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Heparin-binding protein as a Prognostic Biomarker of Sepsis and Disease Severity at the Emergency Department

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Running title: HBP as marker for severe infections

Abstract

OBJECTIVE: Rapid and early detection of patients at risk to develop sepsis remains demanding. Heparin-binding protein (HBP) has previously demonstrated good prognostic properties in detecting organ dysfunction among patients with suspected infections. This study aimed to evaluate the plasma-levels of HBP as a prognostic biomarker for infection-induced organ dysfunction among patients seeking medical attention at the emergency department.

DESIGN: Prospective, international multicenter, convenience sample study

SETTING: Four general emergency departments at academic centers in Sweden, Switzerland and Canada.

PATIENTS: All emergency encounters among adults where one of the following criteria were fulfilled: a) respiratory rate >25 breaths per minute; b) heart rate >120 beats per minute; c) altered mental status; d) systolic blood pressure <100 mm Hg; e) oxygen saturation <90% without oxygen; f) oxygen saturation <93% with oxygen; g) reported oxygen saturation <90%.

INTERVENTION: None

MEASUREMENTS AND MAIN RESULTS: A total of 524 ED patients were prospectively enrolled, of these 236 (45%) were eventually adjudicated to have a non-infectious disease. Three hundred forty-seven patients (66%) had or developed organ dysfunction within 72 hours, 54 patients (10%) were admitted to an intensive care unit (ICU), and 23 patients (4%) died within 72 hours. For the primary outcome, detection of infected-related organ dysfunction within 72 hours, the AUC for HBP was 0.73 (95% C.I. 0.68-0.78) among all patients and 0.82 (95% C.I. 0.76-0.87) among patients confidently adjudicated to either

infection or no infection. Against the secondary outcome, infection leading to admittance to the ICU, death or a persistent high SOFA-score due to an infection (SOFA-score ≥5 at 12-24 hours) HBP had an AUC of 0.87 (95% C.I. 0.79-0.95) among all patients and 0.88 (95% C.I. 0.77-0.99) among patients confidently adjudicated to either infection or non-infection.

CONCLUSIONS: Among patients at the emergency department, HBP demonstrated good prognostic and discriminatory properties in detecting the most severely ill patients with infection.

Keywords: HBP, intensive care, emergency department, sensitivity, specificity, multicenter study

Introduction

Sepsis is a complex and life-threatening condition caused by a dysregulated immune response to infection, which leads to damages at both cell and tissue levels. Sepsis is common and affects approximately 700 / 100 000 every year (1-3), thus being more prevalent than the most common forms of cancer (i.e. prostate, breast, skin, bowel, lung, malignant melanoma and urinary tract cancer) (4). The mortality in sepsis is high, with more than 5 million deaths worldwide annually (5). Early recognition and treatment of sepsis is of outmost importance for the outcome (6).

Predicting which patients presenting with an infection that will progress to develop infection-induced organ failure in the emergency department (ED) is challenging. The symptoms of sepsis can be vague and are often misinterpreted. In a study by *Gille-Johnson et al*, 23% of the patients with symptoms for an infection and intact organ function when presenting at the ED developed severe sepsis within 24 hours (7). This further implicates the need for reliable sepsis biomarkers.

The quest for new useful biomarkers in sepsis has been challenging (8). In the era of multi-omics, biomarkers may be the path towards a more personalized approach to the septic patient (9). Heparin-binding protein (HBP) is a promising candidate (10). It is a constitutively expressed protein stored in the azurophilic granulae and secretory vesicles of neutrophils (11); as such its release results in it being among the first detectable markers of infection. HBP serves many important biological functions but its vascular actions are most relevant to the development of organ failures. HBP has been shown to be the link in neutrophil-derived induction of vascular leakage (12). It has subsequently been shown to reliably predict the development of severe sepsis among febrile patients (10,13). HBP has also been evaluated as

a prognostic or predictive marker in bacterial meningitis, urinary tract infections, infections in lung transplantation and cystic fibrosis patients (14-17).

Prior studies of HBP have focused primarily on patients with suspected infection. To truly validate HBP, as a marker for infection-related organ dysfunction (OD) in a clinical setting, the present study included the full spectrum of patients presenting at the ED, both with and without infections. Thus, the objectives of the current study were to 1) evaluate HBP as a prognostic biomarker for infection-induced organ dysfunction among patients seeking medical attention at the emergency department regardless if there were a suspicion of infection or not; and 2) to compare the prognostic properties of HBP to other biomarkers currently in use.

Material and Methods

This was a prospective, multicenter, observational, convenience sample study of ED patients (ClinicalTrials.gov NCT02366650) conducted at two Swedish (Skåne University Hospital, Lund and Helsingborg Hospital, Helsingborg), one Swiss (Inselspital University Hospital, Bern) and one Canadian (St Paul's Hospital, Vancouver) academic centers. The size of the catchment areas of the respective hospital varied from 200,000 to 1,500,000 inhabitants and the annual visits of the EDs range from 45,000 to 115,000 per year. Patients were recruited during February 2015 and then again from January to March 2016 in Lund (Sweden), February to April 2015 in Bern (Switzerland), March to April 2015 in Vancouver (Canada) and April to December 2015 in Helsingborg (Sweden). The Institutional Review Board for Human Research approved the trial at each center (Lund and Helsingborg 2014/41, Bern KEK 315/14, Vancouver H11-00505).

Patient population

Patients were enrolled upon presentation to the ED when fulfilling the following inclusion criteria: 1) age over 18 years 2) at least one of the following criteria irrespectively of cause to the condition a) respiratory rate >25 breaths per minute; b) heart rate >120 beats per minute; c) altered mental status; d) systolic blood pressure <100 mm Hg; e) oxygen saturation <90% without oxygen; f) oxygen saturation <93% with oxygen; g) reported oxygen saturation <90%. The inclusion criteria were adopted from the RETTS™-triage system, which is system used at the majority of Emergency Departments in Sweden (18). The vital parameters correspond to Red RETTS (demanding immediate attention) or Orange RETTS (demanding attention within 1 hour).

Patients fulfilling the inclusion criteria were asked to participate in the study and to give their written informed consent. If the patient was unable to give an informed consent their next-of-kin was asked for permission. If the patient died without being able to give a consent (and no consent from next-of-kin was available), the use of data and samples was requested at the local ethical committee. The inclusion or exclusion of the data and samples was in accordance with ethical board decision. If patients or their next-of-kin denied or withdrew consent, they were excluded from the study.

Data collection

Patient data collected at enrollment included demographics, vital signs (heart rate, respiratory rate, blood pressure, arterial oxygen saturation [SaO_2], and mental status). Vital signs were documented at enrollment and during the further course, according to the observational study design. The cause for the visit to the ED was noted by the including physician. Laboratory testing (white blood cell count [WBC], platelets, C-reactive protein [CRP], prothrombin time test presented as international normalized ratio [INR], activated partial thromboplastin time

[aPTT], bilirubin, serum creatinine, and plasma lactate) was performed. Microbiological investigations were performed at the discretion of the treating physicians, and results were obtained for this study. The time to and treatment duration of antibiotics was registered. Data on comorbid conditions, concomitant medication, new medications (including intravenous fluids and vasopressors), and if the patient was transferred to an intensive care unit (ICU) were recorded. OD and mortality within the 72-hour study period was monitored.

Sample Collection and Biomarker Assays

Venous blood samples for the determination of biomarkers were drawn in EDTA tubes from patients at enrollment. Samples were centrifuged, stored at –80°C within 2 hours of collection. HBP and procalcitonin (PCT) were subsequently analyzed in a centralized laboratory. HBP was analyzed in duplicate using the Axis-Shield HBP microtiter plate enzyme-linked immunosorbent assay (Axis-Shield Diagnostics, Dundee, United Kingdom) and PCT by the ADVIA Centaur BRAHMS PCT assay (Siemens Healthcare Diagnostics, Surrey, United Kingdom). WBC, CRP, and lactate analyses were performed at the Clinical Chemistry Departments at each site. Prior to analysis each sample was visually screened for hemolysis.

Definition of Outcomes

The primary outcome was the presence or development of infection-induced OD within the 72-hour time period from enrollment. OD within 72 hours from enrollment was defined according to the Sepsis-2 definition since this definition was the definition in use when the study was conducted.

