

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<u>http://bmjopen.bmj.com</u>).

If you have any questions on BMJ Open's open peer review process please email <u>info.bmjopen@bmj.com</u>

BMJ Open

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

Journal:	BMJ Open
Manuscript ID	bmjopen-2023-078687
Article Type:	Original research
Date Submitted by the Author:	10-Aug-2023
Complete List of Authors:	Zuo, Lingyun; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Li, Xiaoyun; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Wang, Luhao; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Yuan, Hao; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Liao, Zihuai; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Zhou, Si; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Wu, JianFeng; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Uiu, YangDong; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Guan, XiangDong; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Liu, YongJun ; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine
Keywords:	INTENSIVE & CRITICAL CARE, Infection control < INFECTIOUS DISEASES, Adult intensive & critical care < INTENSIVE & CRITICAL CARE





I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our <u>licence</u>.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which <u>Creative Commons</u> licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

terez oni

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies



Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

2	
3	
1	
- -	
ر د	
6	
/	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
10	
17 20	
20 21	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	
31	
32	
33	
34	
25	
26	
20	
3/	
38	
39	
40	
41	
42	
43	
44	
45	
46	
47	
48	
49	
50	
51	
51	
52	
ک ۲	
54	
55	
56	
57	
58	
59	
60	

1

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

Lingyun Zuo*, Xiaoyun Li*, Luhao Wang*, Hao Yuan*, Zihuai Liao, Si Zhou, Jianfeng

Wu, Xiangdong Guan, and Yongjun Liu[†]

6

14

15

16

17

18

19

20

21

22

4

5

7 Department of Critical Care Medicine, The First Affiliated Hospital, Sun Yat-sen

8 University, No. 58, Zhongshan 2nd Road, Guangzhou 510080, Guangdong, China

9 Guangdong Clinical Research Center for Critical Care Medicine, No. 58, Zhongshan

10 2nd Road, Guangzhou 510080, Guangdong, China

11 * These authors contributed equally to this work.

12 † Corresponding author: Yongjun Liu, E-mail: liuyjun3@mail.sysu.edu.cn

13 Manuscript words count: 3627 words.

Abstract

23

1

2	
3	
4	
5	
6	
7	
, Q	
0	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	
31	
32	
33	
34	
35	
36	
37	
20	
20	
10	
40	
41	
42	
43	
44	
45	
46	
4/	
48	
49	
50	
51	
52	
53	
54	
55	
56	
57	
58	
59	
60	

24	Objectives: This study aims to investigate the diagnostic value of heparin-binding
25	protein (HBP) in sepsis and develop a sepsis diagnostic model incorporating HBP with
26	key biomarkers and disease-related scores for an early, rapid, and accurate diagnosis of
27	sepsis.
28	Design: Retrospective cohort study.
29	Setting: A comprehensive teaching tertiary hospital in China.
30	Participants: Adult patients (age≥18years) who had tested HBP in intensive care unit
31	(ICU).
32	Main outcome measures: HBP, C-reactive protein (CRP), procalcitonin (PCT), white
33	blood cell count (WBC), interleukin-6 (IL-6), lactate (LAC), acute physiology and
34	chronic health evaluation II (APACHE II) and sequential organ failure assessment
35	(SOFA) score were recorded.
36	Results: From March 2019 and December 2021, 326 patients were enrolled in this
37	study. The patients were categorized into the non-infection group (control group),
38	infection group, sepsis group, and septic shock group as per the Sepsis-3 criteria. The
39	levels of HBP in the sepsis group and septic shock group were 45.7 and 69.0 ng/mL,
40	significantly higher than those in the control group and infection group, 18.0 and 24.0
41	ng/mL, respectively ($p < 0.001$). The AUC value of HBP for diagnosing sepsis was
42	0.733, which was lower than those corresponding to PCT, CRP, and SOFA, but higher
43	than those of IL-6, LAC, and APACHE II. Multivariate binary logistic regression
44	analysis identified HBP, PCT, CRP, IL-6, and SOFA as valuable indicators for

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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.

61 Keywords: HBP, Sepsis, Diagnostic model

67	Backgrour	ıd
----	-----------	----

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.

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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 diseasespecific scoring systems. Currently, there are over 200 sepsis-related biomarkers

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

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

108 Methods

109 Study Population

110 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.

121 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. Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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 (SpO₂), and urine output were collected. Infection biomarkers such as procalcitonin (PCT), white blood cell count (WBC), C-reactive protein (CRP),

Page 8 of 31

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

> 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 íelie 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

BMJ Open

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 íelien and SPSS 25.0.

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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.

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

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*.

196 The APACHE II and SOFA scores in the sepsis and septic shock groups were 197 significantly higher than those in the control and infection groups. The median length 198 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 < p0.001). 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.

208 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). Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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

223	Analysis of the diagnostic accuracy of different biomarkers for sepsis
224	HBP demonstrated promising diagnostic performance for early detection of sepsis,
225	with an AUC of 0.733 (95% CI, 0.678-0.789), which was higher than AUCs
226	corresponding to IL-6, LAC, and APACHE II scores (AUCs of 0.658, 0.632, and 0.688,
227	respectively), but lower than PCT, CRP, and SOFA scores (AUCs of 0.812, 0.775, and
228	0.801, respectively). When the HBP cut-off value was set at 35.2 ng/mL, the sensitivity
229	and specificity for diagnosing sepsis were 65.5% and 74.9%, respectively (Table 2,
230	Supplementary Figure 1).
231	
232	Relationship between HBP and other biomarkers
233	No significant correlation was observed between HBP levels and CRP, PCT, WBC,
234	IL-6, LAC, APACHE II scores, and SOFA scores (Supplementary Figure 2).
235	
236	Sepsis diagnostic model and test
237	Based on the training set, variables were selected through univariate regression
238	analysis for patient demographics (such as gender, age, underlying diseases, infection
239	sites, and pathogens), infection biomarkers (HBP, PCT, WBC, CRP, IL-6, and LAC),
240	APACHE II scores, and SOFA scores. Variables with statistical significance were
241	included in the multivariate regression model (Supplementary Table 2). Furthermore,
242	insignificant variables were removed from the multivariate model to streamline the

Page 13 of 31

BMJ Open

243 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). Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

Discussion

263 Sepsis is a major cause of mortality in critically ill patients, with high morbidity 264 and mortality. Approximately 20%–30% of severely infected patients do not exhibit

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

265	typical symptoms of organ dysfunction upon admission but rapidly progress to sepsis
266	^[6] . Therefore, early identification of sepsis is crucial for developing appropriate and
267	effective treatment strategies and reducing mortality. Clinicians require more specific
268	and sensitive biomarkers to identify the early diagnosis of sepsis. Currently, WBC, CRP,
269	and PCT are proposed commonly in clinical practice as inflammatory biomarkers [7].
270	However, WBC and CRP are nonspecific markers of systemic inflammation and cannot
271	effectively differentiate among bacterial, non-bacterial, and sterile inflammation. PCT
272	has a higher specificity for bacterial infections but performs poorly in predicting sepsis-
273	associated organ dysfunction ^[6, 16] . In recent years, numerous studies have proven that
274	HBP has good predictive performance for infection, sepsis, or organ function
275	assessment, superior to PCT, CRP, and other biomarkers [6, 8, 11, 12, 17, 18].
276	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

Page 15 of 31

BMJ Open

well as skin and soft tissue infections [6, 8, 11, 19, 20]. However, its clinical use has not vet

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

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

sepsis.

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

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

3		
4		
5		
6		
7		
8		
9		
1	0	
1	1	
1	ว	
1	2	
1	л	
1	-+ 	
1	د م	
1	0	
1	/	
1	8	
1	9	
2	0	
2	1	
2	2	
2	3	
2	4	
2	5	
2	6	
2	7	
2	8	
2	g	
ב 2	ñ	
2	1	
с 2	ו ר	
כ ר	2	
3	3	
3	4	
3	5	
3	6	
3	7	
3	8	
3	9	
4	0	
4	1	
4	2	
4	3	
4	4	
4	5	
4	6	
r ⊿	7	
т Л	2 0	
1	0 0	
+ F	2	
Э г	1	
5	1	
5	2	
5	3	
5	4	
5	5	
5	6	
5	7	
5	8	
5	9	

60

309	found that the AUC of HBP for predicting sepsis was 0.85, with a sensitivity of 87%
310	and specificity of 95%, which were significantly higher than those of PCT, CRP, WBC,
311	IL-6, and other biomarkers [7]. Furthermore, HBP had the ability to predict the
312	occurrence of organ dysfunction and circulatory failure at an early stage, providing
313	indications for timely interventions such as fluid resuscitation and antibiotic use, which
314	were indispensable components of sepsis bundle therapy [7, 11, 22]. In addition, the
315	favorable predictive value of HBP was validated in pediatric patients with severe sepsis
316	^[23] . The emergence of this phenomenon was considered to be related to the pathological
317	process in which HBP was involved in vascular leakage and organ dysfunction in septic
318	patients, and its release occurred earlier than CRP, PCT, and other markers [17, 18, 21]. In
319	this study, the AUC for HBP in predicting sepsis was 0.733, which was not superior to
320	PCT, CRP, and SOFA. Previous studies reported varying diagnostic accuracy of HBP
321	for sepsis at different time points ^[17] . Meta-analyses also revealed that HBP often
322	performs better in diagnosing sepsis in emergency department patients compared with
323	ICU patients [13, 14, 17]. Based on the above analysis, it was considered that a correlation
324	between the more severe condition of ICU patients and the complexity of intervention
325	measures may be the reasons. First, most ICU patients had multiple influencing factors
326	such as surgery, trauma, procedures, and infections. Second, patients received broad-
327	spectrum antibiotics, fluid resuscitation, and other sepsis-related treatments in
328	emergency departments or general wards prior to being transferred to the ICU,
329	indicating a relatively advanced stage of the disease. Lastly, ICU patients had complex
330	medication regimens and multiple intervention measures, such as heparin, albumin, and

Page 17 of 31

BMJ Open

331	CRRT, among others ^[24-28] . All of these factors might potentially affect the plasma level
332	of HBP. Furthermore, this phenomenon also reflected the limitations of a single
333	biomarker, as it could not fully reflect the clinical reality and accurately diagnose sepsis.
334	The pathophysiological mechanisms of sepsis are complex. They involve different
335	immune states, sites of infection, and pathogens. The immune response patterns vary,
336	and so do the pathophysiological processes of various biomarkers. Additionally, the
337	severity of organ dysfunction also varies. During its occurrence and progression, there
338	are always dual factors that simultaneously lead to an exaggerated inflammatory
339	response and immune dysfunction. Systemic inflammatory response and immune
340	suppression do not generally exist as simple independent entities but rather co-exist.
341	Therefore, a single biomarker cannot serve as a reliable diagnostic indicator for sepsis
342	^[7, 10] . In this study, we also observed that HBP showed almost no correlation with PCT,
343	CRP, IL-6, LAC, APACHE II, and SOFA scores. This suggested that HBP, as a
344	biomarker, could provide unique information for the diagnosis of sepsis that was
345	independent of other biomarkers. We hypothesized that establishing a diagnostic model
346	combining HBP with PCT, CRP, IL-6, LAC, APACHE II, SOFA scores, and other
347	indicators could become a new approach for early diagnosis of sepsis. Currently,
348	relevant studies have been conducted in this regard. Gibot et al. found that a biological
349	scoring system combining soluble triggering receptor expressed on myeloid cells-1
350	(sTREM-1), PCT, and CD64 had an AUC of 0.95 for diagnosing sepsis, which was
351	higher than any single marker [29]. Furthermore, a prospective observational study
352	suggested that CRP, PCT, and CD64 were good predictive markers for sepsis, and their

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

2	
3	
4	
5	
6	
7	
8	
a	
10	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
∠ । つつ	
∠∠ วว	
23	
24	
25	
26	
27	
28	
29	
30	
31	
22	
22	
22	
34	
35	
36	
37	
38	
39	
40	
41	
42	
<u>⊿</u> २	
11	
44	
45	
46	
47	
48	
49	
50	
51	
52	
53	
51	
55	
22	
50	
57	
58	
59	
60	

1

353	combination further improved the diagnostic accuracy of sepsis [30]. However, many of
354	the biomarkers mentioned in the above studies have not been widely used in clinical
355	practice, making them less practical. In this study, commonly used biomarkers in
356	clinical settings were included. Based on the ROC analysis of various markers, a sepsis
357	diagnostic model using binary logistic regression was constructed. Upon test, the sepsis
358	diagnostic model exhibited an AUC above 0.90, indicating its high clinical applicability
359	Some limitations of this study should be discussed. First, the study population
360	consisted of patients from a comprehensive ICU, and the model might not be applicable
361	to sepsis patients in the emergency department or general wards. Second, in many septic
362	shock patients, the HBP levels exceeded the upper limit of measurement, which could
363	potentially reduce the statistical differences. Lastly, as a single-center retrospective
364	study, the sample size was relatively small, which affected the statistical power.
365	Subsequent research can be conducted in the form of multi-center prospective studies,
366	involving multiple specialties, and monitoring HBP dynamically to further evaluate its
367	predictive value in sepsis patients.

