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Original Article

Sepsis biomarkers for early diagnosis of bacteremia in emergency department

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Abstract

Introduction: We compared the diagnostic values of individual and composite biomarkers used in the prediction of bacteremia in adult emergency department patients.

Methodology: First-hour blood levels of C- reactive protein, procalcitonin, interleukin-6, lactate, lipopolysaccharide-binding protein and white blood cell count were collected from a 30-person control group and 47 adult patients. Patients included in this study were admitted to the emergency department on suspicion of sepsis. We categorized patients according to presence/absence of sepsis and bacteremia. Our control group was categorized as S-B -, septic patients with bacteremia were S+B+, and septic patients without bacteremia were S+B-.

Results: All biomarkers showed a statistically significant elevation when S+B- and S+B+ groups were compared with the S-B-. When S+B+ group was compared with the S+B- group only procalcitonin and lactate levels had statistically significant elevation ($p < 0.005$). Regression analysis demonstrated that lactate and procalcitonin were independently associated with having bacteremia in the state of sepsis and Hosmer-Lemeshow score was 0.772. The areas under the curve (AUC) values of biomarkers procalcitonin, lactate, C-reactive protein, combined 1 (procalcitonin+ lactate), and combined 2 (procalcitonin + lactate + C-reactive protein) were 0.773, 0.744, 0.523, 0.806, and 0.829 respectively.

Conclusions: Combination of tests such as combined 1 or combined 2 were highly predictive of bacteremia in adult septic patients. Combined 2 demonstrated the best predictive performance and could be utilized as a tool to assist diagnosis of bacteremia before culture results are available.

Key words: sepsis; bacteremia; biomarkers; emergency department; early diagnosis.

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Introduction

Sepsis is a serious condition associated with acute organ dysfunction and a high risk of death [1]. It is defined as life-threatening organ dysfunction resulting from an infection [2]. Treatment and identification of sepsis have improved significantly over the years. Nevertheless, the incidence of sepsis still appears to be high, and the condition is among the leading causes of death globally. In 2017, an estimated 48.9 million cases of sepsis were recorded worldwide, and 11.0 million (10.1–12.0) sepsis-related deaths were reported, representing 19.7% (18.2–21.4) of all global deaths [3]. In order to improve the survival of patients with sepsis, early clinical diagnosis and the rapid implementation of a series of measures are fundamental, which have now been protocolized with international consensuses [2]. This has the advantage of standardizing the definition of sepsis across the globe; however, the incidence of loss due to sepsis is far from being over.

Bacteremia is associated with severe sepsis and septic shock, and patients with both bacteremia and sepsis have increased mortality [4]. Moreover, mortality of the patients whose blood cultures were taken in emergency departments have been reported to be high. In a recent cohort study, the mortality rate was 11% among patients who had their blood cultures performed within 72 hours of their arrival at the emergency department. The same study used multivariate cox analysis and found that bacteremia was one of the most significant prognostic factors of mortality in blood cultured patients [5].

If bacteremia is spotted earlier, appropriate antibiotics can be initiated and thus, mortality of septic patients can be reduced significantly. However, distinguishing non-infectious systemic inflammation (SIRS) from bacteremic sepsis is an arduous task. Blood culture is considered the standard for diagnosis [6]. However, blood culture, including both identification and antibiotic drug sensitivity testing, takes at least 12–48 hours. This signifies the importance of biomarkers that would be able to guide physicians to spot bacteremic sepsis before culture results are available. Emergency Department (ED) is the first point of entry for the majority of sepsis suspected of patients. Thus, spotting of bacteremia in the ED setting favors the initiation of early and satisfactory antimicrobial treatment and will fortunately decrease unnecessary antibiotic use in non-bacteremic septic patients [7].

