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HEPARIN BINDING PROTEIN FOR THE EARLY DIAGNOSIS AND PROGNOSIS OF SEPSIS IN THE EMERGENCY DEPARTMENT: THE PROMPT MULTICENTER STUDY

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ABSTRACT—**Background:** The validation of new biomarkers for the diagnosis and risk stratification of patients with sepsis at an early point is essential for successful treatment. Recent publications prompted us to investigate of heparin binding protein (HBP) for the emergency department (ED) admissions. **Materials and Methods:** In this multicenter, cross-sectional study, HBP and procalcitonin (PCT) were measured within the first hour upon admission to the ED in plasma samples of 371 patients with signs of infection. Patients were classified into non-sepsis and sepsis by the Sepsis-3 definitions and were followed up for outcome. **Results:** HBP was significantly higher in patients with sepsis and was positively correlated to PCT and C-reactive protein, absolute neutrophil and monocyte counts, creatinine, bilirubin and lactate. Sensitivity, specificity, positive predictive value, and negative predictive value of HBP more than 19.8 ng/mL for the diagnosis of sepsis was 66.3%, 44.9%, 49.3%, and 62.2%, respectively; and for prediction of early death was 100%, 41.0%, 4.5%, and 100%, respectively. Single HBP and PCT could not predict 28-day mortality; this was performed with sensitivity, specificity, positive predictive value, and negative predictive value 44.8%, 81.8%, 17.3%, and 94.6% when used in combination. **Conclusion:** Admission HBP can be used as a tool for the early diagnosis of sepsis and for the risk of early death in the ED.

KEYWORDS—Biomarker, heparin binding protein, outcome, procalcitonin, sepsis

INTRODUCTION

In 2017, more than 49 million hospitalizations and 11 million deaths worldwide were attributed to sepsis. This accounts for almost 20% of global deaths in 2017 (1). One suggested strategy for the prevention of unfavorable outcome is the early detection of patients at risk guiding early treatment. This strategy may be valuable especially for patients without full blown symptoms (2). This necessitates the development of appropriate diagnostic tools that suggest the early presence of sepsis.

Heparin-binding protein (HBP), also known as CAP37, is a constitutively expressed protein stored in the azurophilic granules and secretory vesicles of neutrophils and it is released early on during the immune response to infections (3). HBP has been suggested to associate with infection-induced organ dysfunction (4). These characteristics render HBP a promising candidate as a biomarker for sepsis.

PROMPT (PROspective, non-interventional, Multi-centre clinical study to assess the clinical validity of the heparin binding protein assay to indicate the presence and outcome of sepsis in Patients with suspected infection following emergency departmenT admission) is a prospective, non-interventional, multi-centre clinical study aiming to assess the clinical validity of HBP for the diagnosis of sepsis and the prediction of outcome over the first 72 h following emergency department

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TG performed lab measurements, critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

AV conceptualized the study design, contributed to the analysis of the data, wrote the manuscript, and gave final approval of the version to be published.

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(ED) admission. This is not the first study where the diagnostic performance of HBP for the diagnosis of sepsis is assayed at the level of the ED (5–9). PROMPT is a confirmatory study in another healthcare setting testing, however, another platform of measurement of HBP than the previous studies and aiming to use HBP as a tool for screening of the risk of early death and not of general unfavorable outcome.

PATIENTS AND METHODS

This was a cross-sectional study that took place between September 26, 2017 and September 1, 2018 at the emergency department (ED) of six hospitals of the network of the Hellenic Sepsis Study Group (ClinicalTrials.gov registration NCT03295825). The protocol was approved by the Ethics Committees of the participating hospitals (Nafplion General Hospital Approval Nr. 23133/27.03.2017; ATTIKON University Hospital Approval Nr. 2383/16-05-2017; Chalkida General Hospital Approval Nr. 30/17-5-2017; Syros General Hospital Approval Nr. 5078/25-05-17; Sparti General Hospital Approval Nr. 7/26-05-2017 and G. Gennimatas Thessaloniki General Hospital Approval Nr. 8237/31-5-2017). The analysis of the gene expression of the 29-mRNA InSep diagnostic tool on 102 patients enrolled in PROMPT has recently been published (10).

Patients were enrolled after written informed consent provided by themselves or by first-degree relatives for individuals unable to consent. Enrolled patients were adults of either gender with suspected infection and presence of at least one of the following:

1. core temperature > 38°C or <36°C;
2. heart rate > 90 bpm;
3. respiratory rate > 20/min and
4. self- reported fever/chills.

No exclusion criteria applied. Infections were defined according to standardized international criteria (Supplemental data, http://links.lww.com/SHK/B387).