The criteria for OD for sepsis-2 were adapted from consensus criteria for sepsis syndrome (18) (19). OD was defined as present when any definition of organ dysfunction was met

within 72 hours from admission in the absence of preexisting pathology that could explain the abnormal results (for definition of organ dysfunction see supplemented table S1, http://links.lww.com/SHK/A852). Plasma lactate elevation was not included as a criterion for OD as its utility was evaluated as a marker in the study. If the systolic blood pressure was <90 mmHg or the drop in blood pressure was >40 mmHg but the patient was sent home within 12 hours from the ED and had not received any fluid, this was not regarded as a circulatory OD (6 patients were redefined by this additional criterion). If patients were discharged from the hospital before the 72-hour period and there was no evidence of OD during the hospitalization, they were assumed to have no subsequent OD.

For the calculation of SOFA-score a modified SOFA-scale was used. The reason was that the majority of patients were treated on regular hospital wards and thus PaO_2/FiO_2 was not available (table S2, http://links.lww.com/SHK/A852). The FiO_2 was estimated using a conversion table (supplemented table S3, http://links.lww.com/SHK/A852).

A *critical disease* was defined as condition leading to death or admittance to the ICU within 24 hours or a SOFA-score ≥5 at 12-24 hours post enrollment. The time when the organ dysfunction developed was evaluated and categorized in 4 groups; (i) present at inclusion, (ii) developing within 0-12 hours, (iii) 12-24 hours, (iv) 24-48 hours, or (v) 48-72 hours after admittance to the ED. A *critical infection* was defined as a *critical disease* caused by an infection.

The presence of an infection was assessed by two independent Infectious Diseases (ID) consultants (F.K. and A.L. or P.Å.) blinded to the results of HBP and PCT. If the classification differed a third ID physician reviewed the data and the patient was discussed by all three physicians to reach consensus. The patients were divided into five groups; (i)

bacterial infection, (ii) probable bacterial infection, (iii) probably not an infection, (iv) no infection and, (v) viral infection

(I) Bacterial infection (hereafter called Infection) was defined as a microbiological or a radiological finding together with symptoms of infections from that site, or a positive blood culture with a relevant pathogen. Patients with chronic obstructive pulmonary disease (COPD) and lung symptoms with concomitant radiological findings were defined as probable infections (II).

(II) Probable bacterial infection (hereafter called probable infection) was defined as the presence of an infection causing the current disease as evaluated by the reviewing physician but lack of criteria for a bacterial infection (i.e. presence of a microbiological, or a radiological finding from the suspected site of infection OR a positive blood culture).

(III) Probably not an infection was defined as most likely a condition not caused by a bacterial infection by the reviewing physician but not fulfilling the criteria no infection (e.g. the patient may have had a positive culture from an irrelevant site, or a dose of antibiotics might have been given).

(IV) No infection was defined as the presence of a clear non-bacterial infection diagnosis, no positive cultures, no use of antibiotics, and survival of at least 12 hours post-inclusion.

(V) Viral infection was defined as:

a. A finding of a viral agent by PCR likely of giving rise to the present disease and the absence of any probable bacterial cause was defined as a viral cause

OR

b. Patients diagnosed with a viral infection by the treating physician, with laboratory and microbiological findings in agreement, and no use of antibiotics but with no certain findings of a viral agent

Statistical Methods

Means, medians, standard deviations (SDs), and interquartile ranges (IQRs) were reported as appropriate. Differences in frequencies between groups were tested with the chi^2-test and Fisher's exact test as appropriate and differences between group medians with the Mann-Whitney U test. The Jonckheere-Tepstra test was used to asses ordered differences between classes. Area under the receiver operating curve (AUCs) were calculated to assess the diagnostic power of each marker. Youden's index was used to calculate the optimal cut-off for HBP. For analysis of risk versus HBP-levels, the relationship was fitted using Local Weighted Scatterplot Smoothing (LOWESS). To assess the performance of HBP in relation to proportion of infected patients only patients able to be confidently adjudicated as to the presence or absence of infection were included (n=332, group I and IV). Samples from the groups I and IV were drawn (with replacement) to mimic cohorts with the proportion of infected patients ranging from 10% to 100% in steps of 1%. For each proportion of infected patients 100 stochastic cohorts (each with 1000 patients) were drawn and the AUC-values for the different biomarkers were calculated and the median was calculated and plotted. To assess the association between biomarkers and infection or organ dysfunction/critical disease, respectively, a logistic regression was then fitted. First, each biomarker was dichotomized according to their respective cut-off value suggested by Youden's index. Next, a logistic regression was fitted using infection and organ dysfunction/critical disease as well as the interaction between them as covariates. In no cases the interaction term was significant and therefore a new logistic regression was fitted without the interaction term and the

corresponding odds ratios (ORs) were calculated. R (R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) was used for statistical computation with the following packages: readxl, ggplot2, grid, gridExtra, clinfun, pROC, epiR, dplyr and Hmisc. P-values lower than 0.05 were regarded as significant.

RESULTS

Patient Characteristics

A total of 718 ED patients were prospectively enrolled. Patients with WBC >50 x 10^9/L or <3 x 10^9/L or missing WBC values were excluded from the study since HBP is a neutrophilic protein and excess or lack of neutrophils may give rise to erroneous results. Furthermore, samples with any sign of hemolysis were excluded, since hemolysis could indicate lysis of white blood cells and hence concomitant release of HBP to the blood. In total, 194 patients (27%) were excluded, of these 146 patients (75%) were excluded due to hemolysis (Figure 1). Details on patient characteristics are presented in Table 1 and characteristics subdivided per site can be found in Table S4 (http://links.lww.com/SHK/A852) and Figure S1 (http://links.lww.com/SHK/A849).

HBP as predictor of infection-related organ dysfunction

HBP-levels were analyzed according to both the likelihood of infection (group I-V) and presence of organ dysfunction (OD) during the 72 hours study period (Figure 2). Overall, infected patients had significantly higher HBP-levels than non-infected patients. HBP levels were highest (median 18.3 ng/mL, IQR 9.3-32.7 mg/mL in infected patients with OD, these being significantly higher values than in infected patients without OD (median 9.4 mg/mL, IQR 7.4-17.6 ng/mL) (p=0.036).

Three hundred thirty-two patients were able to be confidently adjudicated to have presented with infection or non-infectious cause of illness (group I and IV). To investigate how specific HBP was in detecting infection-induced OD, ROC curves were constructed and the AUCs were calculated for HBP, PCT, CRP, WBC, and lactate among these patients. CRP had the highest AUC followed by HBP (Table 2). Suggested cut-off values were calculated using Youden's index and discriminatory values were calculated (Table 2).

To assess how HBP might perform prospectively (without the benefit of post-hoc adjudication as to the presence of infection), sensitivity analyses were performed in the entire cohort (n=524, group I-V). We performed a similar analysis including patients with hemolytic samples (n=670, n=426), as well as subdivision per site) (supplemented table S5 and S6, http://links.lww.com/SHK/A852). Although the AUC was slightly lower in these analyses HBP performed well as a predictor of OD in all analyses (supplemented table S5 and S6, http://links.lww.com/SHK/A852).

HBP levels are related to the severity of the disease

To assess the potential bias that patients with infections were more severely ill at presentation than non-infected patients, they were stratified based on number of dysfunctional organs **(Figure 3).** For every number of dysfunctional organs patients with infection (n=96, group I) had significantly higher HBP-levels than non-infected patients (n=236, group IV). Infected patients with more than 4 dysfunctional organs had the highest HBP levels (median 31.9 ng/mL, IQR 22.9-110.7 ng/mL), with a significant increase in HBP-levels according to number of dysfunctional organs (p=0.00084) In non-infected patients HBP levels were lower overall, though a similar significant increase existed wherein HBP levels were highest in those with more than 4 dysfunctional organs (p=0.019). The same pattern was true when assessing SOFA-scores at admission instead of number of dysfunctional organs (Figure S2,

http://links.lww.com/SHK/A850). Altogether, these analyses suggest that the level of HBP at presentation is increased predominantly as a response to infection as well as to the severity of disease.