368 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.

Page 19 of 31

BMJ Open

375	Notes
376	Acknowledgments. We appreciate Yanzhe Xia from the department pharmacy and
377	Kang Liao from the microbiology laboratory for their professional support of this study
378	and their careful interpretation of medication guidance and each specimen's etiological.
379	Author contributions. Study concept and design: Yongjun Liu, and Lingyun Zuo.
380	Definition of the diagnostic algorithm: Yongjun Liu, Jianfeng Wu and Xiangdong Guan.
381	Acquisition and analysis of data: Lingyun Zuo, Xiaoyun Li, Zihuai Liao, and Si Zhou.
382	Interpretation of data: Luhao Wang and Hao Yuan. Drafting of manuscript: Lingyun
383	Zuo, Xiaoyun Li, Luhao Wang, Hao Yuan and Yongjun Liu. Revision of manuscript:
384	all authors.
385	Potential conflicts of interest. All authors: No reported conflicts. All authors have
386	submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts
387	that the editors consider relevant to the content of the manuscript have been disclosed.
388	Financial support. This research received no specific grant from any funding agency
389	in the public, commercial or not-for-profit sectors.
390	References
391	1. Evans, L., et al., Surviving Sepsis Campaign: International Guidelines for Management of
392	Sepsis and Septic Shock 2021. Crit Care Med, 2021. 49(11): p. e1063-e1143.
393	2. Rudd, K.E., et al., <i>Global, regional, and national sepsis incidence and mortality, 1990-</i>
394	2017: analysis for the Global Burden of Disease Study. Lancet, 2020. 395(10219): p. 200-
395	211.
396	3. Xie, J., et al., <i>The Epidemiology of Sepsis in Chinese ICUs: A National Cross-Sectional</i>

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

2			
3	207		Summer Crit Corres Mart 2020 $49(2)$ = -200 -218
4 r	397		<i>Survey</i> . Crit Care Med, 2020. 48 (3): p. e209-e218.
5 6			
0	398	4.	Kumar, A., et al., Duration of hypotension before initiation of effective antimicrobial
/			
8			
9	399		therapy is the critical determinant of survival in human septic shock. Crit Care Med, 2006.
10			
11	100		
12	400		34 (6): p. 1589-96.
13			
14	401	5	Kuttab H L et al Evaluation and Predictors of Fluid Resuscitation in Patients With Severe
15	401	5.	Kuttab, 11.1., et al., Evaluation and Fredetors of Fluid Resuscitation in Fatients with Severe
16			
17	402		Sepsis and Septic Shock, Crit Care Med. 2019, 47(11); p. 1582-1590.
18			
19			
20	403	6.	Yang, Y., et al., A Promising Candidate: Heparin-Binding Protein Steps onto the Stage of
21			
22	40.4		G : D /: /: Llow ol D : 2010 2010 - 751524/
22	404		Sepsis Prediction. J Immunol Res, 2019. 2019: p. 7515346.
23			
24	405	7	Pierrakos C et al Riomarkers of sensis: time for a reappraisal Crit Care 2020 $24(1)$:
25	405	1.	
20			
27	406		p. 287.
28			
29			
30	407	8.	Linder, A., et al., <i>Heparin-binding protein: an early marker of circulatory failure in sepsis.</i>
31			
32	108		Clin Infact Dis 2000 $40(7)$: n 1044 50
33	408		Chin milect Dis, 2009. 49 (7). p. 1044-30.
34			
35	409	9.	Cai, G., J. Yan, and H. Oiu, <i>[The standardization of diagnosis and treatment of severe</i>
36			
37			
38	410		sepsis/septic shock and its practice]. Zhonghua Nei Ke Za Zhi, 2015. 54(6): p. 484-5.
39			
40	411	10	Shools at al IChinasa amout concerning on diagraphic and management of
41	411	10.	Shock, et al., [Chinese expert consensus on allognosis and management of
42			
43	412		<i>immunosuppression in sepsis1</i> . Zhonghua Wei Zhong Bing Ji Jiu Yi Xue, 2020, 32 (11); p.
44			
45			
46	413		1281-1289.
47			
48	414	11	
40 40	414	11.	Linder, A., et al., Heparin-Binding Protein Measurement Improves the Prediction of Severe
50			
51	415		Infection With Organ Dysfunction in the Emergency Department Crit Care Med 2015
50			- general and a general and and general period period. One one mod, 2010.
52			
55	416		43 (11): p. 2378-86.
54 55			
55	417	10	
50	417	12.	Linder, A., et al., Elevated plasma levels of heparin-binding protein in intensive care unit
5/			
58	418		patients with severe sensis and sentic shock Crit Care 2012 16(3): n R90
59	10		panenio min severe sepsis and septie shoek. On Care, 2012. 10(5). p. 130.
60			

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open

3 4 5	419	13.	Chew, M.S., et al., Increased plasma levels of heparin-binding protein in patients with
6 7 °	420		shock: a prospective, cohort study. Inflamm Res, 2012. 61(4): p. 375-9.
8 9 10	421	14.	Llewelyn, M.J., et al., Sepsis biomarkers in unselected patients on admission to intensive
11 12 13	422		or high-dependency care. Crit Care, 2013. 17(2): p. R60.
14 15	423	15.	Singer, M., et al., The Third International Consensus Definitions for Sepsis and Septic
16 17 18	424		Shock (Sepsis-3). JAMA, 2016. 315(8): p. 801-10.
19 20 21	425	16.	Tang, B.M., et al., Accuracy of procalcitonin for sepsis diagnosis in critically ill patients:
22 23	426		systematic review and meta-analysis. Lancet Infect Dis, 2007. 7(3): p. 210-7.
24 25 26	427	17.	Wu, Y.L., et al., Accuracy of Heparin-Binding Protein in Diagnosing Sepsis: A Systematic
27 28	428		Review and Meta-Analysis. Crit Care Med, 2021. 49(1): p. e80-e90.
29 30 31	429	18.	Fisher, J. and A. Linder, Heparin-binding protein: a key player in the pathophysiology of
32 33 34	430		organ dysfunction in sepsis. J Intern Med, 2017. 281(6): p. 562-574.
35 36	431	19.	Kjolvmark, C., P. Akesson, and A. Linder, <i>Elevated urine levels of heparin-binding protein</i>
37 38 39	432		in children with urinary tract infection. Pediatr Nephrol, 2012. 27(8): p. 1301-8.
40 41	433	20.	Linder, A., et al., Heparin-binding protein: a diagnostic marker of acute bacterial
42 43 44	434		meningitis. Crit Care Med, 2011. 39 (4): p. 812-7.
45 46 47	435	21.	Linder, A., O. Soehnlein, and P. Akesson, Roles of heparin-binding protein in bacterial
48 49	436		<i>infections</i> . J Innate Immun, 2010. 2 (5): p. 431-8.
50 51 52	437	22.	Kahn, F., et al., Heparin-Binding Protein as a Prognostic Biomarker of Sepsis and Disease
53 54	438		Severity at the Emergency Department. Shock, 2019. 52(6): p. e135-e145.
55 56 57	439	23.	Liu, P., et al., Heparin-binding protein as a biomarker of severe sepsis in the pediatric
58 59 60	440		intensive care unit: A multicenter, prospective study. Clin Chim Acta, 2023. 539: p. 26-33.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

441	24.	Fisher, J., et al., Is Heparin-Binding Protein Inhibition a Mechanism of Albumin's Efficacy
442		in Human Septic Shock? Crit Care Med, 2018. 46(5): p. e364-e374.
443	25.	Samuelsson, L., et al., Renal clearance of heparin-binding protein and elimination during
444		renal replacement therapy: Studies in ICU patients and healthy volunteers. PLoS One,
445		2019. 14 (8): p. e0221813.
446	26.	Honore, P.M., S. Redant, and D. De Bels, Reliability of biomarkers of sepsis during
447		extracorporeal therapies: the clinician needs to know what is eliminated and what is not.
448		Crit Care, 2020. 24 (1): p. 553.
449	27.	Xing, L., et al., Activation of M1 macrophages in sepsis-induced acute kidney injury in
450		response to heparin-binding protein. PLoS One, 2018. 13(5): p. e0196423.
451	28.	Fisher, J., et al., Heparin-Binding Protein (HBP): A Causative Marker and Potential Target
452		for Heparin Treatment of Human Sepsis-Induced Acute Kidney Injury. Shock, 2017. 48(3):
453		p. 313-320.
454	29.	Gibot, S., et al., Combination biomarkers to diagnose sepsis in the critically ill patient. Am
455		J Respir Crit Care Med, 2012. 186 (1): p. 65-71.
456	30.	Bauer, P.R., et al., Diagnostic accuracy and clinical relevance of an inflammatory
457		biomarker panel for sepsis in adult critically ill patients. Diagn Microbiol Infect Dis, 2016.
458		84 (2): p. 175-80.
459		
460	Tables	
461	Table 1	. Characteristics of the patients.

3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	
31	
3Z	
33	
35	
36	
37	
38	
39	
40	
41	
42	
43	
44	
45	
46	
47	
48	
49	
50	
51	
52 52	
52	
54	
55	
57	
58	
59	
60	

	Control	Infection	Sepsis	Septic shock	Р
	(n = 93)	(n = 94)	(n = 53)	(n = 86)	
Age, years,	56	63	58	64	0.023
median (IQR)	(45.0–69.0)	(51.0–73.8)	(49.0–70.0)	(53.0–70.0)	
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

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

Fungi	3 (3.2)	17 (18.1)	14 (26.4)	38 (44.1)	< 0.001
APACHE II score,	9.0	12.0	13.0	16.5	< 0.001
median (IQR)					
	(7.0–12.0)	(9.0–16.0)	(9.00–18.0)	(12.0–21.0)	
SOFA score,	2.0	4.0	5.0	10.0	< 0.001
median (IQR)					
	(1.0–5.0)	(2.3–7.0)	(3.0–7.0)	(7.0–13.0)	
Length of ICU stay, days	2.0	5.0	6.0	8.0	
					< 0.001
median (IQR)	(1.0-4.0)	(3.0–7.8)	(3.0–10.0)	(4.0–13.0)	
3-day improvement, n (%)	88 (94.6)	83 (88.3)	47 (88.7)	64 (74.4)	0.001
28-day overall mortality, n (%)	3 (3.2)	9 (9.6)	6 (11.3)	28 (32.6)	< 0.001

462 APACHE II score: acute physiology and chronic health evaluation II score, ICU: intensive care

463 unit, IQR: interquartile range, SOFA score: sequential organ failure assessment score.

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

		Cut-off	Sensitivity	Specificity	PPV	NPV
	AUC (95% CI)	value	(%)	(%)	(%)	(%)
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
РСТ	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

2	
3	
4	
5	
6	
7	
/	
8 Q	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
10	
20	
20 21	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	
31	
32	
22	
27 21	
34 25	
35	
30	
37	
38	
39	
40	
41	
42	
43	
44	
45	
46	
47	
48	
<u>4</u> 0	
50	
50	
51	
52	
53	
54	
55	
56	
57	
58	
59	
60	

ge 25 of 31	BMJ Open
466	APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP:
467	heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: procalcitonin, SOFA:
468	sequential organ failure assessment, WBC: white blood cell count.
469	
470	Figure legends
471	Figure 1. Comparison of plasma levels of biomarkers among different groups. A: HBP, B: PCT,
472	C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and
473	chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood
474	lactic acid, PCT: procalcitonin, IL-6: procalcitonin, SOFA: sequential organ failure assessment,
475	WBC: white blood cell count. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
476	Figure 2. A nomogram predicting the risk of spesis for patients. The value of each of variable was

477 given a score on the point scale axis. A total score could be easily calculated by adding each single Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

score and by projecting the total score to the lower total point scale. We were able to estimate the 478

probability of Spesis. CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin, 479

IL-6: procalcitonin, SOFA: sequential organ failure assessment. 480

481 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.