Five millilitres of whole blood were collected at the ED within the first 1 h from admission into a tube containing ethylenediaminetetraacetic acid (EDTA) for 10 min at 5,000 rpm. Plasma was separated and stored at −80°C until further analysis. HBP and procalcitonin (PCT) were measured in plasma by a fluorescence dry quantitative immunoassay using the Jet-iStar 800 analyzer (Join-Star, Hangzhou, China). The lowest limit of detection was 0.2 ng/mL for HBP and 0.05 ng/mL for PCT.

For each patient the following variables were recorded:

1. demographics;
2. severity scores, namely Acute Physiology and Chronic Health Evaluation (APACHE) II score, Charlson's Comorbidity Index (CCI) and Sequential Organ Failure Assessment (SOFA) score;
3. absolute blood cell counts, biochemistry, and blood gases;
4. comorbidities;
5. type of infection;
6. and microbiology.

The study primary endpoint was the diagnostic performance of HBP for sepsis the first 72 h post-ED admission. To this end, patients were classified as no-sepsis and sepsis if they met the Sepsis-3 criteria for sepsis classification the first 72 h (11). The secondary study endpoint was the prognostic ability of HBP for early death which was defined as death for any reason recorded the first 72 h post-ED admission. Another secondary endpoint was 28-day mortality.

Statistics

The study was powered on the assumptions that almost 40% of ED admissions with the pre-specified inclusion criteria will develop sepsis and that HBP would be increased in 70% of cases with sepsis and in 50% of cases without sepsis. To achieve this with 80% power at the 10% level of significance, 360 patients should be enrolled; 40 more patients were enrolled to adjust for missing data.

Categorical data were presented as frequencies and quantitative variables as mean ± SD (parametric values) or median + interquartile range (non-parametric values). The type of distribution of values (parametric or non-parametric) was analyzed using the Kolmogorov–Smirnov's test. Differences and 95% confidence intervals (CIs) were calculated for all variables between patients with sepsis and patients without sepsis, between 72-h survivors and 72-h non-survivors and between 28-day survivors and 28-day non-survivors. Two group comparisons for quantitative data were performed using the Mann–Whitney U test for non-parametric variables and the Student's t test for parametric variables. In order to define a cut-off of HBP for the early diagnosis of sepsis and for prognosis, Receiver Operator Characteristics (ROC) curve analysis was done. The cut-off was selected by the Youden index. Comparisons between groups of patients above and below the cut-off were done by the Fisher exact test; odds ratios and 95% confidence intervals (CIs) were calculated according to Mantel and Hanszel. The analysis was done for all cases where the information on the primary endpoint was available and for whom HBP measurement was available. One sensitivity analysis was pre-planned for the primary endpoint considering all cases with missing data as sepsis.

Forward step-wise logistic regression analysis was done between variables associated with early death the first 72 h. The selection of variables used for the analysis was done after comparing all variables for which data were captured between 72-h survivors and non-survivors. These variables entered into both univariate and multivariate analyses. In the regression model, 72-h outcome (death/survival) entered as the dependent variable. All quantitative variables that were different between 72-h survivors and non-survivors entered as continuous variables and all qualitative variables that were different between 72-h survivors and non-survivors entered as categorical variables. The selected cut-off of HBP entered as categorical variable. Survival analysis was done by the log-rank test. Any P value below 0.05 was considered statistically significant.

RESULTS

A total of 400 (ED) admissions were enrolled in the study. HBP and PCT were measured in 371 patients; in 29 samples measurement failed. Results were analyzed in 371 patients. Their demographics presented as differences between patients who met Sepsis-3 criteria and patients who did not meet Sepsis-3 criteria the first 72 h postadmission are shown in Table 1. No cases with missing data on the primary endpoint existed and no sensitivity analysis was needed.

HBP was significantly greater in patients with sepsis (Fig. 1A). Following ROC curve analysis it was found that plasma levels of HBP above 19.8 ng/mL had the best trade-off for sensitivity and specificity for diagnosing sepsis (Fig. 1B). In total, 223 patients had higher, and 148 patients had less than or equal to 19.8 ng/mL of HBP. Within 72-h from ED presentation 110 (49.3%) patients with levels greater than 19.8 ng/mL and 56 (37.8%) patients with levels less than or equal to 19.8 ng/mL developed sepsis (Fig. 1C). The odds ratio (OR) for HBP for the diagnosis of sepsis was 1.60 (95% Confidence Interval (CI): 1.05 – 2.44, $P = 0.033$). The early diagnostic potential of HBP can be explained by its positive association with markers of organ function and other inflammatory biomarkers (Fig. 2).