HBP is a good predictor of infection-related admission to intensive care or mortality

An important property of a sepsis biomarker is to correctly identify patients with substantial increased *risk* of rapid clinical deterioration. Thus, to study the prognostic capacity, as opposed to the diagnostic capacity outlined above, a composite outcome was constructed and analyzed as a secondary outcome. This outcome was defined as death within 24 hours or admission to the ICU within 24 hours. However, the decision of admitting a patient to intensive care in part depends on local tradition and the availability of intensive care. Therefore, SOFA-score was added to account for this bias. A SOFA-score ≥5 was previously shown to be correlated with a mortality of more than 25% (19). Hence, a SOFA-score ≥5 at 12-24 hours was included in the composite outcome, hereafter called *critical disease*. An infection giving rise to a *critical disease* was defined as a *critical infection* (Figure 4). Analysis of the prognostic potential for detecting *critical infection* in infected and non-infected patients (group I and IV, n=332) showed that HBP had the highest AUC 0.88 (0.77-0.99, n=332), followed by CRP (0.86 [0.73-0.99], n=301). Suggested cut-off values were calculated using Youden's index and discriminatory values for *critical infection* were calculated (table 3).

To verify the results, sensitivity analyses were performed with the whole cohort (n=524, group I-V), and the total cohort including patients with hemolytic samples (n=670 and n=426) as well as subdivision per site. These analyses were in agreement with the results above (supplemented table S7 and S8, http://links.lww.com/SHK/A852).

An analysis was also made to investigate the relationship between plasma-HBP and the *risk* for *critical infection*. Both when including only confidently adjudicated cases as well as including the whole cohort the risk for *critical infection* increased as HBP-levels increased (Figure S3, http://links.lww.com/SHK/A851). In the whole cohort, an HBP level of over 30 ng/mL resulted in a likelihood ratio of 8.17 (5.36-12.45) of developing *critical infection,* whereas HBP levels less than 15 ng/mL resulted in a negative LR of 0.23 (0.09-0.56) (Table S7, http://links.lww.com/SHK/A852).

Finally, an analysis of patients able to be confidently adjudicated as to the presence of infection (groups I and IV) with HBP >30 ng/mL but no *critical infection* was performed. Of 332 patients, 320 patients (96.4%) did not experience a *critical infection*, and of these 26 (8.1%) had HBP above 30 ng/mL. Characteristics of these patients can be found in supplemented table S9 (http://links.lww.com/SHK/A852).

The performance of HBP is related to the proportion of infected patients in the population at study inclusion

Organ dysfunction, death or admission to the ICU can be caused both by infectious and non-infectious diseases but only infection-related events were regarded as a positive outcome in this study. Thus, the prognostic performance will be affected by the proportions of infected patients in the cohort since only infection-induced events were regarded as positive in this study. The proportion of infected patients will vary between different settings, time of year but also due to different triage systems. In the whole cohort, 34% were infected (excluding virus). To isolate the ability to prognosticate OD or *critical infection* from the ability of detecting infection, a sensitivity analysis was done. Only patients able to be confidently adjudicated as to the presence of infection were included and new samples were drawn from patients with infection and non-infection, respectively, until a new cohort of 1000 patients

was created. The sampling was adjusted so that the proportion of infected patients varied from 10% to 100% in steps of 1%. For each cohort, the AUC for OD and *critical infection* was calculated (Figure 5A and 5B).

In cohorts with a low frequency of infection, CRP was the best biomarker for infection-induced OD but as the proportion of infected patients increased, HBP and PCT exceeded CRP in detecting infection-induced OD. HBP was the best marker in detecting *critical infection* across all frequencies of infection.

To further verify this result and to dissect the individual importance of infection and organ dysfunction a multivariable analysis was performed with each biomarker as the dependent variable and infection and organ dysfunction/critical disease as covariates. The biomarkers were dichotomized according to their respective cut-off level suggested by Youden's index (Table 4). All biomarkers except lactate were significantly associated with infection. Only HBP was significantly associated with OD. For critical disease HBP, CRP, WBC and lactate had a significant association although HBP had the highest odds ratio (OR).

Discussion

In this ED-based prospective observational multicenter study, HBP was a good marker for identification of patients at risk of developing a *critical infection*. Previous studies on the diagnostic and prognostic properties of HBP have focused on cohorts mainly consisting of infected patients. The present study investigated the specificity of HBP among unselected patients with all types of diagnoses. Hence, this study favored inclusion of patients with a broad spectrum of diagnoses but also more severely ill patients.

The primary outcome of the study was detection of any OD due to infection within 72 hours in accordance with a previous study (13). Against this outcome HBP had an AUC of 0.82 but was exceeded by CRP (AUC 0.87). The good performance of CRP is explained by the excellent ability of CRP in differing infectious diagnoses from non-infectious diagnoses. In this cohort, with a high proportion of patients presenting with or developing OD within 72 hours, correct identification of an infection will have a large impact on the AUC (Figure 5). When raising the percentage of patients with infection, the performance of CRP deteriorates. Adjusting the fraction of infected patients to 89%, equivalent to the study by *Linder et al*, CRP had an AUC of 0.62, based on the present cohort, as compared to 0.70 in the previous study. In contrast, HBP had an AUC of 0.72, based on the present cohort, compared to 0.80 in the *Linder et al* cohort (13). This was also verified in a multivariable logistic regression where CRP had a very high OR for infection but was not significantly associated with organ dysfunction. HBP was the only biomarker of those studied that was significantly associated with both infection and organ dysfunction.

OD is, however, a blunt criterion for the definition of a serious infection. Patients with transient OD (e.g. a SaO_2<90% at one occasion) do not necessarily need to be admitted to intermediate or intensive care. The most important feature of a prognostic biomarker is to identify patients in need of special treatment. HBP demonstrated good prognostic properties with an AUC of 0.88 in predicting *critical infections* and outperformed all other markers studied. The cut-off value to detect a *critical infection* was 22.85 ng/mL. This value is in agreement with earlier studies that suggested cut-offs of 15 ng/mL or 30 ng/mL (10,13).

As the severity of disease increased there was a concomitant increase in HBP-levels. Furthermore, higher HBP-levels were associated with higher risk for a *critical infection*. This suggests that it may be useful to have two cut-off levels for HBP. Setting the cut-off to ≥30 ng/mL the positive predictive value was 0.27 and the positive likelihood ratio was 8.17 in

detecting a *critical infection* in the whole cohort. On the other hand, HBP <15 ng/mL had a negative predictive value of 0.99 and a negative likelihood ratio of 0.23. These results indicate that patients with HBP ≥30 ng/mL are at greater risk of having or developing a *critical infection* and will thus require close monitoring and the use of empirical antibiotic therapy might be considered while concomitantly investigating for possible differential diagnoses. For patients with HBP <15 ng/mL the risk of an unfavorable infection-related outcome is low. Patients with HBP between 15 and 30 ng/mL have an intermediate risk and they may benefit from observation for a few hours followed by a new HBP test. However, consecutive HBP measurements was not part of the present study, and therefore this assumption is speculative.

Patients without infection also had higher HBP levels as the number of failing organs increased but from lower levels of HBP compared to infected patients. Indeed, a few patients (n=8) without infection had HBP values above 30 ng/mL. These patients had a significantly increased mortality rate (38% vs 4%) compared to non-infected patients with HBP below 30ng/mL (n=227). It is not surprising that some patients with severe non-infectious diseases indeed have high HBP levels. It has previously been shown that patients with neutrophil-driven vasculitides have high HBP-levels (20). Furthermore, recent work has demonstrated that hypoxia can drive neutrophil activation and release of neutrophil granular proteins (21-23). It is therefore reasonable that a few non-infected patients with several dysfunctional organs may have elevated HBP-levels. Importantly, as can be seen in figure 3, when comparing patients with the same degree of physiological disturbances, infected patients have significantly higher HBP-levels than non-infected patients. Previous studies have also shown that increased plasma- HBP is associated with development of septic acute kidney injury (AKI) in an intensive care cohort (24). This is in agreement with the present study where septic AKI was part of the organ dysfunction criterium. If septic AKI *per se*, as a result of

reduced kidney function, can increase plasma-HBP has not been studied in humans. Interestingly, preliminary data from a mouse model suggest that the main HBP-clearing organ is the liver and not the kidneys (Fisher *et al* manuscript in preparation)

In this study, where the inclusion criteria favored inclusion of more severely ill patients and also non-infected patients, the ability of HBP to identify patients with infection-induced OD was lower than was the ability of CRP, in contrast to previous studies. Still, this study showed that there was an increase in HBP-levels with increasing number of organ dysfunction implying that HBP is a marker of disease severity. In addition to the use of biomarkers, several scores (e.g. qSOFA, NEWS2) are used in the ED to assess patients at risk for sepsis and infection-induced OD. HBP might have a role as an additional criterion when evaluating such scores and we are currently conducting such a study, where the patients from this study are part of the cohort (manuscript in preparation).