448x296mm (300 x 300 DPI)

Page 27 of 31

1	
2	
3	
4	
5	
6	
7	
8	Points
9	PCT 0 20 40 60 80 100 120 140 160 180
10	СКР ² 0 50 100 150 200 250 300 350 НВР
11	0 50 100 150 200 250 300 IL6
12	SOFA 0 2 4 6 8 10 12 14 16 18 20 22
13	Total Points 0 20 40 60 80 100 120 140 160 180 200 220 240 260
14	Prod di Sepais 0.1 0.3 0.5 0.8 0.9 0.99
15	
16	
17	Figure 2. A nomogram predicting the risk of spesis for patients. The value of each of variable was given a
18	score on the point scale axis. A total score could be easily calculated by adding each single score and by
19	projecting the total score to the lower total point scale. We were able to estimate the probability of Spesis.
20	CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin, IL-6: procalcitonin, SOFA:
21	sequential organ failure assessment.
22	423x127mm (300 x 300 DPI)
23	
24	
25	
26	
20	
28	
29	
30	
31	
32	
32	
34	
35	
36	
37	
38	
39	
40	
41	
42	
43	
44	
45	
46	
47	
48	
49	
50	
51	
52	
53	
54	
55	
56	
57	
58	
59	
60	For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Page 28 of 31





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

82x82mm (150 x 150 DPI)

Supplementary Data

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

	Infection	Sepsis	Septic shock	Р
Abdomen,	24.8	44.7	78.0	< 0.
median (IQR)	(14.0–74.5)	(25.9–108.0)	(38.6–156.3.0)	
Respiratory	23.2	55.2	55.7	< 0.
median (IQR)	(10.8–55.3)	(37.8–73.9)	(14.1–300)	
Blood	9.5*	80.4	207.6	< 0.
median (IQR)		(45.1–115.6)	(176.6–238.6)	
Skin and soft tissues	25.5	27.3	61.8	0.02
median (IQR)	(19.1–37.3)	(14.6–41.4)	(36.2–136)	
Other	18.3	45.6	22.6	0.00
median (IQR)	(14.5–22.5)	(27.0–64.3)	(19.5–86.7)	
Multi-infection site	22.7	37.7	39.0	0.33
median (IQR)	(20.9–32.8)	(18.0–110.6)	(23.7–134.6)	

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

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

Variable	β	Ζ	Р	OR (95%CI)
Intercept	-3.833	-7.29	<0.001	0.022 (0.008, 0.061)
РСТ	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)

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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)

7 CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin, IL-6: procalcitonin,







11 Supplementary Figure 1. ROC curves for biomarkers in distinguishing sepsis from non-sepsis. A:



13 physiology and chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding

14 protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: procalcitonin, SOFA: sequential organ





Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.



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







training set, B: test set.

BMJ Open

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

Journal:	BMJ Open
Manuscript ID	bmjopen-2023-078687.R1
Article Type:	Original research
Date Submitted by the Author:	15-Jan-2024
Complete List of Authors:	Zuo, Lingyun; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Li, Xiaoyun; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Wang, Luhao; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Yuan, Hao; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Liao, Zihuai; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Zhou, Si; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Wu, JianFeng; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Guan, XiangDong; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Liu, YongJun ; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine
Primary Subject Heading :	Diagnostics
Secondary Subject Heading:	Diagnostics
Keywords:	INTENSIVE & CRITICAL CARE, Infection control < INFECTIOUS DISEASES, Adult intensive & critical care < INTENSIVE & CRITICAL CARE

SCHOLARONE[™] Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our <u>licence</u>.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which <u>Creative Commons</u> licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

terez oni

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies


1	Heparin-binding protein as a biomarker for early diagnosis
2	of sepsis in the intensive care unit: a retrospective cross-
3	sectional study in China
4	Lingyun Zuo*, Xiaoyun Li*, Luhao Wang*, Hao Yuan*, Zihuai Liao, Si Zhou, Jianfeng
5	Wu, Xiangdong Guan, and Yongjun Liu [†]
6	
7	Department of Critical Care Medicine, The First Affiliated Hospital, Sun Yat-sen
8	University, No. 58, Zhongshan 2nd Road, Guangzhou 510080, Guangdong, China
9	Guangdong Clinical Research Center for Critical Care Medicine, No. 58, Zhongshan
10	2nd Road, Guangzhou 510080, Guangdong, China
11	* These authors contributed equally to this work.
12	† Corresponding author: Yongjun Liu, E-mail: liuyjun3@mail.sysu.edu.cn
13	Manuscript words count: 3213 words.
14	
15	
16	
17	
18	
19	
20	
21	
22	

Abstract

1

2	
3	
4	
5	
6	
0	
/	
8	
9	
10	
11	
12	
12	
13	
14	
15	
16	
17	
18	
19	
20	
∠∪ 21	
21	
22	
23	
24	
25	
26	
27	
27	
28	
29	
30	
31	
32	
33	
31	
54 25	
22	
36	
37	
38	
39	
40	
<u>1</u>	
40	
4Z	
43	
44	
45	
46	
47	
48	
<u>4</u> 0	
50	
50	
51	
52	
53	
54	
55	
56	
50	
5/	
58	
59	
60	

Objectives: This study aims to investigate the diagnostic value of heparin-binding 24 25 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 26 sepsis in the intensive care unit (ICU). 27 **Design:** Clinical retrospective cross-sectional study. 28 Setting: A comprehensive teaching tertiary hospital in China. 29 Participants: Adult patients (age>18years) who had tested HBP or whose blood 30 31 samples had been collected when admitted to ICU. Main outcome measures: HBP, C-reactive protein (CRP), procalcitonin (PCT), white 32 blood cell count (WBC), interleukin-6 (IL-6), lactate (LAC), acute physiology and 33 34 chronic health evaluation II (APACHE II) and sequential organ failure assessment (SOFA) score were recorded. 35 Results: From March 2019 and December 2021, 326 patients were enrolled in this 36 37 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 38 levels of HBP in the sepsis group and septic shock group were 45.7 and 69.0 ng/mL, 39 significantly higher than those in the control group and infection group, 18.0 and 24.0 40 ng/mL, respectively (p < 0.001). The AUC value of HBP for diagnosing sepsis was 41 0.733, which was lower than those corresponding to PCT, CRP, and SOFA, but higher 42 43 than those of IL-6, LAC, and APACHE II. Multivariate logistic regression analysis

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

44 identified HBP, PCT, CRP, IL-6, and SOFA as valuable indicators for diagnosing

45	sepsis. A sepsis diagnostic model was constructed based on these indicators, whose
46	AUC was 0.901, with a sensitivity of 79.7% and specificity of 86.9%.
47	Conclusions : HBP could serve as a biomarker for early diagnosis of sepsis in the ICU.
48	Compared with single indicators, the sepsis diagnostic model constructed with HBP,
49	PCT, CRP, IL-6, and SOFA further enhanced the diagnostic performance of sepsis.
50	
51	Strengths and limitations of this study
52	• This study included a highly heterogeneous population, making it highly
53	applicable to sepsis patients in ICU.
54	• Moreover, most of the biomarkers included in this diagnostic model were widely
55	used in clinical practice, making them easily obtainable, highly reproducible, and
56	operationally feasible.
57	• This study was an ICU single-center retrospective research, the results might not
58	be applicable to sepsis patients in other settings.
59	• The SOFA scores in the study were absolute values automatically obtained by the
60	electronic scoring system, rather than the delta values.
61	• Its design did not allow for the determination of causal relationships.
62	
63	Keywords: HBP, Sepsis, Diagnostic model
64	
65	
66	

67	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.

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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 diseasespecific scoring systems. Currently, there are over 200 sepsis-related biomarkers reported in the literature, among which heparin-binding protein (HBP) is a novel

BMJ Open

89	biomarker. [7] HBP is a serine protease-like protein secreted by neutrophils after
90	infection and has functions such as altering endothelial cell permeability, antimicrobial
91	activity chemotaxis and regulation of cell apontosis [8] It has been identified as an
71	activity, chemotaxis, and regulation of cen apoptosis. [6] it has been identified as an
92	early diagnostic indicator for severe sepsis/septic shock in Chinese Guidelines for the
93	Management of Severe Sepsis/Septic Shock (2014) [9] and Chinese Expert Consensus
94	on Early Prevention and Interruption of Sepsis in Emergency Medicine (2020). [10] In
95	addition, an increasing number of studies had furnished evidence regarding the use of
96	HBP for diagnosing sepsis in recent years. The results demonstrated that HBP could be
97	used for sepsis diagnosis and monitoring the severity. [8, 11, 12] On the other hand, a
98	few studies had indicated that elevated levels of HBP irrespective of infectious etiology
99	and no correlation with severity and outcome. [13] Furthermore, differences and
100	inconsistencies have been noted among various studies in regard to the diagnostic
101	performance of HBP of sepsis. [14, 15] Therefore, it remains controversial to use HBP
102	for the early diagnosis of sepsis. The aim of this study was to analysis the early
103	diagnostic value of HBP in sepsis and to develop a sepsis diagnostic model combining
104	HBP with multiple biomarkers and disease-specific scoring systems retrospectively, in
105	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

Page 7 of 31

BMJ Open

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.

123 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 μ L of supernatants were collected for plasma level of HBP determination using an immunofluorescence dry quantitative method (JetiStar3000, Hangzhou, Joinstar Biomedical Technology Co,.LTD). The procedure strictly followed the instructions provided with the reagent kit, and the quality control was performed well. Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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.

- 140 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

BMJ Open

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. REVIE 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,

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

and other comorbidities.

176 The control group consisted of postoperative recovery patients from various

BMJ Open

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

191 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). Page 11 of 31

BMJ Open

199	When comparing HBP levels among different infection sites in the infection,
200	sepsis, and septic shock groups, statistical differences were observed among the
201	subgroups except for multi-infection site (Supplementary Table 1). As the severity of
202	infection increased, APACHE II and SOFA scores gradually increased, showing
203	statistical differences. However, no statistical difference was observed when comparing
204	the infection group with the sepsis group (Figure 2).
205	
206	Analysis of the diagnostic accuracy of different biomarkers for sepsis
207	HBP demonstrated promising diagnostic performance for early detection of sepsis,
208	with an AUC of 0.733 (95% CI 0.678-0.789), which was significantly higher than WBC
209	(AUC 0.541, 95% CI 0.474-0.607) and higher than the AUCs of IL-6, LAC, and
210	APACHE II scores (0.658, 0.632, and 0.688, respectively) but not statistical
211	significantly. The AUC of HBP was significantly lower than PCT (AUC 0.812, 95%CI
212	0.766-0.857). When the HBP cut-off value was set at 35.2 ng/mL, the sensitivity,
213	specificity, PPV and NPV for diagnosing sepsis were 65.5%, 74.9%, 65.9% and 74.5%,
214	respectively (Table 2, Supplementary Figure 1).
215	
216	Relationship between HBP and other biomarkers
217	No significant correlation was observed between HBP levels and CRP, PCT, WBC,
218	IL-6, LAC, APACHE II scores, and SOFA scores (Supplementary Figure 2).
219	
220	Construction of a sepsis diagnostic model

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

3		
4		
5		
6		
7		
8		
9		
1	0	
1	1	
1	2	
1	3	
1	4	
1	5	
1	6	
1	7	
1	8	
1	9	
2	0	
2	1	
2	2	
2	3	
2	4	
2	5	
2	6	
2	7	
2	8	
2	9	
3	0	
3	1	
3	2	
3 7	ک ۸	
3 7	4 7	
3 ว	с С	
с 2	07	
с С	/ 0	
2 2	o o	
⊃ ∧	9 0	
-+ ⊿	0 1	
- Л	י כ	
1 4	3	
4	4	
4	5	
4	6	
4	7	
4	8	
4	9	
5	0	
5	1	
5	2	
5	3	
5	4	
5	5	
5	6	
5	7	
5	8	
5	9	

60

1 2

221	Based on the training set, variables were selected through univariate logistic
222	regression analysis for patient demographics (such as gender, age, underlying diseases,
223	infection sites, and pathogens), infection biomarkers (HBP, PCT, WBC, CRP, IL-6,
224	and LAC), APACHE II scores, and SOFA scores. Variables with statistical significance
225	$(p \le 0.05)$ were included in the multivariate logistic regression model (Supplementary
226	Table 2). Among the statistically significant variables in the univariate analysis were
227	HBP、PCT、CRP、IL-6、LAC、APACHE II、SOFA. The final multivariate
228	logistic regression results showed that PCT (OR = 1.034, 95%CI 1.009-1.060, $p =$
229	0.009), CRP (OR = 1.011, 95%CI 1.006-1.016, $p < 0.001$), HBP (OR = 1.006,
230	95%CI 1.000-1.012, $p = 0.041$), IL-6 (OR = 1.001 95%CI 1.000-1.001, $p = 0.013$)
231	SOFA (OR = 1.252, 95%CI 1.110-1.412, $p < 0.001$) were significantly associated
232	with sepsis diagnosis. The sepsis diagnostic model was constructed based on the results
233	of logistic regression that was shown in Figure 3.