We then sought to investigate whether HBP could be utilized for prognosticating death within 72 h. Patient demographics according to early death are presented in supplementary Table 1, http://links.lww.com/SHK/B388. HBP was significantly greater in patients who died the first 72 h after admission, compared to survivors (Fig. 3A). Using the same cut-off value of 19.8 ng/mL, it was found that 10 (4.5%) patients with levels greater than 19.8 ng/mL and none of the patients with levels less than or equal to 19.8 ng/mL died within 72 h after admission in the ED (Figs. 3B and C). The comparisons between 72-h survivors and 72-h non-survivors showed that qSOFA score,

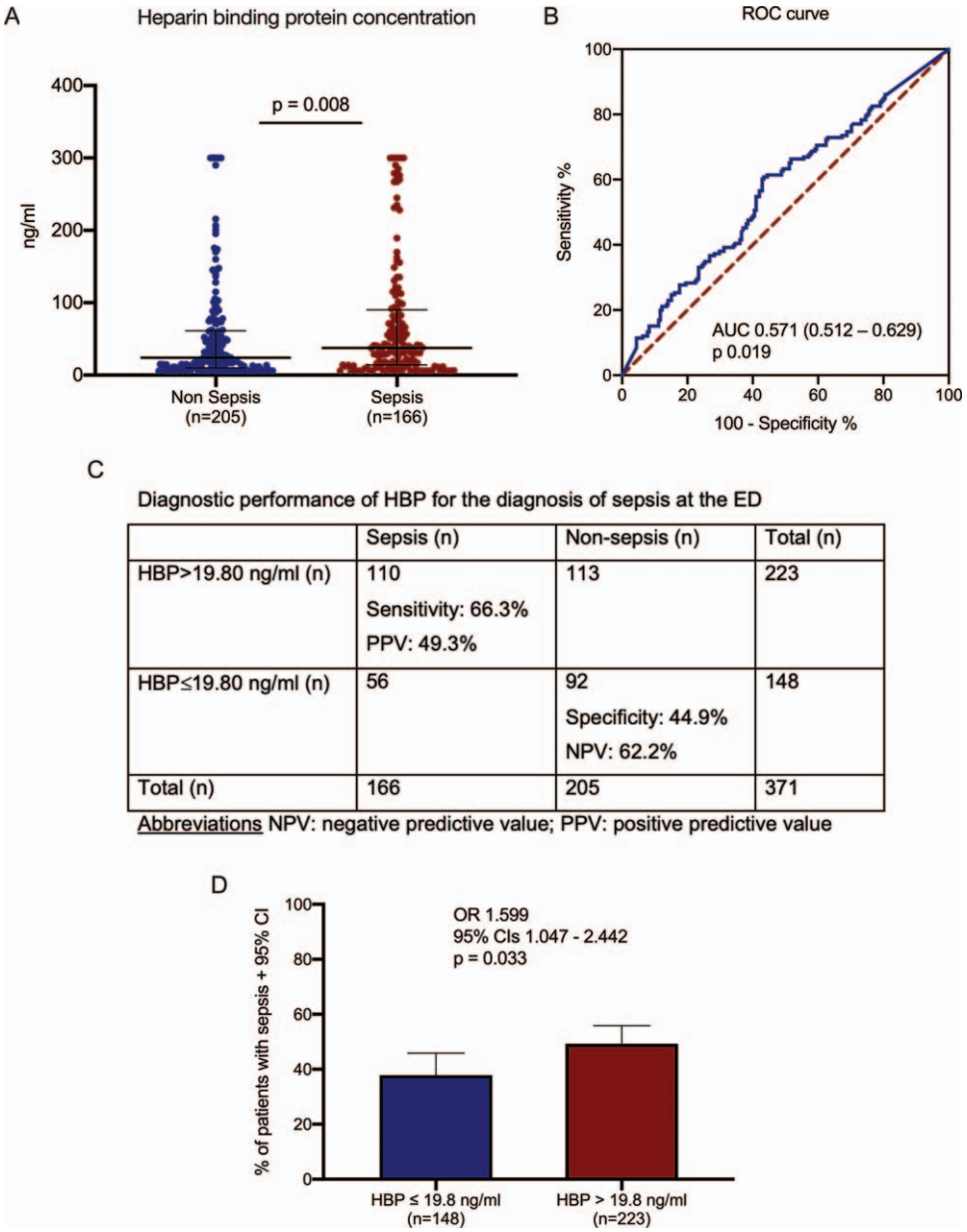
Table 1. **Comparative baseline demographics of patients who were diagnosed with sepsis and of those who were not diagnosed with sepsis**