As the model for sepsis pathogenesis has evolved over the years, many different biomarkers of infections have been used as diagnostic and prognostic tools [8]. Even though there are many biomarkers of the inflammatory response, six of them have demonstrated significant clinical relevance and they are either readily available in routine clinical practice or they could be obtained with ease in the hospital setting. The biomarkers of interest are procalcitonin (PCT), C-reactive protein (CRP), interleukin-6 (IL-6), lactate (LAC), lipopolysaccharide-binding protein (LBP) and white blood cell count (WBC) [9,10]. The usefulness of these biomarkers has been established in pediatric and critical patient groups. However, only a few studies have assessed the usefulness of the aforementioned biomarkers in the emergency department [11]. Considering this, our research aims to determine the efficacy of biomarkers (IL-6, WBC, LAC, LBP, CRP and PCT) for the early diagnosis of bacteremia in patients suspected of sepsis in the ED.

We explore if there is a meaningful difference between sepsis patients with bacteremia and without bacteremia and if the use of any one of the biomarkers or collective use of them could be used to predict bacteremia in septic patients before culture results are available.

Methodology

Study design

We conducted a prospective study on patients suspected of having sepsis who were above 18 years old and were admitted to the ED of Acıbadem University Altunizade Hospital (Istanbul) from 2020 to 2021. Information was collected within the first hour of ED admission. The information collected with clinical assessment included determination of the presence/absence of sepsis using the sepsis-3 criteria, as well as the degree of severity calculation by the sequential organ failure assessment (SOFA) score. Sepsis-3 criteria defines sepsis as having a life-threatening organ dysfunction caused by a dysregulated host response to infection. The SOFA score is used to diagnose sepsis by quantifying organ dysfunction. Sepsis is thus identified as an acute change in total SOFA score ≥ 2 points as a result of the infection.[2]. We categorized patients with SOFA scores ≥ 2 and no ongoing acute comorbidities as septic patients. Blood samples were collected from patients who had ≥ 2 SOFA score. The blood samples were then analyzed for biomarkers and culture. This procedure was carried out in the healthy control population as well. It is crucial to state that all sampling was performed prior to antibiotic administration since antibiotics could have affected culture results by eliminating bacteria.

The study was approved by Acıbadem Healthcare Institution's Medical Research Ethics Committee (ATADEK). Our research is in compliance with the principles of Helsinki. Written informed consent was obtained from the patients involved in this study.

Study population

A total of 47 patients admitted to the ED with suspicion of sepsis and who were evaluated with ≥ 2 SOFA score according to sepsis-3 criteria were included in the study and were considered as sepsis positive. Blood samples of these patients were checked for the presence of bacteremia. A control group of 30 healthy subjects who underwent routine blood testing check-up was included.

The patients and the control group were classified according to the presence/absence of sepsis and bacteremia. The patients who had sepsis with bacteremia were therefore classified as S+B+. Patients who had sepsis without bacteremia were classified as S+B-. Controls who had neither sepsis nor bacteremia were classified as S- B-.

Exclusion criteria

Our exclusion criteria included: patients who recently (within a year) underwent chemotherapy due to haematological or solid organ malignancy, febrile neutropenic patients, recent (within a year) solid-organ and bone marrow transplant patients, and patients who had comorbidities such as ongoing acute heart failure, acute coronary syndrome. The rationale behind this exclusion was that these ongoing comorbidities could create false positive elevations in SOFA scores and

biomarkers levels. We wanted to assess organ dysfunction resulting from sepsis. The exclusion of cultures regarded as contamination is explained in the sampling and laboratory analysis section of methods.