234

235 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

BMJ Open

(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). Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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-

BMJ Open

3	
4	
5	
6	
7	
, 0	
ð	
9	
10	
11	
12	
12	
13	
14	
15	
16	
17	
18	
10	
17	
20	
21	
22	
23	
24	
25	
25	
26	
27	
28	
29	
30	
21	
21	
32	
33	
34	
35	
36	
27	
27	
38	
39	
40	
41	
42	
43	
10	
44	
45	
46	
47	
48	
49	
50	
50	
21	
52	
53	
54	
55	
56	
50	
5/	
58	
50	

286

60

1 2

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 268 stored in the secretory granules of neutrophils and azurophilic granules. It contains a 269 large number of positively charged amino acid residues, which are concentrated on one 270 side of the protein. [18] A hydrophobic pocket structure formed by amino acid residues 271 20-44 exhibits a high affinity for endotoxins. [6] Therefore, HBP is initially discovered 272 273 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 274 infection and inflammatory response. [6, 18] These characteristics make HBP a 275 276 promising novel infection biomarker. Recent studies have reported that HBP could assist in the diagnosis of various diseases, such as respiratory and circulatory failure, 277 sepsis, acute kidney injury, acute lung injury, meningitis, urinary tract infections, as 278 well as skin and soft tissue infections. [6, 8, 11, 19, 20] However, its clinical use has 279 not yet been widely adopted, so further clinical research is required to validate its utility. 280 281 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 282 and whether infected patients had sepsis. Furthermore, its discriminative value was 283 found to be superior to LAC, IL-6, WBC, SOFA, and APACHE II scores. Similar 284

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

Page 15 of 31

BMJ Open

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

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

309	disease course was relatively later; although the detection of HBP or the collection of
310	blood samples occurred upon admission to the ICU, the onset time was still later than
311	emergency cases. Meta-analyses also revealed that HBP often performed better in
312	diagnosing sepsis in emergency department patients compared with ICU patients. [13,
313	14, 17] Unlike previous studies, this research involved ICU patients rather than
314	emergency patients. First, the control group in this study consisted not only of healthy
315	individuals but mostly of surgical postoperative recovery patients. Additionally, ICU
316	patients had more complex conditions, more severe organ damage, and require life
317	support such as ventilators, vasopressors, continuous renal replacement therapy
318	(CRRT), etc. Finally, patients had already received various treatments such as fluid
319	resuscitation and antibiotics in the emergency room or ward. [24-28] In summary, these
320	conditions might have some impact on HBP levels, but this study population was more
321	representative of the actual situations of ICU patients. From another perspective, this
322	phenomenon also reflected the limitations of a single biomarker, as it could not fully
323	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 Page 17 of 31

BMJ Open

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.

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies

353 Notes

354	Acknowledgments. We appreciate Yanzhe Xia from the department pharmacy and
355	Kang Liao from the microbiology laboratory for their professional support of this study
356	and their careful interpretation of medication guidance and each specimen's etiological.
357	Author contributions. Study concept and design: Yongjun Liu, and Lingyun Zuo.
358	Definition of the diagnostic algorithm: Yongjun Liu, Jianfeng Wu and Xiangdong Guan.
359	Acquisition and analysis of data: Lingyun Zuo, Xiaoyun Li, Zihuai Liao, and Si Zhou.
360	Interpretation of data: Luhao Wang and Hao Yuan. Drafting of manuscript: Lingyun
361	Zuo, Xiaoyun Li, Luhao Wang, Hao Yuan and Yongjun Liu. Revision of manuscript:
362	all authors.
363	Potential conflicts of interest. All authors: No reported conflicts. All authors have
364	submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts
365	that the editors consider relevant to the content of the manuscript have been disclosed.
366	Financial support. This research received no specific grant from any funding agency
367	in the public, commercial or not-for-profit sectors.
368	Patient and public involvement. Patients and/or the public were not involved in the
369	design, or conduct, or reporting, or dissemination plans of this research.
370	<i>Ethics approval.</i> This was a retrospective study that did not create any additional
371	risks. Therefore, we did not obtain informed consent from the participants. Regarding
372	the collection of blood samples for HBP testing during the holiday, the participants in
373	our study had previously provided informed consent for the collection of biological
374	samples.

2 3 4 5	375	Prove	nance and peer review. Not commissioned; externally peer reviewed.					
6 7 8	376	Date availability statement. Date are available upon reasonable request.						
9 10	377	References						
11 12 13	378	1.	Evans, L., et al., Surviving Sepsis Campaign: International Guidelines for Management of					
14 15 16	379		Sepsis and Septic Shock 2021. Crit Care Med, 2021. 49(11): p. e1063-e1143.					
17 18	380	2.	Rudd, K.E., et al., Global, regional, and national sepsis incidence and mortality, 1990-					
19 20 21	381		2017: analysis for the Global Burden of Disease Study. Lancet, 2020. 395(10219): p. 200-					
22 23 24	382		211.					
25 26	383	3.	Xie, J., et al., The Epidemiology of Sepsis in Chinese ICUs: A National Cross-Sectional					
27 28 29	384		<i>Survey</i> . Crit Care Med, 2020. 48 (3): p. e209-e218.					
30 31 32	385	4.	Im, Y., et al., <i>Time-to-antibiotics and clinical outcomes in patients with sepsis and septic</i>					
33 34	386		shock: a prospective nationwide multicenter cohort study. Crit Care, 2022. 26 (1): p. 19.					
35 36 37	387	5.	Kuttab, H.I., et al., Evaluation and Predictors of Fluid Resuscitation in Patients With					
38 39 40	388	_	Severe Sepsis and Septic Shock. Crit Care Med, 2019. 47(11): p. 1582-1590.					
41 42	389	6.	Yang, Y., et al., A Promising Candidate: Heparin-Binding Protein Steps onto the Stage of					
43 44	390		<i>Sepsis Prediction</i> . J Immunol Res, 2019. 2019 : p. 7515346.					
45 46 47	391	7.	Pierrakos, C., et al., <i>Biomarkers of sepsis: time for a reappraisal</i> . Crit Care, 2020. 24(1):					
48 49 50	392		p. 287.					
50 51 52	393	8.	Linder, A., et al., <i>Heparin-binding protein: an early marker of circulatory failure in sepsis.</i>					
53 54 55	394		Clin Infect Dis, 2009. 49 (7): p. 1044-50.					
56 57 58	395	9.	Cai, G., J. Yan, and H. Qiu, [The standardization of diagnosis and treatment of severe					
59 60	396		sepsis/septic shock and its practice]. Zhonghua Nei Ke Za Zhi, 2015. 54(6): p. 484-5.					

BMJ Open

2	
- २	
1	
-	
5	
0	
/	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
17	
10	
19	
20	
21	
22	
23	
24	
25	
26	
27	
28	
29	
30	
30	
21	
5Z	
33	
34	
35	
36	
37	
38	
39	
40	
41	
42	
43	
Δ <i>Λ</i>	
44	
40 AC	
40	
4/	
48	
49	
50	
51	
52	
53	
54	
55	
56	
50	
5/	
20	
59	
60	

1

397	10.	Shock, et al., [Chinese expert consensus on diagnosis and management of
398		immunosuppression in sepsis]. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue, 2020. 32(11): p.
399		1281-1289.
400	11.	Linder, A., et al., Heparin-Binding Protein Measurement Improves the Prediction of Severe
401		Infection With Organ Dysfunction in the Emergency Department. Crit Care Med, 2015.
402		43 (11): p. 2378-86.
403	12.	Zhou, Y., et al., Usefulness of the heparin-binding protein level to diagnose sepsis and
404		septic shock according to Sepsis-3 compared with procalcitonin and C reactive protein: a
405		prospective cohort study in China. BMJ Open, 2019. 9(4): p. e026527.
406	13.	Chew, M.S., et al., Increased plasma levels of heparin-binding protein in patients with
407		shock: a prospective, cohort study. Inflamm Res, 2012. 61(4): p. 375-9.
408	14.	Llewelyn, M.J., et al., Sepsis biomarkers in unselected patients on admission to intensive
409		or high-dependency care. Crit Care, 2013. 17(2): p. R60.
410	15.	Katsaros, K., et al., Heparin Binding Protein for the Early Diagnosis and Prognosis of
411		Sepsis in the Emergency Department: The Prompt Multicenter Study. Shock, 2022. 57(4):
412		p. 518-525.
413	16.	Jekarl, D.W., et al., Procalcitonin as a prognostic marker for sepsis based on SEPSIS-3. J
414		Clin Lab Anal, 2019. 33 (9): p. e22996.
415	17.	Wu, Y.L., et al., Accuracy of Heparin-Binding Protein in Diagnosing Sepsis: A Systematic
416		Review and Meta-Analysis. Crit Care Med, 2021. 49(1): p. e80-e90.
417	18.	Fisher, J. and A. Linder, Heparin-binding protein: a key player in the pathophysiology of
418		organ dysfunction in sepsis. J Intern Med, 2017. 281(6): p. 562-574.

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open

2			
3		10	
4	419	19.	Kjolvmark, C., et al., Heparin-binding protein: a diagnostic biomarker of urinary tract
5			
7	420		infection in adults. Open Forum Infect Dis, 2014. 1(1): p. ofu004.
8			
9	421	20	Linder A et al Heparin-hinding protein a diagnostic marker of acute bacterial
10	121	20.	Ender, M., et al., heparationaling protein. a angliostic marker of acate bacteria
11			
12	422		<i>meningitis</i> . Crit Care Med, 2011. 39 (4): p. 812-7.
13			
14	423	21.	Linder, A., O. Soehnlein, and P. Akesson, Roles of heparin-binding protein in bacterial
15 16			
10 17	121		infactions Linnots Immun 2010 2(5): n 421 8
17	424		<i>injections</i> . J filliate fillinuli, 2010. 2 (3). p. 431-8.
10			
20	425	22.	Kahn, F., et al., Heparin-Binding Protein as a Prognostic Biomarker of Sepsis and Disease
21			
22	426		Severity at the Emergency Department Shock 2019 52(6): n e135-e145
23	.20		Solution of the line general D open interim. Subset, $2013 \cdot C^{2}(0)$, p . $0100 \cdot 0110$.
24			
25	427	23.	Liu, P., et al., Heparin-binding protein as a biomarker of severe sepsis in the pediatric
26			
27	428		intensive care unit: A multicenter, prospective study. Clin Chim Acta, 2023. 539: p. 26-33.
28			
29	420	24	Fisher L at al La Hongyin Pinding Protain Lubibition a Machaniam of Albumin's Efficant
30	429	24.	Fisher, J., et al., Is repartit-binding Frotein Innibition a Mechanism of Albumin's Efficacy
32			
33	430		in Human Septic Shock? Crit Care Med, 2018. 46(5): p. e364-e374.
34			
35	431	25	Samuelsson L et al Renal clearance of hengrin-binding protein and elimination during
36			
37	120		
38	432		renal replacement therapy: Studies in ICU patients and healthy volunteers. PLoS One,
39			
40	433		2019. 14 (8): p. e0221813.
41 42			
42 43	434	26	Honore PM S Redant and D De Bels <i>Reliability of biomarkers of sensis during</i>
44	тJт	20.	Honore, T.M., S. Redant, and D. De Beis, Reliability of blomarkers of sepsis during
45			
46	435		extracorporeal therapies: the clinician needs to know what is eliminated and what is not.
47			
48	436		Crit Care, 2020. 24 (1): p. 553.
49			
50	127	27	Ving I at all definition of MI manual ages in sensio induced south hidron initial in
51	437	27.	Aing, L., et al., Activation of M1 macrophages in sepsis-induced deute klaney injury in
52			
55 57	438		response to heparin-binding protein. PLoS One, 2018. 13(5): p. e0196423.
55			
55		• •	
50	439	28	Fisher, J., et al., Heparin-Binding Protein (HBP): A Causative Marker and Potential Target
50 57	439	28.	Fisher, J., et al., Heparin-Binding Protein (HBP): A Causative Marker and Potential Target
50 57 58	439	28.	Fisher, J., et al., <i>Heparin-Binding Protein (HBP): A Causative Marker and Potential Target</i>
50 57 58 59	439 440	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):