	Sepsis (n = 166)	Non-sepsis (n = 205)	Difference (95% CIs)
Male gender (%)	88 (53.0)	106 (51.7)	1.3 (−8.8; 11.4)
Age (years, mean ± SD)	75.2 ± 14.5	52.0 ± 20.4	23.2 (19.4; 26.9)
Number of SIRS criteria (mean ± SD)	1.97 ± 1.05	1.62 ± 0.97	0.35 (0.14; 0.56)
qSOFA score (mean ± SD)	0.84 ± 0.82	0.40 ± 0.55	0.44 (0.29; 0.58)
Charlson's comorbidity index (mean ± SD)	4.69 ± 2.87	1.70 ± 2.43	2.99 (2.45; 3.53)
SOFA score (mean ± SD)	3.29 ± 1.92	0.35 ± 0.47	2.94 (2.67; 3.21)
APACHE II score (mean ± SD)	12.59 ± 5.54	5.18 ± 3.74	7.41 (6.41; 8.41)
White blood cells (/mm^3, mean ± SD)	12,891.7 ± 6675.0	10,422.1 ± 4861.6	2469.6 (1290.3; 3648.9)
Platelets (/mm^3, mean ± SD)	232,582.1 ± 87864.0	248,534.0 ± 80808.4	−15951.9 (−1302.5; 33206.3)
PCT (ng/ml, median-IQR)	0.26 (1.03)	0.10 (0.11)	0.16 (0.92)
CRP (mg/l, median-IQR)	60.1 (107.9)	21.8 (66.8)	38.3 (41.1)
HBP (ng/ml, median-IQR)	36.8 (77.9)	23.9 (51.2)	12.9 (26.7)
Final infection diagnosis (n, %)			
Upper respiratory tract infections	2 (1.2)	62 (30.2)	−29.0 (−35.7; −22.4)
Community-acquired pneumonia	47 (28.3)	14 (6.8)	21.4 (13.9; 29.3)
Acute bronchitis	24 (14.5)	26 (12.7)	1.8 (−5.1; 9.1)
Aspiration pneumonia	22 (13.3)	1 (0.5)	12.8 (7.9; 18.8)
Acute pyelonephritis	38 (22.9)	19 (9.3)	13.6 (6.2; 21.3)
Acute cystitis	0 (0)	9 (4.4)	−4.4 (−8.1; −1.3)
Primary bloodstream infection	4 (2.4)	2 (1.0)	1.4 (−1.4; 5.1)
Acute biliary tract infection	15 (9.0)	6 (2.9)	6.1 (1.3; 11.7)
Intrabdominal abscess	3 (1.8)	6 (2.9)	−1.1 (−4.6; 2.6)
Acute gastroenteritis	3 (1.8)	11 (5.4)	−3.6 (−7.7; 0.1)
Skin and soft tissue infection	8 (4.8)	5 (2.4)	2.4 (−1.6; 7.0)
Pathogens isolated from blood cultures (n, %)			
Escherichia coli	9 (5.4)	0 (0)	5.4 (2.2; 9.9)
Klebsiella pneumoniae	2 (1.2)	0 (0)	1.2 (0; 4.3)
Other Gram-negative bacteria	2 (1.2)	1 (0.5)	0.7 (−1.7; 3.8)
Staphylococcus aureus	0 (0)	2 (1.0)	−0.9 (−3.5; 1.4)
Pathogens isolated from urine cultures (n, %)			
Escherichia coli	17 (10.3)	10 (4.9)	5.2 (1.2; 10.1)
Other Gram-negative bacteria	11 (6.6)	3 (1.5)	
Upper respiratory tract secretions PCR (FilmArray) (n, %)			
Rhinovirus	4 (2.4)	18 (8.8)	−5.2 (−10.1; −1.2)
Influenza B	2 (1.2)	12 (5.9)	−4.7 (−8.8; −0.7)
Other viruses	8 (4.8)	14 (6.8)	−2.0 (−6.9; 3.2)
Co-morbidities (n, %)			
Type 2 diabetes mellitus	50 (30.1)	18 (8.8)	21.3 (13.3; 29.4)
Chronic heart failure	35 (21.1)	7 (3.4)	17.7 (11.2; 24.7)
Coronary heart disease	43 (25.9)	11 (5.4)	20.5 (13.3; 28.1)
Chronic obstructive pulmonary disease	36 (21.7)	12 (5.9)	15.8 (8.9; 23.1)
Chronic renal disease	19 (11.4)	4 (2.0)	9.5 (4.5; 15.4)
Corticosteroid intake	11 (6.6)	9 (4.4)	2.2 (−2.5; 7.5)
Chemotherapy	14 (8.4)	4 (2.0)	6.5 (2.0; 11.8)
Non-metastatic solid tumor	12 (7.2)	3 (1.5)	5.8 (1.7; 10.8)
Metastatic solid tumor	9 (5.4)	10 (4.9)	0.5 (−4.1; 5.6)
Ischemic stroke	16 (9.6)	6 (2.9)	6.7 (1.8; 12.4)
Atrial fibrillation	25 (15.1)	13 (6.3)	8.7 (2.4; 15.5)
Dementia	36 (21.7)	10 (4.9)	16.8 (10.0; 24.3)
Parkinson disease	7 (4.2)	3 (1.5)	2.8 (−0.7; 7.1)
Nephrolithiasis	2 (1.2)	8 (3.9)	−2.7 (−6.4; −0.9)
Gallstones	16 (9.6)	8 (3.9)	5.7 (0.6; 11.5)
Depression	18 (10.8)	14 (6.8)	4.0 (−1.8; 10.3)