Sampling and laboratory analysis

The blood samples were collected from a peripheral vein into vacutainers containing ethylenediaminetetraacetic acid (EDTA) and gel tubes (Becton Dickinson, Franklin Lakes, NJ, USA) within the first hour of ED admission. The complete blood count and WBC (10^6/L) were analyzed using an automated cell counter XN-9000 (Sysmex Co., Kobe, Japan) within 2 hours from sample collection. The CRP (mg/dL) was analyzed using Advia 1800 (Siemens, Erlangen, Germany). The PCT (mmol/L) was analyzed using Immulite XP (Siemens, Erlangen, Germany). The LAC (mmol/L) was analyzed using a rapid 500 blood gas analyzer (Siemens, Erlangen, Germany). The interleukin-6 (IL-6) (pg/mL) and LPB (mg/L) were analyzed using Centauur XPT (Siemens, Erlangen, Germany). The blood culture was analyzed using Bactec Fx (Becton Dickinson, Franklin Lakes, NJ, USA). Two sets of blood bottles were taken. Every set consisted of one aerobic and one anaerobic blood bottle. Microorganism identification was analyzed using MALDI-TOF Microflex (Bruker Co. Stuttgart, Germany). If bacteria such as coagulase negative *Staphylococcus*, *Bacillus*, *Corynebacterium*, *Propionibacterium*, *Aerococcus* and *Micrococcus* subtypes were isolated in only one of the four blood bottles, it was accepted as contamination and excluded from the study. The organisms isolated from cultures are listed in Supplementary Table 1. The independent variables: SOFA score, age, gender, heart rate (HR), mean arterial pressure (MAP), respiratory rate (RR) and basal body temperature (BBT) were clinically monitored and investigated.

Statistical analysis

All data were recorded and analyzed with Statistical Product and Service Solutions (SPSS, 20.0), MedCalc (19.1) and Graphpad Prism 8 (8.4.0) software. A confidence level of 95% and a p value < 0.05 were considered significant.

The Kolmogorov-Smirnov test was carried out to assess the normality of our data. Continuous quantitative variables were described as either mean ± SD for the normally distributed data or median ± interquartile range for the data that were not normally distributed. The qualitative variables were described by absolute and relative frequencies.

In the case of normally distributed data, the group comparisons were performed by ANOVA and post hoc analysis for the multiple comparisons between groups was performed by Tukey's statistics. Group comparisons for the data that departed from normal distribution were made by non-parametric Kruskal-Wallis test, and post hoc analysis for multiple comparisons was carried out by the Man-Whitney-U test. The categorical variable gender was analyzed by the Chi square test.

Table 1. Demographic characteristics, biochemical marker blood levels and SOFA scores.

	Healthy controls S-B- (n = 30)	**Sepsis without bacteremia S+B- (n = 27)**	**Sepsis with bacteremia S+B+ (n = 20)**	***p***
Age (years)	60.2 ± 18.2	61.2 ± 20.4	62.0 ± 18	0.930
Male gender (%)	51.6% (n = 16)	51.8% (n = 14)	85% (n = 17)	0.032
RR (BPM)	22.0 ± 3.0	19.5 ± 5.6	19.7 ± 6.3	0.015
HR (BPM)	72.2 ± 9.4	89.4 ± 18.9	94.5 ± 19.3	< 0.001
MAP (mmHg)	92.7 ± 10.5	81.5 ± 12.5	75 ± 16.1	< 0.001
BBT (C^0)	36.5 ± 0.2	36.8 ± 0.7	37.2 ± 1.0	0.044
Biochemical Markers				
CRP (mg/dL)	0.4 /0.4 (0.22– 0.62)	14.10 /18.75 (5.60-24.35)	12.13 /13.92 (6.47- 20.39)	< 0.001
PCT (ng/mL)	0.02 /0.02 (0.01- 0.03)	0.91 / 5.39 (0.08- 5.47)	6.10 /18.74 (2.08- 20.82)	< 0.001
LBP (mg/l)	4.5 /3.9 (3.3-7.2)	5.7 /8.0 (3.6-11.6)	11.75 /12.2 (5.9-18.1)	0.002
LAC (mmol/L)	0.73 /0.37 (0.58 – 0.95)	1.59 /1.01 (1.12 – 2. 13)	2.19 / 1.94 (1.59- 4.13)	< 0.001
WBC (10^6/L)	6720 /2770 (5410 -8180)	10690 /10850 (8760 -19610)	15055 /12792 (11415 -24207)	< 0.001
IL-6 (pg/mL)	17.00 /20.1 (6.2- 26.3)	1412 /2508 (599- 3107)	1005 /2971 (211.2 -3182)	< 0.001
SOFA score				
SOFA score	1.0 /1.0 (1.0- 2.0)	7.0 / 4.0 (5.0- 9.0)	8.0 /5.5 (5.5-11.0)	< 0.001