The secondary outcome of this study, infection-related mortality or admittance to intensive care due to an infection, was analyzed with an additional *post hoc* criterion, SOFA-score ≥5 after 12-24 hours due to an infection. This addition was made to compensate for not all seriously ill patients being admitted to intensive care. The AUC of HBP was 0.87 and highest among all biomarkers studied.

The strengths of this prospective multicenter study are the large cohort of patients included at four different sites in three different countries, the careful blinding of HBP results when determining the cause of the disease and outcome, and the analysis of HBP at a central unit. All patients were also reviewed by at least two Infectious Diseases consultants to accurately determine the diagnosis. The study had inclusion criteria which focused on the enrollment of patients with a high risk of an impending OD and did not have any criterion focusing on singling out patients with infection. The cohort therefore reflects a wide spectrum of patients

that seek medical attention at the ED. Opposed to previous studies, this design can better determine the accuracy of HBP for identifying severe infections. The limitations of the study are the incomplete sample size for some of the comparative biomarkers, the missing data for a few patients and the hemolysis in 146 samples. However, in a sensitivity analysis inclusion of these patients did only change the AUCs to a very minor extent. Most patients were treated in regular wards and the measurements of vital parameters during follow-up could not be accomplished every hour, which also is a limitation.

In conclusion, this is the first study evaluating the prognostic performance of HBP among patients, irrespective of the suspicion of an infection. HBP had a good performance in detecting infection-induced organ dysfunction but was outperformed by CRP against this outcome due to the excellent ability of CRP in detecting an infection. However, in a multivariable analysis CRP was not shown to be significantly associated with organ dysfunction but only to infection. This is opposed to HBP, which was associated with both infection and organ dysfunction. HBP was furthermore shown to be the best biomarker to prognosticate the development of a *critical infection*. When using a cut-off of 22.85 ng/mL, HBP prognosticated a *critical infection* with a sensitivity of 78% and a specificity of 86%. The results further suggest that as HBP increases there is a concomitant increase in the risk for a *critical infection*. This may facilitate clinical decision-making. Based on this, it may be useful to have two cut-off levels of HBP; one lower for negative prospective purposes and one higher where special treatment should be initiated.

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Potential conflicts of interest. B.C., P.Å., and A.L. are listed as inventors on a patent on the use of HBP as a diagnostic tool in sepsis filed by Hansa Medical AB. All other authors: No relevant conflicts of interest. Axis Shield Ltd analyzed the PCT and distributed free of charge ELISA-kits for determining HBP.

References

1. Rhee C, Dantes R, Epstein L, Murphy D J, Seymour C W, Iwashyna T J, Kadri S S, Angus D C, Danner R L, Fiore A E, *et al.*:. Incidence and Trends of Sepsis in US Hospitals Using Clinical vs Claims Data, 2009-2014. *JAMA* 318**(**13**):** 1241–1249, 2017.

2. Henriksen D P, Laursen C B, Jensen T G, Hallas J, Pedersen C, Lassen A T:. Incidence rate of community-acquired sepsis among hospitalized acute medical patients-a population-based survey. *Critical care medicine* 43**(**1**):** 13–21, 2015.

3. Mellhammar L, Wullt S, Lindberg A, Lanbeck P, Christensson B, Linder A:. Sepsis Incidence: A Population-Based Study. *Open Forum Infect Dis* 3**(**4**):** ofw207, 2016.

4. Bergman O, Fredholm L, Jaresand M, Johansson E:. *Cancerfondsrapporten 2015*. (Cancerfonden, 2015).

5. Fleischmann C, Scherag A, Adhikari N K J, Hartog C S, Tsaganos T, Schlattmann P, Angus D C, Reinhart K, International Forum of Acute Care Trialists:. Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *American journal of respiratory and critical care medicine* 193**(**3**):** 259–272, 2016.

6. Kumar A, Roberts D, Wood K E, Light B, Parrillo J E, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, *et al*:. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Critical care medicine* 34**(**6**):** 1589–1596, 2006.

7. Gille-Johnson P, Hansson K E, Gårdlund B:. Severe sepsis and systemic inflammatory response syndrome in emergency department patients with suspected severe infection. *Scandinavian journal of infectious diseases* 45**(**3**):** 186–193, 2013.

8. Pierrakos C, Vincent J L:. Sepsis biomarkers: a review. *Crit Care* 14**(**1**):** R15, 2010.

9. van Engelen T S R, Wiersinga W J, Scicluna B P, van der Poll T:. Biomarkers in Sepsis. *Crit Care Clin* 34**(**1**):** 139–152, 2018.

10. Linder A, Christensson B, Herwald H, Björck L, Åkesson P:. Heparin-binding protein: an early marker of circulatory failure in sepsis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 49**(**7**):** 1044–1050, 2009.

11. Tapper H, Karlsson A, Mörgelin M, Flodgaard H, Herwald H:. Secretion of heparin-binding protein from human neutrophils is determined by its localization in azurophilic granules and secretory vesicles. *Blood* 99**(**5**):** 1785–1793, 2002.

12. Gautam N, Olofsson A M, Herwald H, Iversen L F, Lundgren-Åkerlund E, Hedqvist P, Arfors K E, Flodgaard H, Lindbom L:. Heparin-binding protein (HBP/CAP37): a

missing link in neutrophil-evoked alteration of vascular permeability. *Nature medicine* 7**(**10**):** 1123–1127, 2001.

13. Linder A, Arnold R, Boyd J H, Zindovic M, Zindovic I, Lange A, Paulsson M, Nyberg P, Russell J A, Pritchard D, *et al*:. Heparin-Binding Protein Measurement Improves the Prediction of Severe Infection With Organ Dysfunction in the Emergency Department. *Critical care medicine* 43**(**11**):** 2378–2386, 2015.

14. Linder A, Åkesson P, Brink M, Studahl M, Björck L, Christensson B:. Heparin-binding protein: a diagnostic marker of acute bacterial meningitis. *Critical care medicine* 39**(**4**):** 812–817, 2011.

15. Kjölvmark C, Akesson P, Linder A:. Elevated urine levels of heparin-binding protein in children with urinary tract infection. *Pediatr Nephrol* 27**(**8**):** 1301–1308, 2012.

16. Stjärne Aspelund A, Hammarström H, Inghammar M, Larsson H, Hansson L, Christensson B, Påhlman L I:. Heparin-binding protein, lysozyme, and inflammatory cytokines in bronchoalveolar lavage fluid as diagnostic tools for pulmonary infection in lung transplanted patients. *Am J Transplant* 18**(**2**):** 444–452, 2018.

17. Hovold G, Palmcrantz V, Kahn F, Egesten A, Påhlman L I:. Heparin-binding protein in sputum as a marker of pulmonary inflammation, lung function, and bacterial load in children with cystic fibrosis. *BMC Pulm Med* 18**(**1**):** 104, 2018.

18. Widgren B R, Jourak M:. Medical Emergency Triage and Treatment System (METTS): a new protocol in primary triage and secondary priority decision in emergency medicine. *J Emerg Med* 40**(**6**):** 623–628, 2011.

19. Lie K C, Lau C-Y, Van Vinh Chau N, West T E, Limmathurotsakul D, for Southeast Asia Infectious Disease Clinical Research Network:. Utility of SOFA score, management and outcomes of sepsis in Southeast Asia: a multinational multicenter prospective observational study. *J Intensive Care* 6**(**1**):** 9, 2018.

20. Kahn R, Herwald H, Müller-Esterl W, Schmitt R, Sjögren A C, Truedsson L, Karpman D:. Contact-system activation in children with vasculitis. *Lancet* 360**(**9332**):** 535–541, 2002.

21. Fritzsching B, Zhou-Suckow Z, Trojanek J B, Schubert S C, Schatterny J, Hirtz S, Agrawal R, Muley T, Kahn N, Sticht C, *et al*:. Hypoxic Epithelial Necrosis Triggers Neutrophilic Inflammation via IL-1 Receptor Signaling in Cystic Fibrosis Lung Disease. *American journal of respiratory and critical care medicine* 191**(**8**):** 902–913, 2015.

