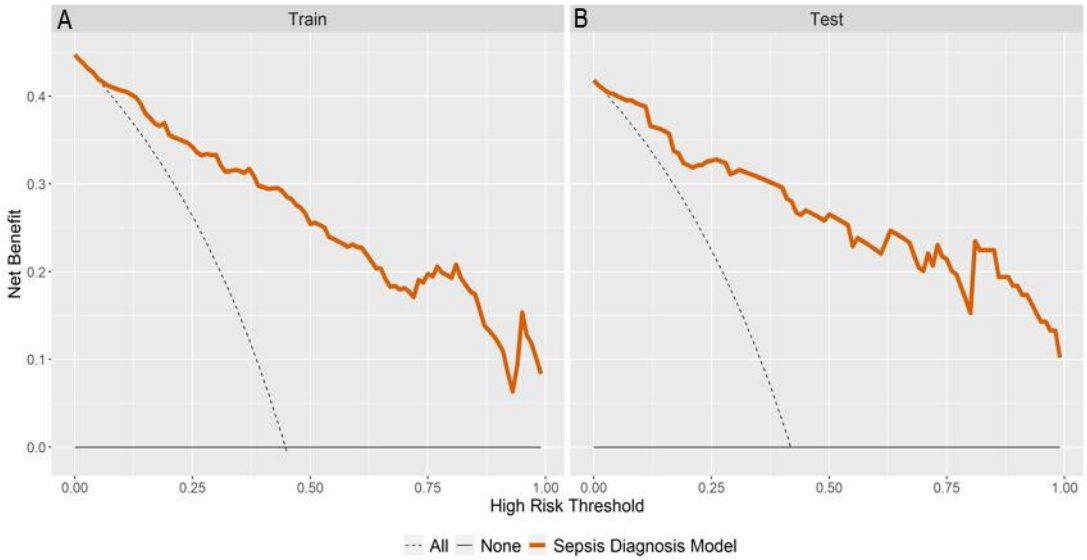
Supplementary Figure 2. The correlations of HBP with CRP (A), PCT (B), WBC (C), LAC (D), APACHE II (E), SOFA (F), and IL-6(G). APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment, WBC: white blood cell count.



Supplementary Figure 3. ROC curve analysis of the sepsis training model and test model.



Supplementary Figure 4. Calibration test of the sepsis diagnostic model. A: training set, B: test set.



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.