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

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. gr. Bauer, P.R., et al., Diagnostic accuracy and clinical relevance of an inflammatory 30. 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 $(n = 86)$	Р
Age, years,	56	63	58	64	0.023
median (IQR)	(45.0–69.0)	(51.0–73.8)	(49.0–70.0)	(53.0–70.0)	
Sex, male, n (%)	50 (53.8)	64 (68.1)	34 (64.2)	53 (61.6)	0.237
Comorbidity, n (%)					
Hypertension	30 (32.3)	38 (40.4)	15 (28.3)	29 (33.7)	0.459
Diabetes	15 (16.1)	25 (26.6)	10 (18.9)	15 (17.4)	0.281
Cardiovascular	21 (22.6)	24 (25.5)	5 (9.4)	15 (17.4)	0.100
Liver disease	3 (3.2)	3 (3.2)	3 (5.7)	5 (5.8)	0.739
Malignant tumor	34 (36.6)	36 (38.3)	18 (34.0)	42 (48.8)	0.243
Others	26 (28.0)	47 (50.0)	15 (28.3)	37 (43.0)	0.005
Source of infection, n (%)					
Abdomen		31 (33.0)	30 (56.6)	63 (73.3)	< 0.001
Respiratory	-	46 (48.9)	17 (32.1)	23 (26.7)	0.006
Blood	-	4 (4.3)	8 (15.1)	16 (18.6)	0.009
Skin and soft tissues	-	16 (17.0)	5 (9.4)	8 (9.3)	0.220
Others	-	6 (6.4)	8 (15.1)	5 (5.8)	0.109
Pathogens, n (%)					
Escherichia coli	3 (3.2)	9 (9.6)	9 (17.0)	24 (27.9)	< 0.001
Klebsiella genus	1 (1.1)	8 (8.5)	8 (15.1)	14 (16.3)	0.003
Other Enterobacteriaceae	2 (2.2)	2 (2.1)	4 (7.6)	9 (10.5)	0.030
Pseudomonas aeruginosa	1 (1.1)	5 (5.3)	7 (13.2)	9 (10.5)	0.015
Acinetobacter baumannii	1 (1.1)	7 (7.5)	4 (7.6)	4 (4.7)	0.112
Stenotrophomonas maltophilia	1 (1.1)	2 (2.1)	1 (1.9)	11 (12.8)	0.001
Enterococcus	1 (1.1)	8 (8.5)	9 (17.0)	19 (22.1)	< 0.001
Other Gram-negative bacteria	1 (1.1)	0 (0.0)	2 (3.8)	9 (10.5)	0.001
Staphylococcus	1 (1.1)	12 (12.8)	5 (9.4)	7 (8.1)	0.024
Streptococcus	2 (2.2)	1 (1.1)	1 (1.9)	3 (3.5)	0.752
Anaerobic bacteria	1 (1.1)	1 (1.1)	1 (1.9)	4 (4.7)	0.377
Fungi	3 (3.2)	17 (18.1)	14 (26.4)	38 (44.1)	< 0.001
APACHE II score,	9.0	12.0	13.0	16.5	< 0.001
median (IQR)	(7.0–12.0)	(9.0–16.0)	(9.00–18.0)	(12.0–21.0)	
SOFA score*,	2.0	4.0	5.0	10.0	< 0.001
median (IQR)	(1.0-5.0)	(2.3 - 7.0)	(3.0 - 7.0)	(7.0 - 13.0)	

Length of ICU stay, days 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

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

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

** * 1 1		Cut-off	Sensitivity	Specificity	PPV	NPV	Р
variable	AUC (95% CI)	value	(%)	(%)	(%)	(%)	
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
РСТ	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

471 APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP:

472 heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA:

473 sequential organ failure assessment, WBC: white blood cell count. The *P* values between AUCs

474 compared to HBP.

BMJ Open

481	Figure legends
482	Figure 1. The flow diagram of participants. HBP: heparin-binding protein, ICU: intensive care unit.
483	Figure 2. Comparison of plasma levels of biomarkers among different groups. A: HBP, B: PCT,
484	C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and
485	chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood
486	lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment,
487	WBC: white blood cell count. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
488	Figure 3. A nomogram predicting the risk of sepsis for patients. The value of each of variable was
489	given a score on the point scale axis. A total score could be easily calculated by adding each single
490	score and by projecting the total score to the lower total point scale. We were able to estimate the
491	probability of sepsis. CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin,
492	IL-6: interleukin-6, SOFA: sequential organ failure assessment.





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

338x190mm (54 x 54 DPI)



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.

448x296mm (300 x 300 DPI)

BMJ Open: first published as 10.1136/bmjopen-2023-078687 on 10 June 2024. Downloaded from http://bmjopen.bmj.com/ on June 7, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES)

Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

1	
2	
3	
5	
4	
5	
6	
7	
, 0	Points 0 10 20 30 40 50 60 70 80 90 10
8	PCT
9	CRP 0 20 40 60 80 100 120 140 160 180
10	HBP 0 50 100 150 200 250 300 350
11	
10	
12	Total Points 0 20 40 40 80 100 120 140 180 20 220 240 240
13	Prob of Sepsis 0,1 0,3 0,5 0,8 0,9 0,99
14	
15	
16	
17	Figure 3 A nomogram predicting the risk of sensis for patients. The value of each of variable was given a
17	score on the point scale axis. A total score could be assily calculated by adding each single score and by
18	scole doin the point scale dxis. A total scole could be easily calculated by adding each single scole and by
19	projecting the total score to the lower total point scale, we were able to estimate the probability of sepsis.
20	CRP: C-reactive protein, HBP: heparin-binding protein, PC1: procactionin, 12-6: interieukin-6, SOFA:
21	sequential organ failure assessment.
21	
22	423x12/mm (300 x 300 DPI)
23	
24	
25	
25	
26	
27	
28	
29	
20	
30	
31	
32	
33	
34	
25	
55	
36	
37	
38	
30	
10	
40	
41	
42	
43	
11	
45	
45	
46	
47	
48	
10	
49	
50	
51	
52	
53	
55	
54 	
55	
56	
57	
58	
50	
50	For peer review only - http://bmionon.hmi.com/site/about/guidelines.yhtml
60	For peer review only "http://binjopen.binj.com/site/about/guidennes.xittmi

1 Supplementary Data

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

	Infection	Sepsis	Septic shock	Р
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

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

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

7 sepsis diagnosis.

	Univariate logistic reg	Multivariate logistic regression		
Variable	analysis	analysis		
_	OR (95%CI)	Р	OR (95%CI)	Р
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		

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.



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

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







BMJ Open

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

Journal:	BMJ Open
Manuscript ID	bmjopen-2023-078687.R2
Article Type:	Original research
Date Submitted by the Author:	26-Mar-2024
Complete List of Authors:	Zuo, Lingyun; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Li, Xiaoyun; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Wang, Luhao; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Yuan, Hao; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Liao, Zihuai; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Zhou, Si; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Wu, JianFeng; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Guan, XiangDong; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Liu, YongJun ; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine
Primary Subject Heading :	Diagnostics
Secondary Subject Heading:	Diagnostics
Keywords:	INTENSIVE & CRITICAL CARE, Infection control < INFECTIOUS DISEASES, Adult intensive & critical care < INTENSIVE & CRITICAL CARE

SCHOLARONE[™] Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our <u>licence</u>.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which <u>Creative Commons</u> licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

terez oni

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

1	Heparin-binding protein as a biomarker for early diagnosis
2	of sepsis in the intensive care unit: a retrospective cross-
3	sectional study in China
4	Lingyun Zuo*, Xiaoyun Li*, Luhao Wang*, Hao Yuan*, Zihuai Liao, Si Zhou, Jianfeng
5	Wu, Xiangdong Guan, and Yongjun Liu [†]
6	
7	Department of Critical Care Medicine, the First Affiliated Hospital, Sun Yat-sen
8	University, No. 58, Zhongshan 2nd Road, Guangzhou 510080, Guangdong, China
9	Guangdong Clinical Research Center for Critical Care Medicine, No. 58, Zhongshan
10	2nd Road, Guangzhou 510080, Guangdong, China
11	* These authors contributed equally to this work.
12	† Corresponding author: Yongjun Liu, E-mail: liuyjun3@mail.sysu.edu.cn
13	Manuscript words count: 3167 words.
14	
15	
16	
17	
18	
19	
20	
21	

2
3
4
5
6
7
/ 0
ð
9
10
11
12
13
14
15
16
17
18
19
20
20 21
∠ ı วว
22
23
24
25
26
27
28
29
30
31
32
32
27
25
22
36
3/
38
39
40
41
42
43
44
45
46
47
т/ ЛО
+0 40
49
50
51
52
53
54
55
56
20
57
57 58
57 58 59

23 Abstract **Objectives**: This study aims to investigate the diagnostic value of heparin-binding 24 25 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 26 27 sepsis in the intensive care unit (ICU). **Design:** Clinical retrospective cross-sectional study. 28 Setting: A comprehensive teaching tertiary hospital in China. 29 30 **Participants:** Adult patients (age \geq 18years) who underwent HBP testing or whose 31 blood samples were collected when admitted to the ICU. Main outcome measures: HBP, C-reactive protein (CRP), procalcitonin (PCT), white 32 blood cell count (WBC), interleukin-6 (IL-6), lactate (LAC), acute physiology and 33 34 chronic health evaluation II (APACHE II), and sequential organ failure assessment (SOFA) score were recorded. 35 Results: Between March 2019 and December 2021, 326 patients were enrolled in this 36 37 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 38 HBP levels in the sepsis group and septic shock group were 45.7 and 69.0 ng/mL, 39 respectively, which were significantly higher than those in the control group (18.0 40 ng/mL) and infection group (24.0 ng/mL) (p < 0.001). The AUC value of HBP for 41 diagnosing sepsis was 0.733, which was lower than those corresponding to PCT, CRP, 42 and SOFA but higher than those of IL-6, LAC, and APACHE II. Multivariate logistic 43

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

44 regression analysis identified HBP, PCT, CRP, IL-6, and SOFA as valuable indicators

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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
67 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.

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

89	(HBP) is a novel biomarker. [7] HBP is a serine protease-like protein secreted by
90	neutrophils after infection that has functions such as altering endothelial cell
91	permeability, antimicrobial activity, chemotaxis, and regulation of cell apoptosis. [8] It
92	has been identified as an early diagnostic indicator for severe sepsis/septic shock in
93	Chinese Guidelines for the Management of Severe Sepsis/Septic Shock (2014) [9] and
94	Chinese Expert Consensus on Early Prevention and Interruption of Sepsis in
95	Emergency Medicine (2020). [10] In addition, an increasing number of studies have
96	recently provided evidence regarding the use of HBP for diagnosing sepsis. The results
97	demonstrate that HBP could be used for sepsis diagnosis and severity monitoring. [8,
98	11-14] On the other hand, a few studies have indicated that elevated levels of HBP
99	irrespective of infectious etiology and no correlation with severity and outcome. [15]
100	Furthermore, differences and inconsistencies have been noted among various studies
101	regarding the diagnostic performance of HBP in sepsis. [16, 17] Therefore, it remains
102	controversial to use HBP for the early diagnosis of sepsis. This study aimed to analyze
103	the early diagnostic value of HBP in sepsis and develop a sepsis diagnostic model
104	combining HBP with multiple biomarkers and disease-specific scoring systems
105	retrospectively to facilitate early identification and diagnosis of sepsis in the ICU.

107 Methods

Study population

109 This study included 2080 patients who were admitted to the ICU of the First110 Affiliated Hospital of Sun Yat-sen University, China, from March 2019 to December

Page 7 of 32

BMJ Open

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.

123 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 μ L of supernatants were collected for plasma level of HBP determination using an immunofluorescence dry quantitative method (JetiStar3000, Hangzhou, Joinstar Biomedical Technology Co., LTD). The procedure strictly followed the instructions provided with the reagent kit, and the quality control was performed well. Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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

BMJ Open

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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.

140 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 cutoff 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

BMJ Open

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. Lien

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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.

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

> 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).

192 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

BMJ Open

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).

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

221	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 - 1.016)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.