22. Hoenderdos K, Lodge K M, Hirst R A, Chen C, Palazzo S G C, Emerenciana A, Summers C, Angyal A, Porter L, Juss J K, *et al*:. Hypoxia upregulates neutrophil degranulation and potential for tissue injury. *Thorax* 71**(**11**):** 1030–1038, 2016.

23. Montgomery S T, Mall M A, Kicic A, Stick S M:. Hypoxia and sterile inflammation in cystic fibrosis airways: mechanisms and potential therapies. *The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology* 49**(**1**):** 1600903–13, 2017.

24. Tverring J, Vaara S T, Fisher J, Poukkanen M, Pettila V, Linder A, FINNAKI Study Group:. Heparin-binding protein (HBP) improves prediction of sepsis-related acute kidney injury. *Ann. Intensive Care* 7**(**1**):** 105, 2017.

25. Severinghaus J W:. Simple, accurate equations for human blood O2 dissociation computations. *J Appl Physiol Respir Environ Exerc Physiol* 46**(**3**):** 599–602, 1979.

Legends

Figure 1. Flow chart of patients in the study cohort.

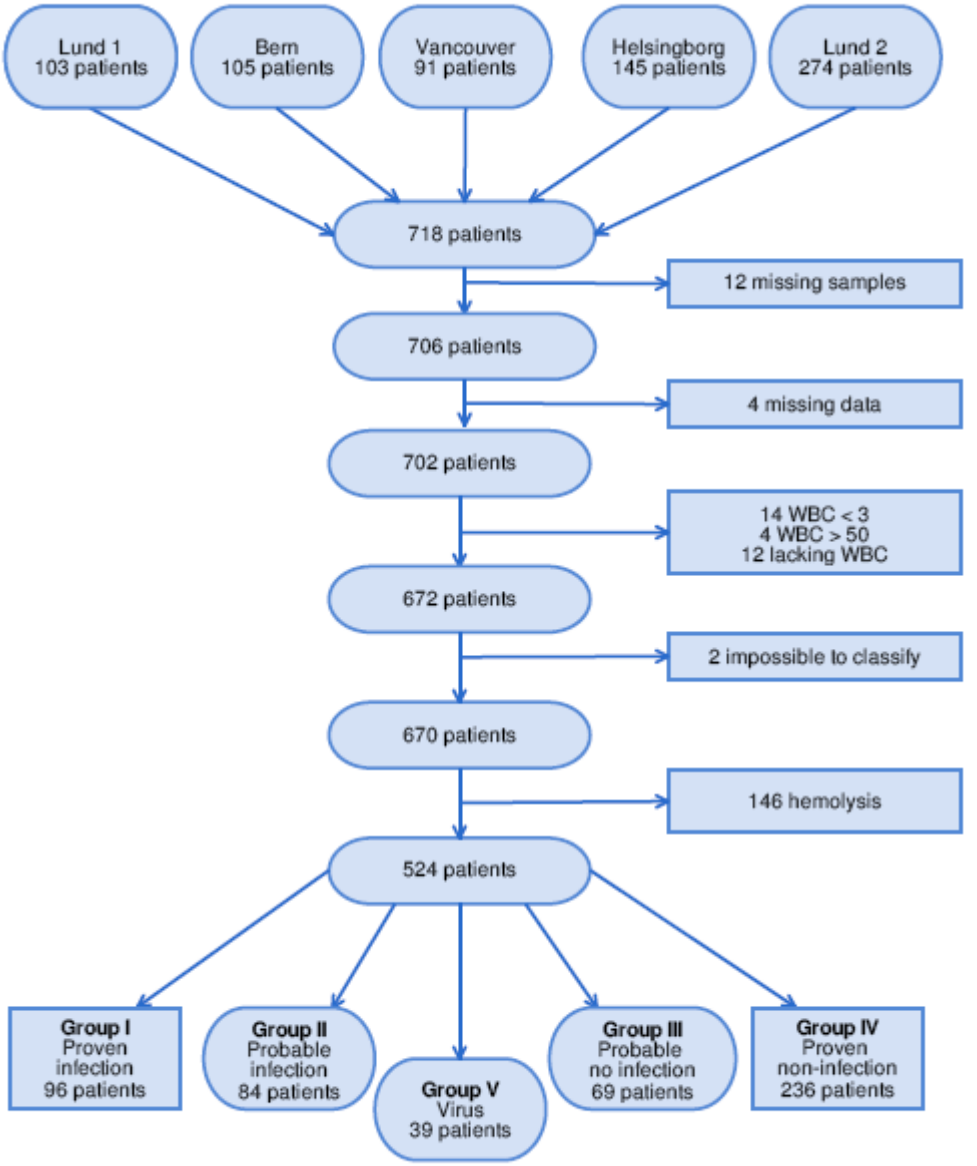

Figure 2. HBP as a marker for infection-induced organ dysfunction

Levels of HBP are shown for patients with and without OD and divided in 5 classes depending on the likelihood of infection. Boxes represent the first, second and third quartile with whiskers extending to the 10th and 90th percentile. The difference between groups was tested with the Mann-Whitney U-test. In the figure HBP-values >200 ng/mL was plotted at 200 ng/mL. Horizontal lines represent 15 and 30 ng/mL which are cut-off levels suggested by previous studies.

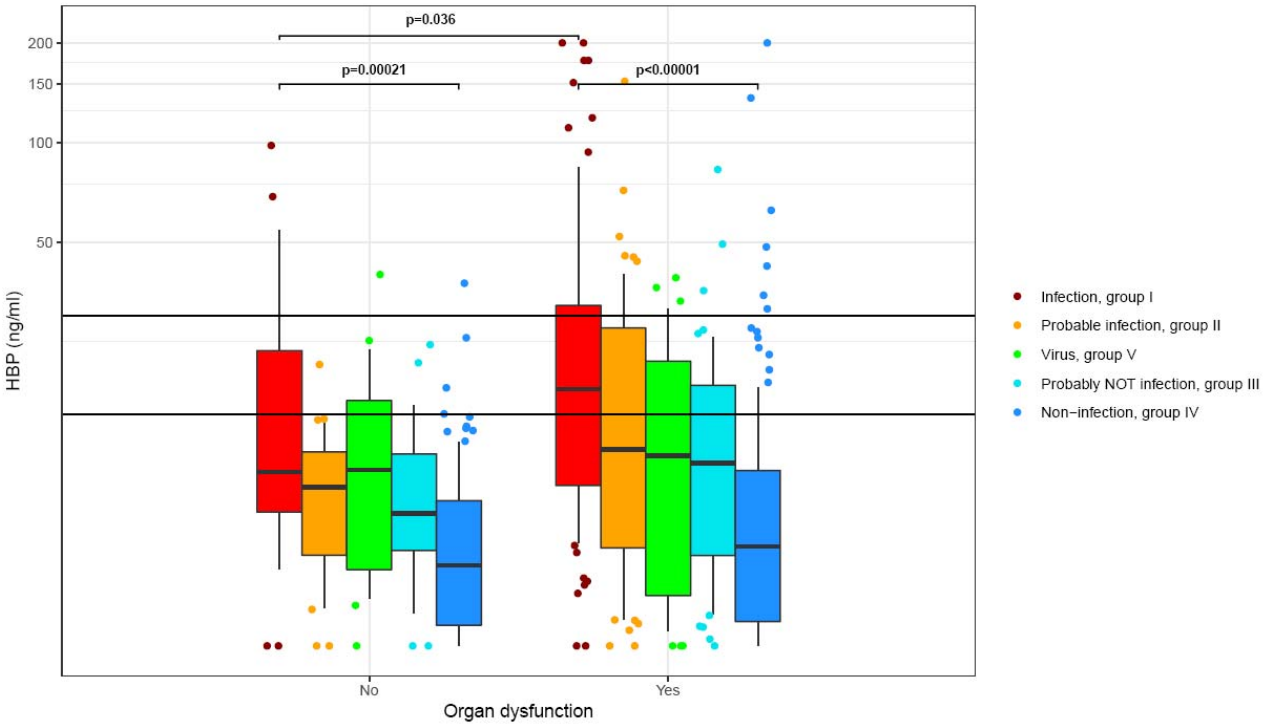

Figure 3. HBP-levels increase with severity of disease

Infected and non-infected patients (i.e. group I and IV from Figure 1) were included in this plot (n=332). The boxplots show the different numbers of failing organs within 72 hours. Boxes represent the first, second and third quartile with whiskers extending to the 10th and 90th percentile. The Jonckheere-Terpstra test for trend was used to asses if HBP-levels increase as number of failing organs increase. The difference between groups was tested with the Mann-Whitney U-test. In the figure HBP-values >200 ng/mL was plotted at 200 ng/mL. Horizontal lines represent 15 and 30 ng/mL which are cut-off levels suggested by previous studies.