p value indicates results of ANOVA (for normally distributed variables) and Kruskal-Wallis Test (for data that is not normally distributed). Continuous quantitative variables are described as mean ± standard deviation for the normally distributed data or median ± interquartile range and 25th percentile and 75th percentile for the data that is not normally distributed. (CRP, PCT, LBP, LAC, WBC, IL-6 and SOFA score are not normally distributed). CRP: C-reactive protein; PCT: procalcitonin; IL-6: interleukin-6; LAC: lactate; LBP: lipopolysaccharide-binding protein; WBC: white blood cell count; SOFA: sequential organ failure assessment; HR: heart rate; MAP: mean arterial pressure; RR: respiratory rate; BBT: basal body temperature; BPM: breathes per minute/beats per minute; SD: standard deviation: IQR: interquartile range; S-B-: control group; S+B-: septic patients without bacteremia; S+B+: septic patients with bacteremia.

Since there was significant variation within groups and a steep variance between the groups, Lns of CRP, IL-6 and PCT were taken to avert the effects of variance and outliers and to help visualize Figure 1.

Spearmen's Test was used for correlation analysis due to the non-parametric nature of biochemical markers.

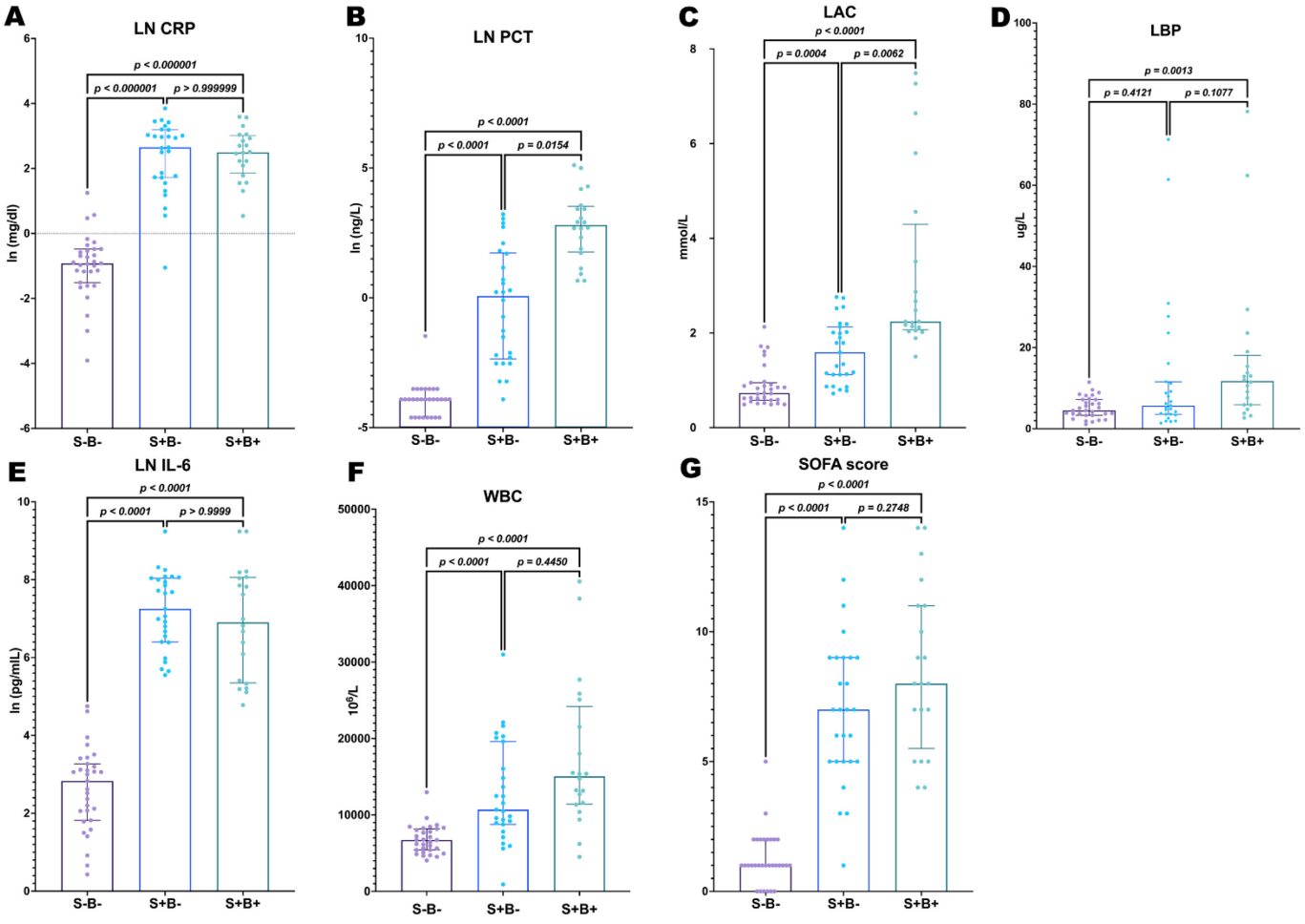
The area under the curve (AUC) of the receiver operating characteristic (ROC) curves of each of the biomarkers (LAC, LBP, IL-6, WBC, CRP and PCT SOFA Score) were calculated for the presence of positive blood cultures globally. We determined the cut-off points that offered the greatest sensitivity and specificity for each biomarker. Moreover, we analyzed the ROC curves that reached statistical significance by calculating the positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-). Finally, each of the AUC obtained from all the scales was compared using non-parametric tests.