5 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

BMJ Open

(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). Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

255 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

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

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 Page 15 of 32

BMJ Open

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

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

Page 16 of 32

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

310	sepsis at different time points. [19] In this study, the disease course was relatively later.
311	Although detecting HBP or collecting blood samples occurred upon admission to the
312	ICU, the onset time was still later than that in emergency cases. Meta-analyses also
313	revealed that HBP often performed better in diagnosing sepsis in emergency department
314	patients compared with ICU patients. [15, 16, 19] Unlike previous studies, this study
315	involved ICU patients rather than emergency patients. First, the control group in this
316	study consisted of surgical postoperative recovery patients without infection.
317	Additionally, ICU patients have more complex conditions, have more severe organ
318	damage, and require life support, such as ventilators, vasopressors, and continuous renal
319	replacement therapy (CRRT). Finally, the patients already received various treatments,
320	such as fluid resuscitation and antibiotics in the emergency room or ward. [29-33] In
321	summary, these conditions might have some impact on HBP levels, but this study
322	population was more representative of the actual situation of ICU patients. From
323	another perspective, this phenomenon also reflects the limitations of a single biomarker,
324	as it could not fully reflect the clinical reality and accurately diagnose sepsis in the ICU.
325	The pathophysiological mechanisms that underlie sepsis are complex. They are
326	involved in different immune states, sites of infection, and pathogens. Immune response
327	patterns vary, as do the pathophysiological processes of various biomarkers. During its
328	occurrence and progression, dual factors that simultaneously lead to an exaggerated
329	inflammatory response and immune dysfunction. Systemic inflammatory responses and
330	immune suppression do not generally exist as simple independent entities but rather
331	coexist. Therefore, a single biomarker cannot serve as a reliable diagnostic indicator for

Page 17 of 32

BMJ Open

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.

344 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. Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies

351 Notes

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

> Acknowledgments. We appreciate Yanzhe Xia from the department of pharmacy and Kang Liao from the microbiology laboratory for their professional support of this study and their careful interpretation of medication guidance and each specimen's etiology. Author contributions. Study concept and design: Yongjun Liu, and Lingvun 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. Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts

Financial support. This research received no specific grants from any funding agency
in the public, commercial or not-for-profit sectors.

that the editors consider relevant to the content of the manuscript have been disclosed.

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

Ethics approval. This retrospective study did not introduce any additional risks.

369 Therefore, informed consent was not obtained from all the participants. Regarding the

370 collection of blood samples for HBP testing during holidays, the participants in our

371 study were previously provided informed consent for collecting biological samples.

Provenance and peer review. Not commissioned; externally peer reviewed.

Date availability statement. Date are available upon reasonable request.

1 2	
3 4 5	374
6 7	375
8 9 10	376
11 12 13	377
14 15	378
16 17 18	379
19 20	380
21 22 23	381
24 25 26	382
27 28	383
29 30 31	384
32 33	385
34 35 36	386
37 38	387
40 41	388
42 43 44	389
45 46	390
47 48 49	391
50 51	392
52 53 54	393
55 56 57	
57 58 59	
60	

374	Refere	nces
375	1.	Evans, L., et al., Surviving Sepsis Campaign: International Guidelines for Management of
376		Sepsis and Septic Shock 2021. Crit Care Med, 2021. 49(11): p. e1063-e1143.
377	2.	Rudd, K.E., et al., Global, regional, and national sepsis incidence and mortality, 1990-
378		2017: analysis for the Global Burden of Disease Study. Lancet, 2020. 395(10219): p. 200-
379		211.
380	3.	Xie, J., et al., The Epidemiology of Sepsis in Chinese ICUs: A National Cross-Sectional
381		<i>Survey</i> . Crit Care Med, 2020. 48 (3): p. e209-e218.
382	4.	Im, Y., et al., Time-to-antibiotics and clinical outcomes in patients with sepsis and septic
383		shock: a prospective nationwide multicenter cohort study. Crit Care, 2022. 26(1): p. 19.
384	5.	Kuttab, H.I., et al., Evaluation and Predictors of Fluid Resuscitation in Patients with Severe
385		Sepsis and Septic Shock. Crit Care Med, 2019. 47(11): p. 1582-1590.
386	6.	Yang, Y., et al., A Promising Candidate: Heparin-Binding Protein Steps onto the Stage of
387		Sepsis Prediction. J Immunol Res, 2019. 2019: p. 7515346.
388	7.	Pierrakos, C., et al., Biomarkers of sepsis: time for a reappraisal. Crit Care, 2020. 24(1):
389		p. 287.
390	8.	Linder, A., et al., Heparin-binding protein: an early marker of circulatory failure in sepsis.
391		Clin Infect Dis, 2009. 49 (7): p. 1044-50.
392	9.	Cai, G., J. Yan, and H. Qiu, [The standardization of diagnosis and treatment of severe
393		sepsis/septic shock and its practice]. Zhonghua Nei Ke Za Zhi, 2015. 54(6): p. 484-5.

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

3	
Δ	
5	
2	
0	
/	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
17	
18	
19	
20	
21	
22	
23	
24	
25	
26	
27	
27	
20	
29	
30	
31	
32	
33	
34	
35	
36	
27	
20	
20	
39	
40	
41	
42	
43	
44	
45	
46	
47	
-77 ΛQ	
40	
49	
50	
51	
52	
53	
54	
55	
56	
57	
52	
20	
59	
bU	

1 2

394	10.	Shock, et al., [Chinese expert consensus on diagnosis and management of
395		immunosuppression in sepsis]. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue, 2020. 32(11): p.
396		1281-1289.
397	11.	Linder, A., et al., Heparin-Binding Protein Measurement Improves the Prediction of Severe
398		Infection With Organ Dysfunction in the Emergency Department. Crit Care Med, 2015.
399		43 (11): p. 2378-86.
400	12.	Zhou, Y., et al., Usefulness of the heparin-binding protein level to diagnose sepsis and
401		septic shock according to Sepsis-3 compared with procalcitonin and C reactive protein: a
402		prospective cohort study in China. BMJ Open, 2019. 9(4): p. e026527.
403	13.	Tang J, Yuan H, Wu YL, et al. The Predictive Value of Heparin-Binding Protein and D-
404		Dimer in Patients with Sepsis. Int J Gen Med. 2023 Jun 6; 16:2295-2303.
405	14.	Li S, Xu Y, Wu Y, et al. Heparin-Binding Protein: A Prognostic Biomarker Associated
406		with Severe or Complicated Community-Acquired Pneumonia in Children. J Inflamm
407		Res. 2023 Jan 26; 16:321-331.
408	15.	Chew, M.S., et al., Increased plasma levels of heparin-binding protein in patients with
409		shock: a prospective, cohort study. Inflamm Res, 2012. 61(4): p. 375-9.
410	16.	Llewelyn, M.J., et al., Sepsis biomarkers in unselected patients on admission to intensive
411		or high-dependency care. Crit Care, 2013. 17(2): p. R60.
412	17.	Katsaros, K., et al., Heparin Binding Protein for the Early Diagnosis and Prognosis of

- 6 414 p. 518-525.

413

19

Sepsis in the Emergency Department: The Prompt Multicenter Study. Shock, 2022. 57(4):

BMJ Open

2			
3			
4	415	18.	Jekarl, D.W., et al., Procalcitonin as a prognostic marker for sepsis based on SEPSIS-3. J
5			
6	416		Clin I ah Anal 2019 33 (9): n e22996
7	410		Chil Edo Anal, 2019. 55 (9). p. 622996.
8			
9	417	19.	Wu, Y.L., et al., Accuracy of Heparin-Binding Protein in Diagnosing Sepsis: A Systematic
10			
11	110		Pavian and Mota Anglusia Crit Core Mod 2021 40(1): p. 200 200
12	410		Review and Meta-Analysis. Chi Cale Med, 2021. 49(1). p. e80-e90.
13			
14	419	20.	Fisher, J. and A. Linder, <i>Heparin-binding protein: a key player in the pathophysiology of</i>
15			
16			
17	420		organ dysfunction in sepsis. J Intern Med, 2017. 281(6): p. 562-574.
18			
19	421	21.	Kiolymark, C., et al., Heparin-binding protein: a diagnostic biomarker of urinary tract
20			
21			
22	422		<i>infection in adults</i> . Open Forum Infect Dis, 2014. 1 (1): p. ofu004.
23			
24	123	22	Linder A et al Hangrin-binding protein: a diagnostic marker of acute bacterial
25	420	22.	Ender, A., et al., Tepartit-binding protein. a augnostic marker of acute bacteria
20			
2/	424		meningitis. Crit Care Med, 2011. 39(4): p. 812-7.
20			
29	105	22	Souidalei M. Matallidia S. Crigoropoulou S. et al. Integration of honorin hinding matein
20 21	425	23.	Sandaki M, Metanidis S, Grigoropoulou S, et al. Integration of neparin-binding protein
30			
32	426		and interleukin-6 in the early prediction of respiratory failure and mortality in pneumonia
34			
35	407		
36	427		by SARS-CoV-2 (COVID-19). Eur J Clin Microbiol Infect Dis. 2021 Jul;40(7):1405-
37			
38	428		1412.
39			
40			
41	429	24.	Kong D, Lei Z, Wang Z, et al. A novel HCP (heparin-binding protein -C reactive protein-
42			
43	430		procalcitonin) inflammatory composite model can predict severe acute pancreatitis. Sci
44			
45			
46	431		Rep. 2023 Jun 9;13(1):9440.
47			
48	132	25	Kong V. Ve V. Ma I. et al. Accuracy of henarin-binding protein for the diagnosis of
49	702	23.	Kong 1, 10 1, Ma J, et al. Accuracy of heparin-binding protein for the diagnosis of
50			
51	433		nosocomial meningitis and ventriculitis. Crit Care. 2022 Mar 8;26(1):56. (诊断脑炎)
52			
53	121	26	Linder A O Soehnlein and P Akesson Roles of hencerin hinding protein in basterial
54	-0-	20.	Emaci, M., O. Soomnom, and T. Akosson, Roles of neparin-binaing protein in bacterial
55			
56	435		<i>infections</i> . J Innate Immun, 2010. 2 (5): p. 431-8.
57			
58			
59			
60			

BMJ Open

3 4 5	436	27.	Kahn, F., et al., Heparin-Binding Protein as a Prognostic Biomarker of Sepsis and Disease
6 7	437		Severity at the Emergency Department. Shock, 2019. 52(6): p. e135-e145.
8 9 10	438	28.	Liu, P., et al., Heparin-binding protein as a biomarker of severe sepsis in the pediatric
11 12	439		intensive care unit: A multicenter, prospective study. Clin Chim Acta, 2023. 539: p. 26-33.
13 14 15	440	29.	Fisher, J., et al., Is Heparin-Binding Protein Inhibition a Mechanism of Albumin's Efficacy
16 17 18	441		in Human Septic Shock? Crit Care Med, 2018. 46(5): p. e364-e374.
19 20	442	30.	Samuelsson, L., et al., Renal clearance of heparin-binding protein and elimination during
21 22 23	443		renal replacement therapy: Studies in ICU patients and healthy volunteers. PLoS One,
24 25 26	444		2019. 14 (8): p. e0221813.
27 28	445	31.	Honore, P.M., S. Redant, and D. De Bels, Reliability of biomarkers of sepsis during
29 30 31	446		extracorporeal therapies: the clinician needs to know what is eliminated and what is not.
32 33	447		Crit Care, 2020. 24 (1): p. 553.
34 35 36	448	32.	Xing, L., et al., Activation of M1 macrophages in sepsis-induced acute kidney injury in
37 38 30	449		response to heparin-binding protein. PLoS One, 2018. 13(5): p. e0196423.
40 41	450	33.	Fisher, J., et al., Heparin-Binding Protein (HBP): A Causative Marker and Potential Target
42 43 44	451		for Heparin Treatment of Human Sepsis-Induced Acute Kidney Injury. Shock, 2017. 48(3):
45 46	452		p. 313-320.
47 48 49	453	34.	Gibot, S., et al., Combination biomarkers to diagnose sepsis in the critically ill patient. Am
50 51 52	454		J Respir Crit Care Med, 2012. 186(1): p. 65-71.
53 54	455	35.	Bauer, P.R., et al., Diagnostic accuracy and clinical relevance of an inflammatory
55 56 57	456		biomarker panel for sepsis in adult critically ill patients. Diagn Microbiol Infect Dis, 2016.
58 59 60	457		84 (2): p. 175-80.

1 2 3 4 5 6	458			
7 8 9 10 11 12 12				
14 15 16 17 18 19				Protected by co
20 21 22 23 24 25 26				pyright, including
20 27 28 29 30 31 32				Enseignemen for uses related to
33 34 35 36 37 38 39				t Superieur (ABES) text and data mini
40 41 42 43 44 45) . ng, Al training, and
46 47 48 49 50 51 52				d similar technolog
53 54 55 56 57 58				gies.
59 60				

Tables

Table 1. Characteristics of the patients.