APACHE, acute physiology and chronic health evaluation; CI, confidence interval; CRP, C-reactive protein; HBP, heparin binding protein; IQR, interquartile range; PCR, polymerase chain reaction; PCT, procalcitonin; SIRS, systemic inflammatory response syndrome; SOFA, sequential organ failure assessment.

CCI, age, SOFA score, and APACHE II score are higher in non-survivors. These variables entered as continuous variables in the step-wise logistic regression analysis model. The inclusion of these scores in the model was considered clinical meaningful since they are calculated at the ED. HBP more than 19.8 ng/mL entered as categorical variable and it was an independent predictor of early death (Table 2).

Finally, it was investigated if HBP concentrations at the ED could prognosticate for 28-day outcome (Supplementary Table 2, http://links.lww.com/SHK/B388). Twenty-two (9.8%) patients with levels greater than 19.8 ng/mL and 7 (4.7%) patients with levels less than or equal to 19.8 ng/mL died within 28 days after admission ($P = 0.078$). PCT is used at the ED at a cut-off of 0.25 ng/mL (12). Using this cut-off, it was



	Sepsis (n)	Non-sepsis (n)	Total (n)
HBP>19.80 ng/ml (n)	110 Sensitivity: 66.3% PPV: 49.3%	113	223
HBP≤19.80 ng/ml (n)	56	92 Specificity: 44.9% NPV: 62.2%	148
Total (n)	166	205	371

Abbreviations NPV: negative predictive value; PPV: positive predictive value

Fig. 1. **Diagnostic performance of heparin binding protein (HBP) for the prediction of sepsis.** (A) Concentrations of HBP in plasma upon admission in the emergency department among patients who will or will not develop sepsis within 72 h after admission. Comparison by the Mann–Whitney *U* test; *P*-value is given. (B) ROC curve analysis of HBP as a diagnostic tool for Sepsis in the ED. (C) Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of HBP in plasma upon admission in the emergency department above 19.8 ng/mL for sepsis diagnosis. (D) Risk of developing sepsis among patients without and with increased HBP above 19.8 ng/mL. The odds ratio (OR), 95% confidence intervals (CIs) and the *P* value of the indicated comparison are given.

found that 15 patients above this cut-off (11.6%) and that 14 (5.8%) below this cut-off died ($P = 0.066$). However, when combining both HBP and PCT it was found that 28-day mortality was significantly increased among patients with increased levels of both HBP and PCT compared to patients showing an increase in only one of the biomarkers (Figs. 4A and B). Mortality among patients with increased HBP and PCT was significantly higher compared to all other patients (17.3% versus 5.6%, $P = 0.001$) (Fig. 4C).

DISCUSSION

The present study shows that HBP could be a useful biomarker for the early diagnosis of sepsis and for the risk stratification the first 72 h post-ED admission. Moreover, HBP integration with PCT could be a significant combined tool for predicting the long-term outcome of patients admitted to the ED with signs of infection. Despite the fact that the sensitivity and negative predictive value of HBP for the prediction of early death were 100%, the findings should be interpreted with caution since the number of patients who died was limited.