We created composite biomarkers using different methods such as logistic regression and linear discriminant analysis. We combined LAC with PCT at the optimal cut-off of PCT and created the composite biomarker combined 1. We combined CRP, PCT, LAC at the optimal cut-off of PCT and created a composite biomarker combined 2.

Results

Demographic characteristics, (age, gender, RR, HR, MAP, BBT), biochemical marker blood levels (CRP, PCT, LBP, LAC, WBC, IL-6) and SOFA scores of each respective group (S-B-, S+B+, S+B-) were measured and are summarized in Table 1.

Figure 1. Differences between biochemical marker plasma levels between groups, and the differences of the SOFA scores between groups is demonstrated.



p values are provided on top of pairwise comparison lines and indicate whether a difference between pointed groups is statistically significant or not. **A.** C-reactive protein; **B.** Procalcitonin; **C.** Lactate; **D.** Lipopolysaccharide binding protein; **E.** Intertleukin-6; **F.** White blood cell count; **G.** Sequential organ failure assessment score. PCT: Procalcitonin; IL-6: Interleukin-6; LAC: Lactate; LBP: Lipopolysaccharide-binding protein; WBC: White blood cell count; SOFA: Sequential organ failure assessment; S-B-: Control group; S+B-: Septic patients without bacteremia; S+B+: Septic patients with bacteremia

There were statistically significant differences between group means of HR, RR, MAP, BBT, CRP, PCT, LBP, LAC, WBC, IL-6 and SOFA score ($p < 0.05$). Differences between group means of age were not statistically significant ($p > 0.05$) (Table 1).

The significance of the differences of biochemical marker plasma levels (CRP, PCT, LBP, LAC, WBC, IL-6) between groups, and the significance of the differences of the SOFA scores between groups are presented in Figure 1. Moreover, the distribution of the aforementioned parameters within groups are further demonstrated in Figure 1. Figure 1A shows C-reactive protein; figure 1B shows procalcitonin; figure 1C shows lactate; figure 1D shows lipopolysaccharide binding protein; figure 1E shows intertleukin-6; figure 1F shows white blood cell count and figure 1G shows sequential organ failure assessment score.