	Control (n = 93)	Infection $(n = 94)$	Sepsis $(n = 53)$	Septic shock (n = 86)	Р
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
<i>Fungi</i> APACHE II score, median (IQR)	3 (3.2) 9.0 (7.0 - 12.0)	17 (18.1) 12.0 (9.0 - 16.0)	14 (26.4) 13.0 (9.00 - 18.0)	38 (44.1) 16.5 (12.0 - 21.0)	<0.001 <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

BMJ Open

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

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

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

Variable		Cut-off	Sensitivity	Specificity	PPV	NPV	Р
Variable	AUC (95% CI)	value	(%)	(%)	(%)	(%)	
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
РСТ	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

467 APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP:

468 heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA:

469 sequential organ failure assessment, WBC: white blood cell count. The *P* values between AUCs

470 compared to HBP.

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

472 Figure legends

- **Figure 1.** The flow diagram of participants. HBP: heparin-binding protein, ICU: intensive care unit.
- 474 Figure 2. Comparison of plasma levels of biomarkers among different groups. A: HBP, B: PCT,
- 475 C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and
- 476 chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood
- 477 lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment,
- 478 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.

338x190mm (54 x 54 DPI)

BMJ Open: first published as 10.1136/bmjopen-2023-078687 on 10 June 2024. Downloaded from http://bmjopen.bmj.com/ on June 7, 2025 at Agence Bibliographique de I Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open





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: Creactive 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.

448x296mm (300 x 300 DPI)

Page 29 of 32

1	
2	
3	
4	
5	
6	
7	
8	Points
9	PCT 0 20 40 60 80 100 120 140 160 180
10	CRP 0 50 100 150 200 250 300 350
11	0 50 100 150 200 250 300 IL6
12	0 500 1500 2500 3600 4500 SOFA 0 2 4 6 8 10 12 14 16 18 20 22
12	Total Points 0 20 40 60 80 100 120 140 160 180 200 220 240 260
14	Prob of Sepsis 0.1 0.3 0.5 0.8 0.9 0.99
15	
15	
17	Figure 3. A nomogram predicting the risk of sepsis for patients. The value of each of variable was given a
17	score on the point scale axis. A total score could be easily calculated by adding each single score and by
10	projecting the total score to the lower total point scale. We were able to estimate the probability of sepsis.
20	CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin, IL-6: interleukin-6, SOFA:
20	sequential organ failure assessment.
21	(122v127mm (200 v 200 DDI)
22	423X12711111 (SUU X SUU DP1)
25	
24	
25	
26	
27	
28	
29	
30	
31	
32	
33	
34	
35	
36	
37	
38	
39	
40	
41	
42	
43	
44	
45	
46	
47	
48	
49	
50	
51	
52	
53	
54	
55	
56	
57	
58	
59	
60	For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

1 Supplementary Data

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

	Infection	Sepsis	Septic shock	Р
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

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

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

7 sepsis diagnosis.

	Univariate logistic regression		Multivariate logistic regression		
Variable	analysis		analysis		
	OR (95%CI)	Р	OR (95%CI)	Р	
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			



- 16 protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ
- 17 failure assessment, WBC: white blood cell count.



For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml



36 range(x-axis), the sepsis diagnostic model surpasses both Treat-all and Treat-no.

BMJ Open

BMJ Open

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

Journal:	BMJ Open
Manuscript ID	bmjopen-2023-078687.R3
Article Type:	Original research
Date Submitted by the Author:	27-May-2024
Complete List of Authors:	Zuo, Lingyun; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Li, Xiaoyun; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Wang, Luhao; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Yuan, Hao; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Liao, Zihuai; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Zhou, Si; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Wu, JianFeng; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Guan, XiangDong; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine Liu, YongJun ; Sun Yat-sen University First Affiliated Hospital, Critical Care Medicine
Primary Subject Heading :	Diagnostics
Secondary Subject Heading:	Diagnostics
Keywords:	INTENSIVE & CRITICAL CARE, Infection control < INFECTIOUS DISEASES, Adult intensive & critical care < INTENSIVE & CRITICAL CARE

SCHOLARONE[™] Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our <u>licence</u>.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which <u>Creative Commons</u> licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

terez oni

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

1	Heparin-binding protein as a biomarker for the diagnosis of
2	sepsis in the intensive care unit: a retrospective cross-
3	sectional study in China
4	Lingyun Zuo*, Xiaoyun Li*, Luhao Wang*, Hao Yuan*, Zihuai Liao, Si Zhou, Jianfeng
5	Wu, Xiangdong Guan, and Yongjun Liu $^{\scriptscriptstyle \dagger}$
6	
7	Department of Critical Care Medicine, the First Affiliated Hospital, Sun Yat-sen
8	University, No. 58, Zhongshan 2nd Road, Guangzhou 510080, Guangdong, China
9	Guangdong Clinical Research Center for Critical Care Medicine, No. 58, Zhongshan
10	2nd Road, Guangzhou 510080, Guangdong, China
11	* These authors contributed equally to this work.
12	† Corresponding author: Yongjun Liu, E-mail: liuyjun3@mail.sysu.edu.cn
13	Manuscript words count: 3228 words.
14	
15	
16	
17	
18	
19	
20	
21	

2	
3	
4	
5	
6	
7	
/	
8	
9	
10	
11	
12	
13	
14	
14	
15	
16	
17	
18	
19	
20	
21	
21 22	
∠∠ วว	
23	
24	
25	
26	
27	
28	
29	
20	
20	
51	
32	
33	
34	
35	
36	
37	
20	
20	
39	
40	
41	
42	
43	
44	
45	
46	
17	
47	
48	
49	
50	
51	
52	
53	
54	
55	
55	
20	
5/	
58	
59	
60	

23	Abstract
24	Objectives: This study aims to investigate the diagnostic value of heparin-binding
25	protein (HBP) in sepsis and develop a sepsis diagnostic model incorporating HBP with
26	key biomarkers and disease-related scores for rapid, and accurate diagnosis of sepsis in
27	the intensive care unit (ICU).
28	Design: Clinical retrospective cross-sectional study.
29	Setting: A comprehensive teaching tertiary hospital in China.
30	Participants: Adult patients (age \geq 18years) who underwent HBP testing or whose
31	blood samples were collected when admitted to the ICU.
32	Main outcome measures: HBP, C-reactive protein (CRP), procalcitonin (PCT), white
33	blood cell count (WBC), interleukin-6 (IL-6), lactate (LAC), acute physiology and
34	chronic health evaluation II (APACHE II), and sequential organ failure assessment
35	(SOFA) score were recorded.
36	Results: Between March 2019 and December 2021, 326 patients were enrolled in this
37	study. The patients were categorized into a non-infection group (control group),
38	infection group, sepsis group, and septic shock group based on the final diagnosis. The
39	HBP levels in the sepsis group and septic shock group were 45.7 and 69.0 ng/mL,
40	respectively, which were significantly higher than those in the control group (18.0
41	ng/mL) and infection group (24.0 ng/mL) ($p < 0.001$). The AUC value of HBP for
42	diagnosing sepsis was 0.733, which was lower than those corresponding to PCT, CRP,
43	and SOFA but higher than those of IL-6, LAC, and APACHE II. Multivariate logistic
44	regression analysis identified HBP, PCT, CRP, IL-6, and SOFA as valuable indicators

Page 4 of 32

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

45	for diagnosing sepsis. A sepsis diagnostic model was constructed based on these
46	indicators, with an AUC of 0.901, a sensitivity of 79.7%, and a specificity of 86.9%.
47	Conclusions : HBP could serve as a biomarker for the diagnosis of sepsis in the ICU.
48	Compared with single indicators, the sepsis diagnostic model constructed using HBP,
49	PCT, CRP, IL-6, and SOFA further enhanced the diagnostic performance of sepsis.
50	
51	Strengths and limitations of this study
52	• This study included a highly heterogeneous population, making it highly applicable
53	to patients with sepsis in the ICU.
54	• Moreover, most of the biomarkers included in this diagnostic model are widely
55	used in clinical practice, making them easily obtainable, highly reproducible, and
56	operationally feasible.
57	• This was an ICU single-center retrospective study, and the results might be
58	inapplicable to sepsis patients in other settings.
59	• The SOFA scores in the study were absolute values automatically obtained by the
60	electronic scoring system rather than the delta values.
61	• Its design dose not allow for the determination of causal relationships.
62	
63	Keywords: HBP, Sepsis, Diagnostic model
64	
65	
66	Background

Page 5 of 32

BMJ Open

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.

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

89	neutrophils after infection that has functions such as altering endothelial cell
90	permeability, antimicrobial activity, chemotaxis, and regulation of cell apoptosis. [8] It
91	has been identified as an early diagnostic indicator for severe sepsis/septic shock in
92	Chinese Guidelines for the Management of Severe Sepsis/Septic Shock (2014) [9] and
93	Chinese Expert Consensus on Early Prevention and Interruption of Sepsis in
94	Emergency Medicine (2020). [10] In addition, an increasing number of studies have
95	recently provided evidence regarding the use of HBP for diagnosing sepsis. The results
96	demonstrate that HBP could be used for sepsis diagnosis and severity monitoring. [8,
97	11-14] On the other hand, a few studies have indicated that elevated levels of HBP
98	irrespective of infectious etiology and no correlation with severity and outcome. [15]
99	Furthermore, differences and inconsistencies have been noted among various studies
100	regarding the diagnostic performance of HBP in sepsis. [16, 17] Therefore, it remains
101	controversial to use HBP for the early diagnosis of sepsis. This study aimed to analyze
102	the diagnostic value of HBP in sepsis and develop a sepsis diagnostic model combining
103	HBP with multiple biomarkers and disease-specific scoring systems retrospectively to
104	facilitate identification and diagnosis of sepsis in the ICU.

106 Methods

107 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
BMJ Open

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.

122 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 μ L of supernatants were collected for plasma level of HBP determination using an immunofluorescence dry quantitative method (JetiStar3000, Hangzhou, Joinstar Biomedical Technology Co., LTD). The procedure strictly followed the instructions provided with the reagent kit, and the quality control was performed well. Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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

BMJ Open

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

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.

- 139 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 cutoff 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

BMJ Open

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. (elie 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.

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

The control group consisted of patients who recovered postoperatively from

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

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

191 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). Page 11 of 32

BMJ Open

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

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

221	Based on the training set, variables were selected using univariate logistic
222	regression analysis for patient demographics (such as gender, age, underlying diseases,
223	infection sites, and pathogens), infection biomarkers (HBP, PCT, WBC, CRP, IL-6,
224	and LAC), APACHE II scores, and SOFA scores. Variables with statistical significance
225	(p < 0.05) were included in the multivariate logistic regression model (Supplementary
226	Table 2). Statistically significant variables in the univariate analysis were HBP, PCT,
227	CRP, IL-6, LAC, APACHE II, and SOFA scores. The final multivariate logistic
228	regression results showed that PCT (OR = $1.034, 95\%$ CI $1.009-1.060, p = 0.009$), CRP
229	(OR = 1.011, 95% CI 1.006–1.016, $p < 0.001$), HBP (OR = 1.006, 95% CI 1.000–
230	1.012, $p = 0.041$), IL-6 (OR = 1.001 95% CI 1.000–1.001, $p = 0.013$), SOFA (OR =
231	1.252, 95% CI 1.110–1.412, $p < 0.001$) were significantly associated with sepsis
232	diagnosis. The sepsis diagnostic model was constructed based on the results of logistic
233	regression, as illustrated in Figure 3.

235 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

BMJ Open

(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). Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

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

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

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 Page 15 of 32

BMJ Open

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 cvtoskeleton, 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,

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

and SOFA. Previous studies have reported varying diagnostic accuracies of HBP for

the AUC of HBP in predicting sepsis was 0.733, which was not superior to PCT, CRP,

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

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

The pathophysiological mechanisms that underlie sepsis are complex. They are

Page 17 of 32

BMJ Open

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

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

349 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,

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

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

356 Notes

Acknowledgments. We appreciate Yanzhe Xia from the department of pharmacy and Kang Liao from the microbiology laboratory for their professional support of this study and their careful interpretation of medication guidance and each specimen's etiology. 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.

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. *Financial support.* This research received no specific grants from any funding agency
in the public, commercial or not-for-profit sectors.

Patient and public involvement. Patients and/or the public were not involved in the

372 design, or conducting, or reporting, or dissemination plans of this research.

Ethics approval. This retrospective study did not introduce any additional risks.