The biological plausibility of HBP as a biomarker of sepsis and infection associated death is supported by its early release from neutrophils during immune response against pathogens and by its causative link to several processes related to infection-induced organ dysfunction, such as vascular leak (5). This is supported in our study by the positive correlation to

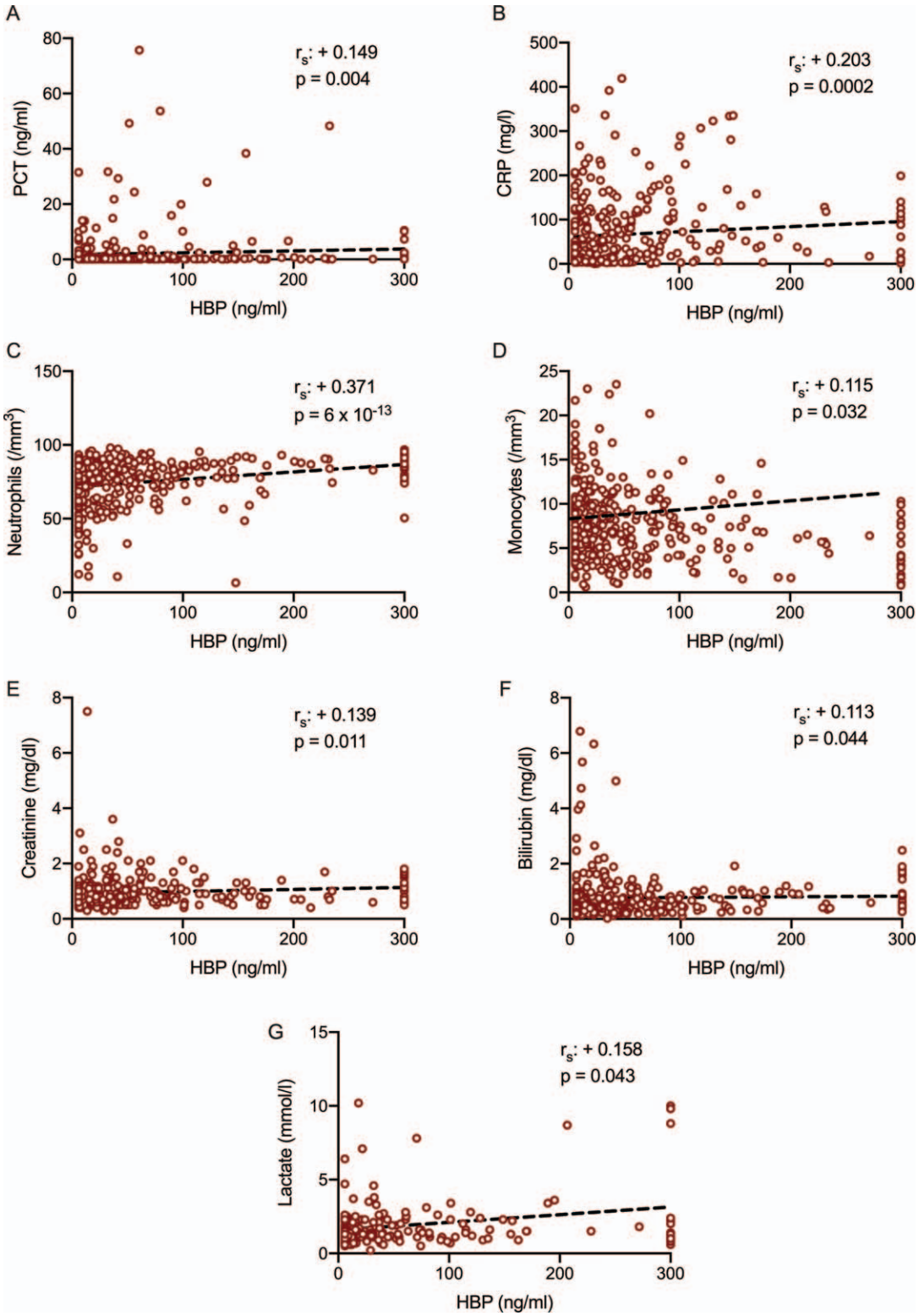
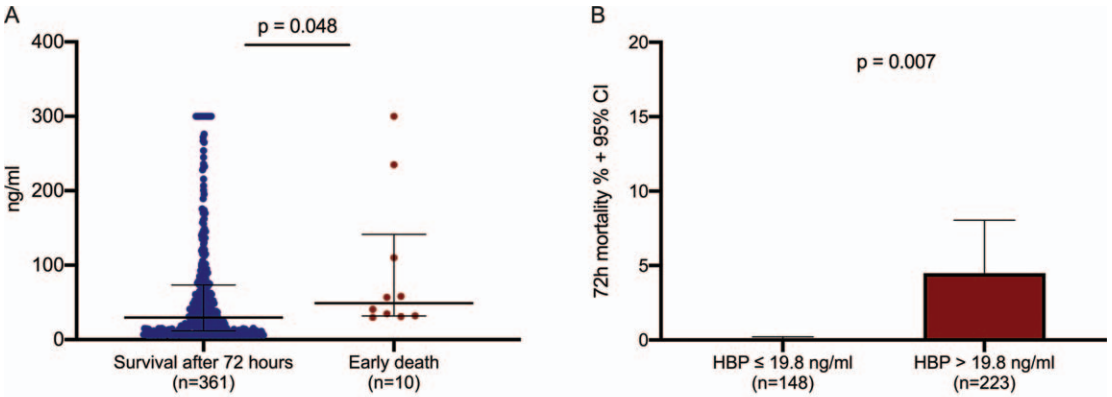