The differences between blood levels of CRP, LAC, LBP, WBC, PCT, IL6 and differences in SOFA score, RR, HR, MAP, BBT were statistically significant ($p < 0.05$) between S+B+ and S-B- groups.

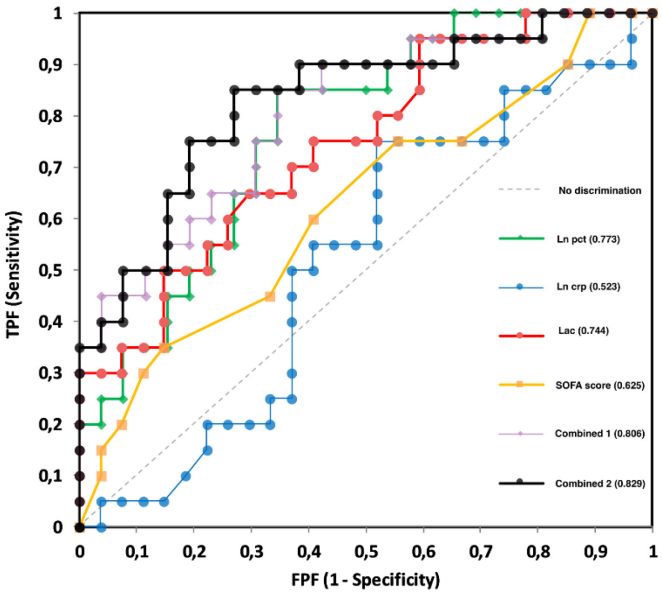
In the case of S+B- vs S-B-groups, differences between blood levels of CRP, LAC, WBC, PCT, IL6 and differences between SOFA score, RR, HR, MAP, BBT were found to be statistically significant ($p < 0.05$). The difference in LBP levels was not significant ($p > 0.05$).

In the case of S+B+ vs S+B- groups, only PCT and LAC blood levels had a significant difference ($p < 0.05$). Differences between blood levels of IL-6, CRP, WBC, LBP and differences in HR, RR, MAP, BBT and SOFA scores were statistically insignificant ($p > 0.05$).

Logistic regression analysis was carried out to check for causality between serum biomarker levels measured within the first hour of admission to ED and having bacteremia (Table 2). The two predictor variables LAC and PCT were found to contribute to the model in logistic regression analysis. The model was statistically significant ($p < 0.001$). The Hosmer and Lemeshow test was carried out to check the fitness of the model and the significance was 0.772. Cox and Snell R square of Model was 0.418, and Nagelke R square of the model was 0.560.

We performed ROC curve analyses of LAC, IL-6 LBP, PCT, CRP, WBC and SOFA score in all 47 adult patients who had sepsis to compare their discriminative power in the prediction of the presence of bacteremia in septic patients. The AUCs of the biomarkers that were found to be statistically significant for predicting bacteremia is shown in Figure 2 and Table 3. The biomarkers that are not included in the table were not statistically significant. The non-composite biomarker that obtained the best AUC was PCT with a value of 0.773 (95% CI 0.639-0.907), followed by LAC with an AUC of 0.744 (95% CI 0. 603-0.886) and CRP with a value of 0.523 (95% CI 0.353- 0.694). Other than biomarkers, SOFA score reached a statistical significance for predicting bacteremia with AUC of

Figure 2. ROC curve graphics of selected biomarkers.



PCT: Procalcitonin; LAC: Lactate; SOFA: Sequential organ failure assessment; AUC: Area under the curve; ROC: Receiver operating characteristic; CI: Confidence interval. Combined 1: PCT+ LAC; Combined 2: PCT+LAC+CRP.

Table 2. Logistic regression for biomarkers.