374 Therefore, informed consent was not obtained from all the participants. Regarding the

BMJ Open

3 4 5	375	collection of blood samples for HBP testing during holidays, the participants in our							
6 7	376	study v	study were previously provided informed consent for collecting biological samples.						
8 9 10	377	Proven	Provenance and peer review. Not commissioned; externally peer reviewed.						
11 12 13	378	Date a	Date availability statement. Date are available upon reasonable request.						
14 15	379	Referer	ices						
16 17 18	380	1.	Evans, L., et al., Surviving Sepsis Campaign: International Guidelines for Management of						
19 20 21	381		Sepsis and Septic Shock 2021. Crit Care Med, 2021. 49(11): p. e1063-e1143.						
22 23	382	2.	Rudd, K.E., et al., Global, regional, and national sepsis incidence and mortality, 1990-						
24 25 26	383		2017: analysis for the Global Burden of Disease Study. Lancet, 2020. 395(10219): p. 200-						
27 28	384		211.						
29 30 31	385	3.	Xie, J., et al., The Epidemiology of Sepsis in Chinese ICUs: A National Cross-Sectional						
32 33 34	386		Survey. Crit Care Med, 2020. 48(3): p. e209-e218.						
35 36	387	4.	Im, Y., et al., Time-to-antibiotics and clinical outcomes in patients with sepsis and septic						
37 38 39	388		shock: a prospective nationwide multicenter cohort study. Crit Care, 2022. 26(1): p. 19.						
40 41	389	5.	Kuttab, H.I., et al., Evaluation and Predictors of Fluid Resuscitation in Patients with Severe						
42 43 44	390		Sepsis and Septic Shock. Crit Care Med, 2019. 47(11): p. 1582-1590.						
45 46 47	391	6.	Yang, Y., et al., A Promising Candidate: Heparin-Binding Protein Steps onto the Stage of						
47 48 49	392		Sepsis Prediction. J Immunol Res, 2019. 2019: p. 7515346.						
50 51 52	393	7.	Pierrakos, C., et al., <i>Biomarkers of sepsis: time for a reappraisal</i> . Crit Care, 2020. 24(1):						
53 54	394		p. 287.						
55 56 57	395	8.	Linder, A., et al., Heparin-binding protein: an early marker of circulatory failure in sepsis.						
58 59 60	396		Clin Infect Dis, 2009. 49 (7): p. 1044-50.						

Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open

4
5
6
7
8
9
10
11
12
13
14
15
16
17
10
10
20
20
∠ I วว
22
23
24
25
26
2/
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59

60

1 2 3

397	9.	Cai, G., J. Yan, and H. Qiu, [The standardization of diagnosis and treatment of severe
398		sepsis/septic shock and its practice]. Zhonghua Nei Ke Za Zhi, 2015. 54(6): p. 484-5.
399	10.	Shock, et al., [Chinese expert consensus on diagnosis and management of
400		<i>immunosuppression in sepsis]</i> . Zhonghua Wei Zhong Bing Ji Jiu Yi Xue, 2020. 32 (11): p.
401		1281-1289.
402	11.	Linder, A., et al., Heparin-Binding Protein Measurement Improves the Prediction of Severe
403		Infection With Organ Dysfunction in the Emergency Department. Crit Care Med, 2015.
404		43 (11): p. 2378-86.
405	12.	Zhou, Y., et al., Usefulness of the heparin-binding protein level to diagnose sepsis and
406		septic shock according to Sepsis-3 compared with procalcitonin and C reactive protein: a
407		prospective cohort study in China. BMJ Open, 2019. 9(4): p. e026527.
408	13.	Tang J, Yuan H, Wu YL, et al. The Predictive Value of Heparin-Binding Protein and D-
409		Dimer in Patients with Sepsis. Int J Gen Med. 2023 Jun 6; 16:2295-2303.
410	14.	Li S, Xu Y, Wu Y, et al. Heparin-Binding Protein: A Prognostic Biomarker Associated
411		with Severe or Complicated Community-Acquired Pneumonia in Children. J Inflamm
412		Res. 2023 Jan 26; 16:321-331.
413	15.	Chew, M.S., et al., Increased plasma levels of heparin-binding protein in patients with
414		shock: a prospective, cohort study. Inflamm Res, 2012. 61(4): p. 375-9.
415	16.	Llewelyn, M.J., et al., Sepsis biomarkers in unselected patients on admission to intensive
416		or high-dependency care. Crit Care, 2013. 17(2): p. R60.
417	17.	Katsaros, K., et al., Heparin Binding Protein for the Early Diagnosis and Prognosis of
418		Sepsis in the Emergency Department: The Prompt Multicenter Study. Shock, 2022. 57(4):

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

BMJ Open

3 4	419		p. 518-525.
5 6 7	420	18.	Jekarl, D.W., et al., Procalcitonin as a prognostic marker for sepsis based on SEPSIS-3. J
8 9 10	421		Clin Lab Anal, 2019. 33 (9): p. e22996.
10 11 12	422	19.	Wu, Y.L., et al., Accuracy of Heparin-Binding Protein in Diagnosing Sepsis: A Systematic
13 14 15	423		Review and Meta-Analysis. Crit Care Med, 2021. 49(1): p. e80-e90.
16 17 18	424	20.	Fisher, J. and A. Linder, Heparin-binding protein: a key player in the pathophysiology of
19 20	425		organ dysfunction in sepsis. J Intern Med, 2017. 281(6): p. 562-574.
21 22 23	426	21.	Kjolvmark, C., et al., Heparin-binding protein: a diagnostic biomarker of urinary tract
24 25 26	427		infection in adults. Open Forum Infect Dis, 2014. 1(1): p. ofu004.
26 27 28	428	22.	Linder, A., et al., Heparin-binding protein: a diagnostic marker of acute bacterial
29 30 31	429		meningitis. Crit Care Med, 2011. 39(4): p. 812-7.
32 33	430	23.	Saridaki M, Metallidis S, Grigoropoulou S, et al. Integration of heparin-binding protein
34 35 36	431		and interleukin-6 in the early prediction of respiratory failure and mortality in pneumonia
37 38	432		by SARS-CoV-2 (COVID-19). Eur J Clin Microbiol Infect Dis. 2021 Jul;40(7):1405-
39 40 41	433		1412.
42 43 44	434	24.	Kong D, Lei Z, Wang Z, et al. A novel HCP (heparin-binding protein -C reactive protein-
45 46	435		procalcitonin) inflammatory composite model can predict severe acute pancreatitis. Sci
47 48 49	436		Rep. 2023 Jun 9;13(1):9440.
50 51	437	25.	Kong Y, Ye Y, Ma J, et al. Accuracy of heparin-binding protein for the diagnosis of
52 53 54	438		nosocomial meningitis and ventriculitis. Crit Care. 2022 Mar 8;26(1):56.
55 56	439	26.	Linder, A., O. Soehnlein, and P. Akesson, Roles of heparin-binding protein in bacterial
58 59	440		<i>infections</i> . J Innate Immun, 2010. 2 (5): p. 431-8.

BMJ Open

3			
4	441	27.	Kahn, F., et al., Heparin-Binding Protein as a Prognostic Biomarker of Sepsis and Disease
5			
6	442		Severity at the Emergency Department Shock 2019 52(6): p e135-e145
/ Q			
0 9	442	20	Lie D et el Hennie his disconstain an a his marken of anoma servici in the addition
10	443	28.	Liu, P., et al., Heparin-binaing protein as a biomarker of severe sepsis in the peatatric
11			
12	444		intensive care unit: A multicenter, prospective study. Clin Chim Acta, 2023. 539: p. 26-33.
13			
14	445	29.	Fisher, J., et al., Is Heparin-Binding Protein Inhibition a Mechanism of Albumin's Efficacy
15		_,,	
16 17	110		in Human Cantie Charles Cait Care Mad 2010 4((5): a -2(4 -274
17 18	446		in Human Septic Snock? Crit Care Med, 2018. 46(5): p. e364-e374.
10			
20	447	30.	Samuelsson, L., et al., Renal clearance of heparin-binding protein and elimination during
21			
22	448		renal replacement therapy: Studies in ICU patients and healthy volunteers. PLoS One.
23			
24	440		$2010 \ 14(9) = -0221912$
25	449		2019. 14 (8). p. e0221815.
20 27			
28	450	31.	Honore, P.M., S. Redant, and D. De Bels, Reliability of biomarkers of sepsis during
29			
30	451		extracorporeal therapies: the clinician needs to know what is eliminated and what is not.
31			
32	152		Crit Care 2020 24(1): n 553
33	732		Chi Caic, 2020. 24(1). p. 555.
34 35			
36	453	32.	Xing, L., et al., Activation of MI macrophages in sepsis-induced acute kidney injury in
37			
38	454		response to heparin-binding protein. PLoS One, 2018. 13(5): p. e0196423.
39			
40	455	33.	Fisher, J., et al., Heparin-Binding Protein (HBP): A Causative Marker and Potential Target
41			
42 43	156		for Hangrin Treatment of Human Sensis-Induced Acute Kidney Injury Shock 2017 18(3):
44	450		for meparin Treatment of manual sepsis-maacea Acate Kianey injury. Shock, 2017. 40 (5).
45			
46	457		p. 313-320.
47			
48	458	34.	Gibot, S., et al., Combination biomarkers to diagnose sepsis in the critically ill patient. Am
49			
50 51	459		L Respir Crit Care Med 2012 186 (1): p 65-71
52	109		o respir ent euro meu, 2012. 100(1). p. 00 / 1.
53	160	25	
54	460	33.	Bauer, r.K., et al., Diagnostic accuracy and clinical relevance of an inflammatory
55			
56	461		biomarker panel for sepsis in adult critically ill patients. Diagn Microbiol Infect Dis, 2016.
57			
50 50	462		84 (2): p. 175-80.
60			
-			

463		

464 Tables

465 Table 1. Characteristics of the patients.

	Control (n = 93)	Infection $(n = 94)$	Sepsis (n = 53)	Septic shock (n = 86)	Р
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

BMJ Open

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.

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

Variable	AUC (95% CI)	Cut-off	Sensitivity	Specificity	PPV	NPV	Р
		value	(%)	(%)	(%)	(%)	
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
РСТ	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

472 APACHE II: acute physiology and chronic health evaluation II, CRP: C-reactive protein, HBP:

473 heparin-binding protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA:

474 sequential organ failure assessment, WBC: white blood cell count. The *P* values between AUCs

475 compared to HBP.

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

BMJ Open

477 Figure legends

- **Figure 1.** The flow diagram of participants. HBP: heparin-binding protein, ICU: intensive care unit.
- 479 Figure 2. Comparison of plasma levels of biomarkers among different groups. A: HBP, B: PCT,
- 480 C: WBC, D: CRP, E: IL-6, F: LAC, G: APACHE II, H: SOFA. APACHE II: acute physiology and
- 481 chronic health evaluation II, CRP: C-reactive protein, HBP: heparin-binding protein, LAC: blood
- 482 lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ failure assessment,
- 483 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.

338x190mm (54 x 54 DPI)

BMJ Open: first published as 10.1136/bmjopen-2023-078687 on 10 June 2024. Downloaded from http://bmjopen.bmj.com/ on June 7, 2025 at Agence Bibliographique de I Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, Al training, and similar technologies.

BMJ Open





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: Creactive 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.

448x296mm (300 x 300 DPI)

Page 29 of 32

1	
2	
3	
4	
5	
6	
7	
8	Points
9	PCT 0 20 40 60 80 100 120 140 160 160
10	CRP 0 50 100 150 200 250 300 350
11	0 50 100 150 200 250 300 IL6
12	SOFA 0 2 4 6 8 10 12 14 16 18 20 22
13	Total Points 0 20 40 60 80 100 120 140 160 180 200 220 240 260
14	Proo or Sepsis 0.1 0.3 0.5 0.8 0.9 0.99
15	
16	
17	Figure 3. A nomogram predicting the risk of sepsis for patients. The value of each of variable was given a
18	score on the point scale axis. A total score could be easily calculated by adding each single score and by
10	projecting the total score to the lower total point scale. We were able to estimate the probability of sepsis.
20	CRP: C-reactive protein, HBP: heparin-binding protein, PCT: procalcitonin, IL-6: interleukin-6, SOFA:
21	sequential organ failure assessment.
22	423x127mm (300 x 300 DPI)
22	
23	
25	
26	
20	
27	
20	
30	
31	
32	
32	
34	
35	
36	
37	
38	
39	
40	
40	
42	
43	
44	
45	
46	
47	
48	
40	
50	
50	
52	
53	
54	
55	
56	
57	
58	
59	
60	For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Enseignement Superieur (ABES) Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

1 Supplementary Data

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

	Infection	Sepsis	Septic shock	Р
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

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

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

7 sepsis diagnosis.

	Univariate logistic reg	Multivariate logistic regression		
Variable	analysis	analysis		
	OR (95%CI)	Р	OR (95%CI)	Р
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		



- 16 protein, LAC: blood lactic acid, PCT: procalcitonin, IL-6: interleukin-6, SOFA: sequential organ
- 17 failure assessment, WBC: white blood cell count.



For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml



36 range(x-axis), the sepsis diagnostic model surpasses both Treat-all and Treat-no.