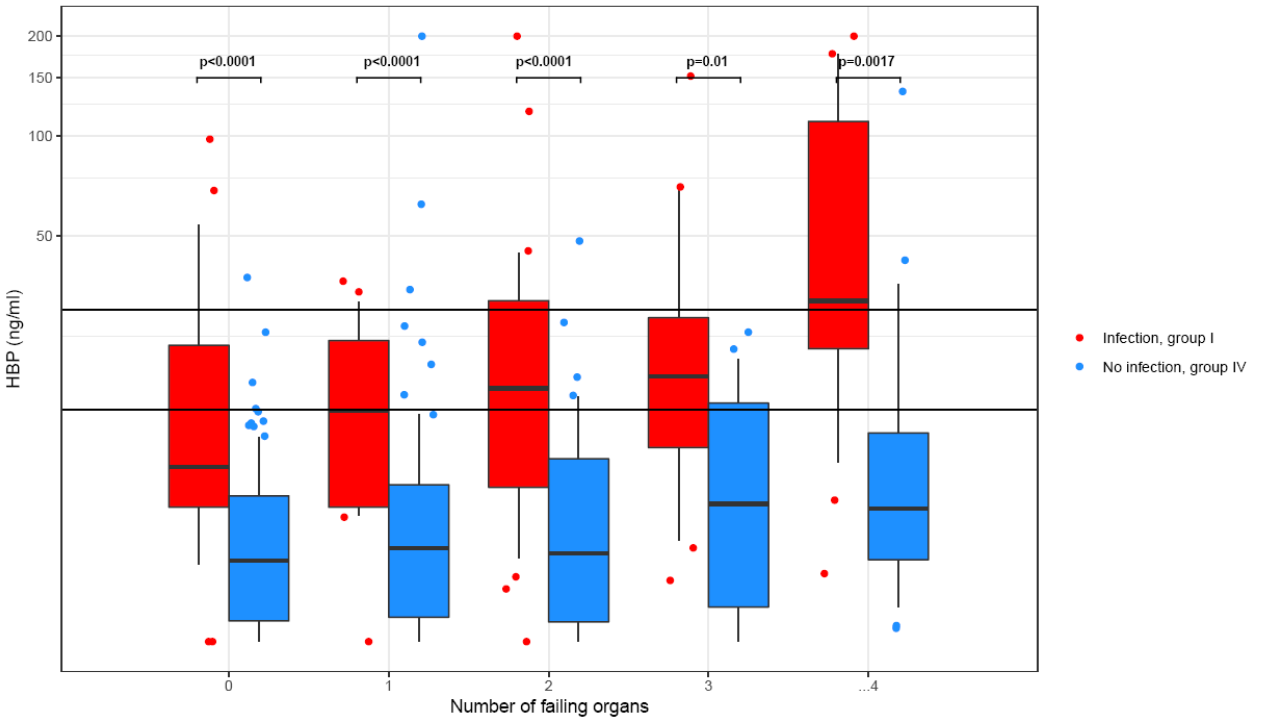

Figure 4 HBP as a prognostic test for *critical infection*

Infected and non-infected patients (group I and IV) were included in this plot (n=332). Levels of HBP are shown for patients with and without *critical disease*. Boxes represent the first, second and third quartile with whiskers extending to the 10th and 90th percentile. The difference between groups was tested with the Mann-Whitney U-test. In the figure HBP-values >200 ng/mL was plotted at 200 ng/mL. Horizontal lines represent 15 and 30 ng/mL which are cut-off levels suggested by previous studies.

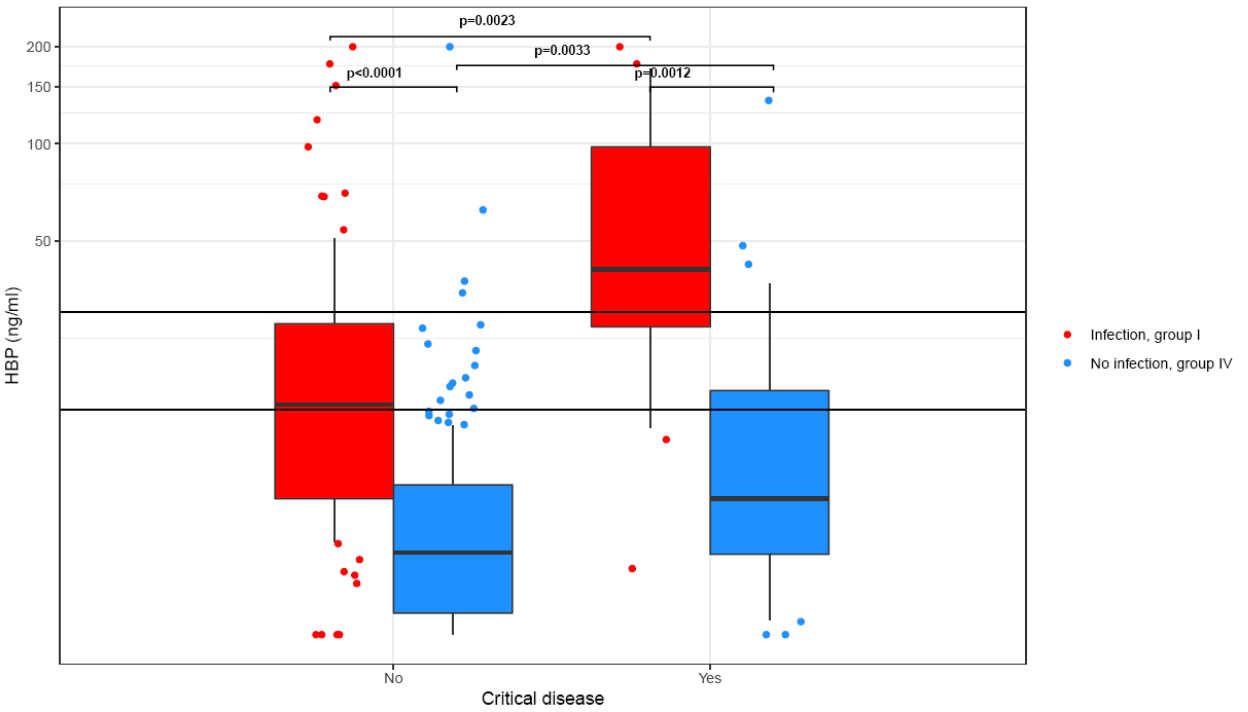

Figure 5 The discriminatory properties of biomarkers change with the frequency of infection in the cohort.

Infected and non-infected patients (group I and IV) were included in this plot (n=332, group I and IV). Samples from the two populations (infected, group I and non-infected, group IV) were drawn to mimic cohorts with the proportion of infected patients ranging from 10% to 100% in steps of 1%. For each proportion of infected patients 100 stochastic cohorts were drawn with replacement and the AUC-values for the different biomarkers were calculated and the median was calculated and plotted. The vertical line represents the proportion of infected patients in the original whole cohort (group I-V, n=524).