Fig. 2. **Association of heparin binding protein (HBP) with other biomarkers of organ dysfunction. Concentrations of HBP were measured in plasma of patients upon admission in the emergency department.** Correlation of levels of HBP with (A) procalcitonin (PCT); (B) C-reactive protein (CRP); (C) absolute neutrophil count; (D) absolute monocyte count; (E) creatinine, (F) bilirubin, and (G) lactate. Spearman rank correlation coefficients (r_s), interpolation lines and *P* values are provided.

creatinine, bilirubin and lactate, and to absolute counts of key immune cell types, such as neutrophils and monocytes. Moreover, a positive correlation with already established biomarkers of sepsis, such as PCT and CRP could be demonstrated.

The performance of HBP as a biomarker in sepsis has been evaluated in several clinical studies. In a cohort of 118 patients admitted at the ED, HBP was increased in patients with sepsis compared to others (6). Additionally, HBP was



C Prognostic performance of HBP for early death

	Early death (n)	Survival after 72 hours (n)	Total (n)
HBP>19.80 ng/ml (n)	10 Sensitivity: 100% PPV: 4.5%	213	223
HBP≤19.80 ng/ml (n)	0	148 Specificity: 41.0% NPV: 100%	148
Total (n)	10	361	371

Abbreviations NPV: negative predictive value; PPV: positive predictive value

Fig. 3. **Diagnostic performance of heparin binding protein (HBP) for the prediction of the outcome of sepsis.** (A) Concentrations of HBP in plasma upon admission in the emergency department among patients who will or will not (early death) survive 72 h after admission. Comparison by the Mann–Whitney *U* test; *P*-value is given. (B) Risk of early death among patients without and with increased HBP above 19.8 ng/mL. The odds ratio, 95% confidence intervals (CIs) and the *P* value of the indicated comparison are given. (C) Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of HBP in plasma upon admission in the emergency department above 19.8 ng/mL for prediction of early death.

increased in patients with severe compared to uncomplicated sepsis. In another cohort of 93 patients with sepsis, HBP was increased in septic shock. At a cut-off value of 28.1 ng/mL, HBP could diagnose sepsis with a sensitivity of 84.9%, a specificity of 78.3%, a positive predictive value (PPV) of 94.0% and a negative predictive value (NPV) of 65.9% (7). In a study of 674 patients admitted at the ED with infection, HBP more than 30 ng/mL had 78% sensitivity to predict progression into severe sepsis within the first 72 h (8). Finally, in a cohort of 524 patients admitted at the ED, HBP levels above 15 ng/mL were prognostic of organ dysfunction (9). HBP has also been used at similar cut-off values as a prognostic marker in COVID-19 related sepsis (13, 14). The use of combination of biomarkers to diagnose patients with sepsis and to stratify them in the ED is considered as the best approach for tackling the difficulties which arise from the atypical clinical presentation or by the delay in microbiological verification of sepsis (15).

The value of HBP as a biomarker in sepsis is supported by its reproducibility, which has already been established due to

1. the fact that HBP can be accurately measured using different assays, such as a fluorescence dry quantitative immunoassay and an enzyme immunosorbent assay; and

Table 2. **Forward step-wise logistic regression analysis of variables associated with early death the first 72 h**

	Univariate analysis		Multivariate analysis	
	OR (95% CIs)	*P*	OR (95% CIs)	*P*
HBP >19.8 ng/ml	7.44 (1.01–58.72)	0.048	48.59 (1.41–167.9)	0.032
qSOFA score	5.16 (2.60–10.24)	<0.0001	4.44 (1.52–13.00)	0.007
Charlsons' comorbidity index	1.20 (1.00–1.43)	0.044	*	
Age	1.13 (1.04–1.22)	0.002	*	
SOFA score	1.98 (1.51–2.61)	<0.0001	*	
APACHE II score	1.35 (1.19–1.52)	<0.0001	1.33 (1.15–1.53)	<0.0001

Variables included in analysis are those that are different between survivors and non-survivors coming from supplementary Table 2, http://links.lww.com/SHK/B388.
*Variables excluded from analysis after 5 steps.
APACHE, acute physiology and chronic health evaluation; HBP, heparin binding protein; OR, odds ratio; SOFA, sequential organ failure assessment.