Variables	B	*p* value	Odds ratio (%95 CI)
PCT	0.653	**0.012**	1.921 (CI 1.156 – 3.193)
IL-6	-0.648	0.101	0.523 (CI 0.241 – 1.135)
CRP	-0.724	0.099	0.485 (CI 0.205-1.147)
LBP	-0.28	0.324	0.973 (CI 0.921-1.028)
WBC	-0.205	0.492	0.815 (CI 0.454-1.462)
LAC	0.952	**0.027**	2.590 (CI 1.116-6.015)
Constant	5.712	0.188	302.510

Unstandardized regression weight (B), significance (*p* value), odds ratio, confidence intervals for odds ratios are provided in the table. *p* values that are significant are written in bold characters. CRP: C-reactive protein; PCT: procalcitonin; IL-6: interleukin-6; LAC: lactate; LBP: lipopolysaccharide-binding protein; WBC: white blood cell count; CI: confidence interval.

0.625 (CI 0.460 – 0.790). PCT with the value of 0.850 had the highest sensitivity and SOFA score with the value of 0.852 had the highest specificity. The composite biomarker combined 1: (LAC+PCT) achieved an AUC of 0.806 (CI 0.790-933) with the sensitivity of 0.850 and a specificity of 0.654. Composite biomarker combined 2 (LAC+CRP+PCT) achieved the highest overall AUC of 0.829 (CI 707-909) with the sensitivity of 0.850 and specificity of 0.731.

Discussion

Our study analyzed the efficacy of different biomarkers for the diagnosis of bacteremia in patients who were suspected to have sepsis in ED. Our patients were predominantly male. Although the male to the female difference in other groups is negligible, the S+B+ group had an overwhelming male majority. Cohen *et al.* found that men were at higher risk of bloodstream and surgical site infections, possibly due to differences in propensity for skin colonization [12]. This may explain why the S+B+ group had a male majority and may pave the way for further studies to check if the male gender is more susceptible to bacteremia. Both our patient and control groups had advanced age. It is important to specify that susceptibility of developing bacteremia is higher in population higher than 65 years of age [13]. In our research, 42.5% of patients with suspicion of sepsis had positive blood culture. In a research carried out by Buisson *et al*. the incidence of bacteremia in ICU was found to be 9.8% (95% CI: 9.2 to 10.5) [14]. Our incidence greatly exceeds that number because we included patients who were suspected of sepsis. Research shows that sepsis and bacteremia are correlated. Studies have found that the blood cultures will be positive in about 50% of patients with severe sepsis/septic shock [15]. Patino *et al.* carried out a similar study and found 38% of blood cultures were positive in patients suspected of sepsis [16].

Among patients with suspected sepsis, the mean values of PCT and LAC were significantly higher in sepsis with bacteremia (S+B+) group compared with sepsis without bacteremia group (S+ B-). So, bacteremia must be suspected in high values of either PCT or LAC independently. PCT had a tenfold increase in diagnostic odds ratio, and LAC had a four-and-a-half-fold increase in diagnostic odds ratio.

The SOFA score and serum CRP levels showed no significant differences between sepsis with bacteremia (S+B+) group and sepsis without bacteremia group (S+ B-) Even though CRP was often investigated in other research as a potential biomarker for bacteremia. In our research, CRP had an AUC value of 0.523 with a cut off value of 2.94 ng/mL and a nearly three folds increase in diagnostic odds ratio. Therefore, it can be stated that CRP performed poorly. This confirms what was found in other researches which indicated that CRP on its own is not a suitable marker for diagnosing bacteremia in the ED [17]. Our AUC values and cut-off values show parallel results with the research carried out by Lin *et al.* [17]. Lin *et al*. had an AUC value of 0.72, 0.69, 0.56 for PCT, LAC and CRP, respectively.

As far as the SOFA score is concerned, it had an AUC value of 0.625, a cut-off value of 9 and a three-fold increase in the diagnostic odds ratio. Based on these findings, we can state that SOFA score is not a suitable parameter to diagnose bacteremia in the ED. This finding is understandable considering that SOFA score is associated with mortality rather than bacteremia [18]. However, it is important to indicate that there are other studies contrary to our finding. Routsi *et al.* found that the admission SOFA score is independently associated with the occurrence of ICU-acquired bacteremia [19].