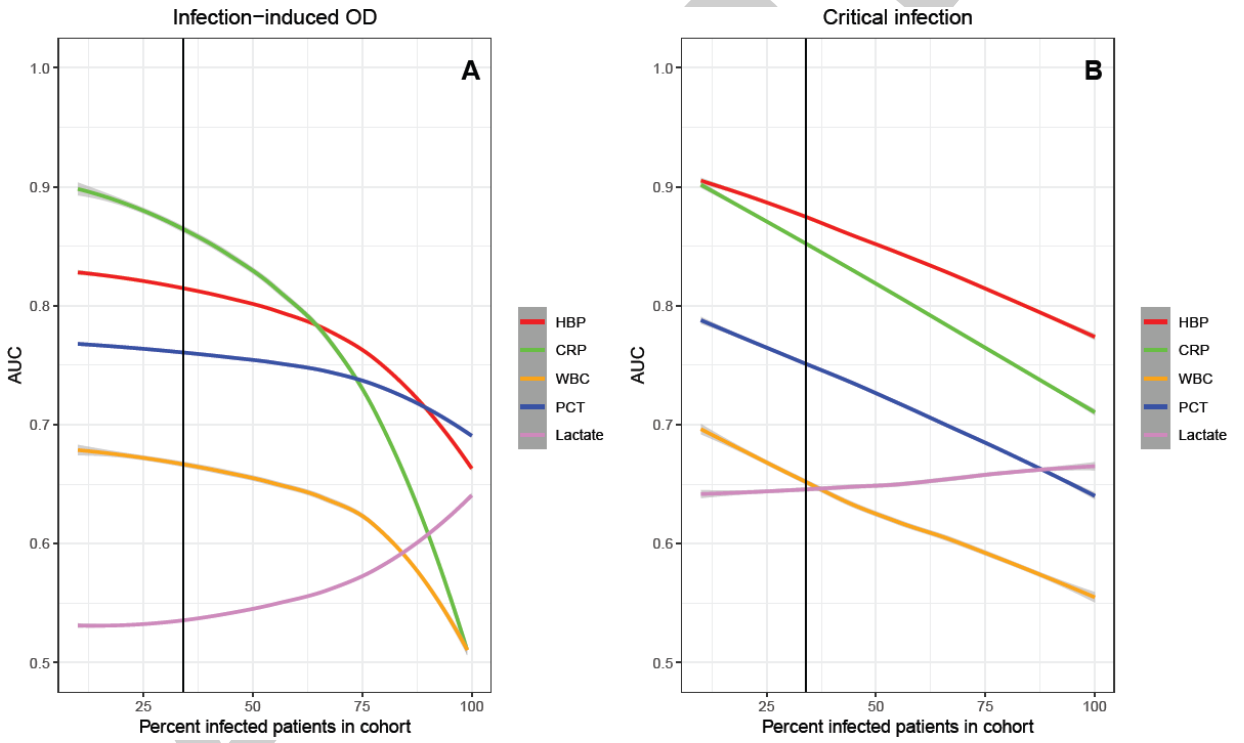

Table 1. Baseline characteristics

	N	Infection N = 96	Probable infection N = 84	Virus N = 39	Probable NOT infection N = 69	No infection N = 236	Combined N = 524	*p-value*
Age	524	76.4 (65.38 – 84.00)	72.2 (61.3– 81.2)	70.0 (52.8– 83.2	62.1 (62.1– 81.90)	71.0 (54.5– 81.6)	72.2 (58.0– 82.0)	0.12
Females	524	47% (45)	52% (44)	56% (22)	41% (28)	50% (117)	49% (256)	0.50
Medications								
Warfarin	520	15% (14)	11% (9)	18% (7)	19% (13)	24% (55)	19% (98)	0.09
Steroids before admission	520	12% (12)	16%(13)	13% (5)	13% (9)	7% (17)	11% (56)	0.17
Heparin/LMWH before admission	519	4% (4)	5% (4)	3% (1)	1% (1)	3% (6)	3% (16)	0.71
Immunosuppression before admission	520	3% (3)	1% (1)	5% (2)	0% (0)	3% (7)	2% (13)	0.40
Comorbidities								
Diabetes	524	22% (21)	18% (15)	15% (6)	30% (21)	17% (40)	20% (103)	0.15
Cardiovascular	524	51% (49)	50% (42)	31% (12)	49% (34)	47% (112)	48% (249)	0.27
Renal	524	27% (26)	10% (8)	13% (5)	16% (11)	12% (29)	15% (79)	**0.01**
Respiratory	524	25%	36%	21%	39%	20%	26%	**0.006**

		(24)	(30)	(8)	(27)	(48)	(137)	
COPD	524	5% (5)	26% (22)	13% (5)	30% (21)	6% (14)	13% (67)	**<0.00001**
Liver	524	10% (10)	2% (2)	3% (1)	4% (3)	3% (8)	5% (24)	0.09
Malignancy	524	12% (12)	10% (8)	13% (5)	16% (11)	11% (27)	12% (63)	0.79
Immunodeficiency	524	2% (2)	2% (2)	5% (2)	0% (0)	1% (3)	2% (9)	0.27
No comorbidities	524	25% (24)	21% (18)	38% (15)	19% (13)	33% (77)	28% (147)	**0.046**
Outcome								
ICU-admittance within 72 hours	524	11% (11)	6% (5)	8% (3)	14% (10)	11% (25)	10% (54)	0.49
Mortality within 72 hours	524	2% (2)	4% (3)	0% (0)	9% (6)	5% (12)	4% (23)	0.21
Positive blood culture	524	44% (42)	0% (0)	0% (0)	0% (0)	0% (0)	8% (42)	**<0.00001**
SOFA at inclusion	524	2 (1–4)	1.5(1–3)	2 (1–3)	2 (1–3)	1(0–2)	1 (0–3)	**<0.00001**
Number of dysfunctional organs	524							**<0.00001**
0		22%% (21)	31% (26)	33% (13)	26% (18)	42% (99)	34% (177)	
1		21% (20)	32% (27)	46% (18)	20% (14)	31% (72)	29% (151)	
2		27% (26)	17% (14)	8% (3)	35% (24)	14% (34)	19% (101)	

3		17% (16)	8% (7)	5% (2)	4% (3)	6% (14)	8% (42)	
4		5% (5)	7% (6)	3% (1)	6% (4)	1% (3)	4% (19)	
5		1% (1)	0% (0)	5% (2)	0% (0)	1% (2)	1% (5)	
6		3 (3)	1% (1)	0% (0)	0% (0)	0% (0)	1% (4)	
7		4%(4)	4% (3)	0% (0)	9% (6)	5% (12)	5% (25)	
Diagnoses	**524**							
Infections								
Central nervous		1% (1)	0% (0)	0% (0)	0% (0)	0% (0)	0% (1)	
COPD exacerbation		0% (0)	14% (12)	3% (1)	0% (0)	0% (0)	2% (13)	
Endocarditis		4% (4)	0% (0)	0% (0)	0% (0)	0% (0)	1% (4)	
Gastrointestinal		3% (3)	11% (9)	10% (4)	0% (0)	0% (0)	3% (16)	
Genitourinary		25% (24)	14% (12)	0% (0)	0% (0)	0% (0)	7% (36)	
Influenza		0% (0)	0% (0)	46% (18)	0% (0)	0% (0)	3% (18)	
Other infection		2% (2)	11% (9)	0% (0)	0% (0)	0% (0)	2% (11)	
Respiratory		50% (48)	37% (31)	10% (4)	0% (0)	0% (0)	16% (83)	
Skin/soft tissue		5% (5)	6% (5)	0%	0% (0)	0% (0)	2% (10)	

				(0)				
Unspecified sepsis		9% (9)	6% (5)	0% (0)	0% (0)	0% (0)	3% (14)	
Viral		0% (0)	1% (1)	31% (12)	0% (0)	0% (0)	2% (13)	
Non-infectious causes								
Acute myocardial infarction		0% (0)	0% (0)	0% (0)	0% (0)	1% (3)	1% (3)	
Cerebrovascular		0% (0)	0% (0)	0% (0)	3% (2)	6% (15)	3% (17)	
CNS		0% (0)	0% (0)	0% (0)	0% (0)	2% (4)	1% (4)	
Diabetes		0% (0)	0% (0)	0% (0)	1% (1)	2% (4)	1% (5)	
Gastro		0% (0)	0% (0)	0% (0)	9% (6)	10% (24)	6% (30)	
Head trauma		0% (0)	0% (0)	0% (0)	1% (1)	2% (4)	1% (5)	
Heart rhythm		0% (0)	0% (0)	0% (0)	0% (0)	19% (46)	9% (46)	
Intoxication		0% (0)	0% (0)	0% (0)	1% (1)	4% (10)	2% (11)	
Kidney		0% (0)	0% (0)	0% (0)	1% (1)	3% (6)	1% (7)	
Liver		0% (0)	0% (0)	0% (0)	0% (0)	0% (1)	0% (1)	

Lung		0% (0)	0% (0)	0% (0)	38% (26)	6% (13)	7% (39)	
Lung embolic		0% (0)	0% (0)	0% (0)	4% (3)	3% (7)	2% (10)	
Orthopedic		0% (0)	0% (0)	0% (0)	1% (1)	6% (13)	3% (14)	
Other		0% (0)	0% (0)	0% (0)	25% (17)	20% (48)	12% (65)	
Seizures		0% (0)	0% (0)	0% (0)	3% (2)	5% (11)	2% (13)	
Unspecified heart		0% (0)	0% (0)	0% (0)	12% (8)	9% (21)	6% (29)	
Vascular		0% (0)	0% (0)	0% (0)	0% (0)	3% (6)	1% (6)	

Continuous variables are displayed with median and inter-quartile range. Categorical variables are displayed with proportions and numbers within brackets.