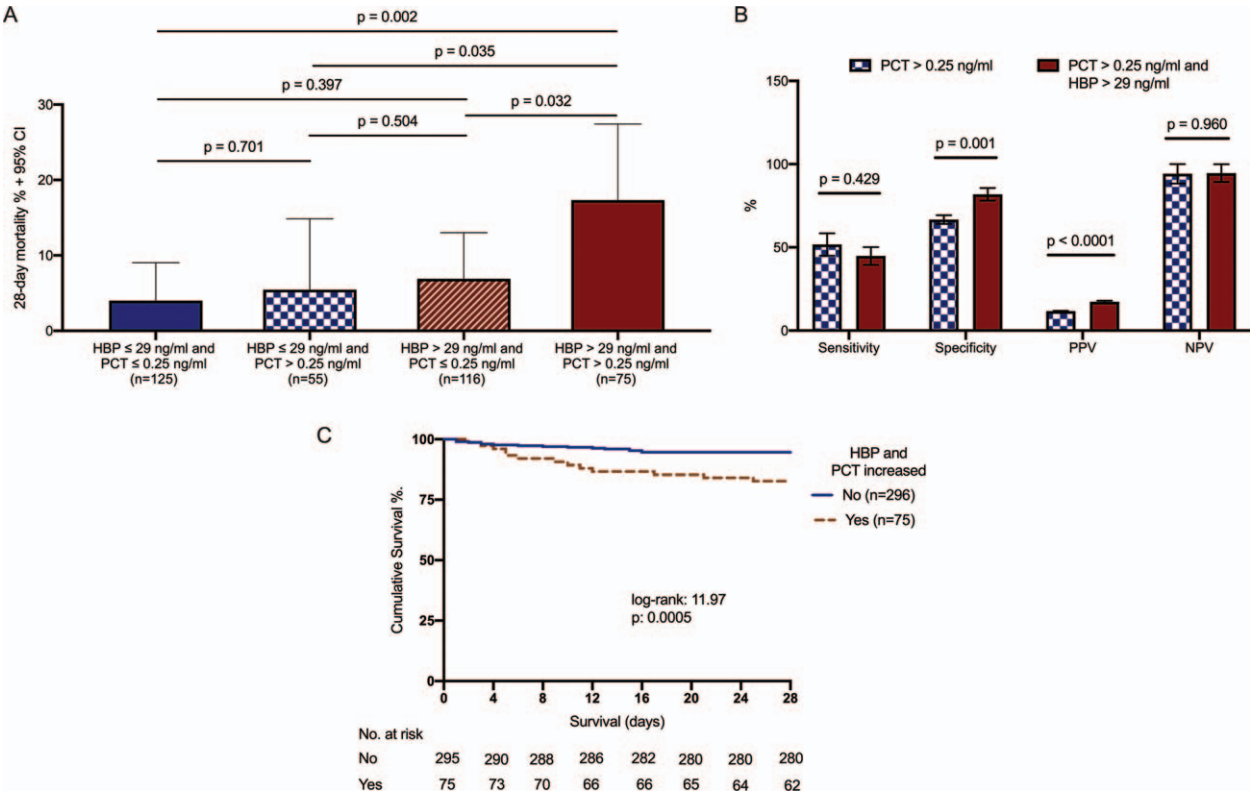


Fig. 4. **Integration of heparin binding protein (HBP) with procalcitonin (PCT) for the prediction of long-term outcome of sepsis. Concentrations of HBP and PCT were measured in plasma of patients with signs of infection upon admission in the emergency department.** (A) 28- day mortality among patients without any increase of admission HBP or PCT; among patients with increase of only PCT or HBP and among patients with increase of PCT and HBP. The *P* values of significance of the indicated comparisons are given. (B) Comparative diagnostic performance of PCT and of PCT/HBP combination for the prediction of death 28 days after admission in the ED. (C) Kaplan–Meier analysis of 28-day survival in association with plasma HBP and PCT admission levels; the log-rank test and *P*-value are given.

2. the fact that several clinical studies, including this study, report similar cut-off values of 15 to 30 ng/mL for the diagnosis of sepsis and for outcome prediction.

Our findings propose the utility of HBP as a tool for sepsis diagnosis and moreover as part of a novel prediction score for identifying patients with signs of infections who are at risk by taking into account the admission levels of PCT and HBP. Using different cut-offs of these biomarkers, the score may predict the likelihood for the development of sepsis, for early death and for 28-day outcome, thereby helping in triage and in decision making in the ED. However, it needs to be taken into consideration that the values of HBP may vary over time, particularly in patients at septic shock in need of vasopressors, and repeated measurement may be needed (16).

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