Non-categorical variables (Age, SOFA, Number of OD) are tested with Kruskal-Wallis tests and categorical variables with Pearson's chi-squared test or Fisher's exact test, as appropriate.

Table 2. Discriminatory Values of Biomarkers for infection-induced organ dysfunction

AUC, cut-off values, sensitivity, specificity, positive and negative likelihood ratio, positive predictive value and negative predictive value were calculated as well as the the 95% C.I. among infected and non-infected patients (n=332, group I and IV). The prevalence of *infection-induced organ dysfunction* was 23%. For HBP, WBC, CRP and lactate the cut-off values suggested by Youden's index were used. For PCT commonly used cut-off values were used. The thresholds for HBP were also set to 15 ng/mL and 30 ng/mL, as suggested in prior publications(10,13).

	n	AUC	Cut-off	Sensitivity (95% C.I)	Specificity (95% C.I)	LR+ (95% C.I)	LR- (95% C.I)	PPV (95% C.I)	NPV (95% C.I)
HBP	332	0.82 (0.76-0.87)	13.7	0.64 (0.53-0.75)	0.86 (0.81-0.90)	4.58 (3.24-6.48)	0.41 (0.30-0.56)	0.58 (0.46-0.68)	0.89 (0.84-0.93)
HBP	332	0.82 (0.76-0.87)	15	0.62 (0.50-0.73)	0.88 (0.83-0.92)	5.11 (3.51-7.43)	0.43 (0.32-0.58)	0.60 (0.49-0.71)	0.89 (0.84-0.92)
HBP	332	0.82 (0.76-0.87)	30	0.30 (0.20-0.42)	0.96 (0.92-0.98)	7.04 (3.60-13.78)	0.73 (0.63-0.85)	0.68 (0.49-0.83)	0.82 (0.77-0.86)
PCT	326	0.76 (0.70-0.82)	0.5	0.59 (0.47-0.70)	0.80 (0.75-0.85)	3.01 (2.19-4.12)	0.51 (0.39-0.68)	0.47 (0.37-0.58)	0.87 (0.82-0.91)
PCT	326	0.79 (0.70-0.82)	2	0.36 (0.25-0.48)	0.96 (0.93-0.98)	9.04 (4.59-17.8)	0.67 (0.56-0.79)	0.73 (0.56-0.86)	0.83 (0.79-0.87)
WBC	332	0.67 (0.59-0.75)	10.8	0.62 (0.50-0.73)	0.70 (0.63-0.75)	2.03 (1.57-2.62)	0.55 (0.41-0.74)	0.38 (0.29-0.47)	0.86 (0.81-0.90)
CRP	301	0.87 (0.83-0.92)	20.1	0.91 (0.81-0.96)	0.77 (0.71-0.82)	3.88 (3.03-4.96)	0.12 (0.06-0.25)	0.56 (0.46-0.65)	0.96 (0.92-0.98)
Lactate	219	0.53 (0.45-0.62)	2.0	0.53 (0.40-0.67)	0.57 (0.49-0.65)	1.25 (0.92-1.68)	0.81 (0.60-1.11)	0.31 (0.22-0.41)	0.77 (0.69-0.84)

Table 3. Discriminatory Value of Biomarkers for the diagnosis of *critical infection*

AUC, cut-off values, sensitivity, specificity, positive and negative likelihood ratio, positive predictive value and negative predictive value were calculated as well as the the 95% C.I. among infected and non-infected patients (n=332, group I and IV). The prevalence of *critical infection* was 4%. For HBP, WBC, CRP and lactate the cut-off values suggested by Youden's index were used. For PCT commonly used cut-off values were used. The thresholds for HBP were also set to 15 ng/mL and 30 ng/mL, as suggested in prior publications(10,13). The prevalence of *critical infection* was 4%.

	n	AUC	Cut-off	Sensitivity (95% C.I)	Specificity (95% C.I)	LR+ (95% C.I)	LR- (95% C.I)	PPV (95% C.I)	NPV (95% C.I)
HBP	332	0.88 (0.77-0.99)	15	0.83 (0.52-0.98)	0.79 (0.74-0.83)	3.92 (2.82-5.45)	0.21 (0.06-0.75)	0.13 (0.06-0.22)	0.99 (0.97-1.00)
HBP	332	0.88 (0.77-0.99)	22.85	0.83 (0.52-0.98)	0.87 (0.83-0.90)	6.35 (4.35-9.27)	0.19 (0.05-0.68)	0.19 (0.10-0.33)	0.99 (0.97-1.00)
HBP	332	0.88 (0.77-0.99)	30	0.67 (0.35-0.90)	0.92 (0.88-0.95)	8.21 (4.76-14.13)	0.36 (0.16-0.81)	0.24 (0.11-0.41)	0.99 (0.97-1.00)
PCT	326	0.76 (0.60-0.93)	0.5	0.75 (0.43-0.95)	0.73 (0.68-0.78)	2.80 (1.93-4.08)	0.34 (0.13-0.91)	0.10 (0.05-0.18)	0.99 (0.96-1.00)
PCT	326	0.76 (0.60-0.93)	2	0.50 (0.21-0.79)	0.90 (0.86-0.93)	5.06 (2.63-9.77)	0.55 (0.31-0.98)	0.16 (0.06-0.32)	0.98 (0.96-0.99)
WBC	332	0.67 (0.48-0.85)	17.55	0.42 (0.15-0.72)	0.93 (0.90-0.96)	6.06 (2.77-13.24)	0.63 (0.39-1.01)	0.19 (0.06-0.38)	0.98 (0.95-0.99)
CRP	301	0.86 (0.73-0.99)	120	0.83 (0.52-0.98)	0.87 (0.82-0.90)	6.18 (4.20-9.09)	0.19 (0.05-0.68)	0.20 (0.10-0.34)	0.99 (0.97-1.00)
Lactate	219	0.64 (0.47-0.82)	2.65	0.50 (0.19-0.81)	0.76 (0.70-0.82)	2.09 (1.07-4.07)	0.66 (0.35-1.23)	0.09 (0.03-0.20)	0.97 (0.93-0.99)

Tabell 4. Predictors of elevated biomarkers.

Among infected and non-infected patients (n=332, group I and IV) a logistic regressions were fitted using diochotimized biomarkers as outcomes with infection and organ dysfunction (A) or infection and *critical disease* (B) as covariates. The biomarkers were dichotomized according to their respective cut-off level suggested by the calculation of Youden's index for each case. The corresponding odds ratios (ORs) were calculated and are displayed for infection and organ dysfunction (A) and for infection and *critical disease* (B).

Table 4A. Infection and Organ dysfunction

Biomarker	Cut-off	OR Infection	OR Organ dysfunction
HBP	13.7	**8.97 (5.11-16.1)**	**3.01 (1.56-6.15)**
CRP	20.1	**38.7 (18.9-87.2)**	0.97 (0.50-1.86)
PCT	0.15	**5.46 (3.24-9.39)**	1.57 (0.94-2.63)
WBC	10.8	**3.76 (2.28-6.28)**	1.12 (0.68-1.85)
Lactate	2.0	0.85 (0.48-1.53)	1.77 (0.96-3.36)

Table 4B. Infection and Critical disease

Biomarker	Cut-off	OR Infection	OR Critical disease
HBP	22.85	**15.6 (7.4-36.2)**	**8.98 (3.57-23.9)**
CRP	120	**51.9 (19.5-183.3)**	**3.45 (1.11-11.6)**
PCT	1	**6.03 (3.35-11.1)**	1.73 (0.71-3.97)
WBC	17.55	**7.86 (3.28-20.7)**	**6.91 (2.60-18.4)**
Lactate	2.65	0.99 (0.51-1.91)	**2.51 (1.12-5.52)**