We combined LAC with PCT at the optimal cut-off of PCT; and created the composite biomarker combined 1, which demonstrated better predictive performance for bacteremia in septic patients with an AUC value of 0.806. Furthermore, the composite biomarker combined

Table 3. Table 3: ROC curve of selected biomarkers.

	AUC	95% CI	Cut off	TP proportion (Sensitivity)	TN proportion (Specificity)	Likelihood ratio (+)	Likelihood ratio (-)	Odds ratio
PCT	0.773	0.639 - 0.907	0.55	0.85	0.654	2.46	0.23	10.704
CRP	0.523	0.353 - 0.694	2.94	0.75	0.481	1.45	0.52	2.786
LAC	0.744	0.603 - 0.886	2.01	0.65	0.704	2.19	0.5	4.411
SOFA score	0.625	0.46 - 0.790	9	0.35	0.852	2.36	0.76	3.096
Combined 1	0.806	0.679 - 0.933	….	0.85	0.654	2.46	0.23	10.704
Combined 2	0.829	0.707 - 0.951	….	0.85	0.731	3.16	0.21	15.381

Areas under the curve and confidence intervals of the different biomarkers; best cut-off points for greatest sensitivity and joint specificity; likelihood ratios and odds ratios are demonstrated. PCT: procalcitonin; LAC: lactate; SOFA: sequential organ failure assessment; AUC: area under the curve; ROC: receiver operating characteristic; CI: confidence interval; Combined 1: PCT+ LAC; Combined 2: PCT+LAC+CRP

2 which is three index tests; CRP, PCT, and LAC combined at the optimal cut-off of PCT, had improved the diagnostic performance of predicting unspecified bacteremia in septic patients with the highest AUC value of 0.829. To the best of our knowledge, there are not many research publications that investigate whether a combination of tests such as the combination of LAC + PCT or PCT+ CRP or a combination of all three of them (CRP + LAC + PCT) would outmatch procalcitonin as a single test in prediction of bacteremia in adult patients in the setting of an ED. Ljungstorm *et al*. used PCT and a biomarker combination of PCT LAC, CRP, WBC for the prediction of bacteremia in adult patients suspected of sepsis who were admitted to the ED at Skaraborg Hospital, Sweden. Composite four biomarkers performed slightly better (AUC: 0.78; (95% CI 0.74– 0.81), $p < 0.001$) than PCT (AUC: 0.74; (95% CI 0.70–0.78) $p < 0.001$) [20]. Our findings combined with other relevant studies indicate that the diagnostic discriminatory power of predicting bacteremia in adult patients in the setting of ED care is improved when combining information from several biomarkers. [20,21] Therefore, collective use of them may be more beneficial. However, there is a drawback to the multi-marker approach, and it is the relatively higher cost of composite biomarkers compared to single biomarkers. When our composite biomarkers were compared with the most successful single biomarker, PCT, it was shown that they had a similar predictive value. This may raise some concerns over the cost-effectiveness of the multi-marker approach.

Clearly, our results support the use of the aforementioned biomarkers as a complementary diagnostic tool for bacteremia amongst patients who are suspected of sepsis. Isolated increases in PCT and LAC blood levels can be used to assist the diagnosis of bacteremia with sepsis as they are independently associated with having bacteremia. But as stated earlier, when predictive values of both LAC, PCT and CRP are combined, it offers the best result. This collective or solitary use of biomarkers could indicate bacteremia in septic patients and could lessen the impact of bacteremia in sepsis-related mortality because as stated earlier various studies demonstrated that rapid diagnosis and early administration of appropriate antibiotic therapy significantly improves the outcomes of septic patients [22].

Limitations

The main limitation of this study is that we did not analyze the biomarkers for Gram –ve and Gram +ve bacteria discriminately. By doing that, we could have offered a better diagnostic value and better treatment chances. Also, all our data came from a single hospital. This could have led to sampling bias. The number of patients that fulfilled the enrollment criteria was not large. Therefore, it will be beneficial to increase the sample size during further analysis to increase the credibility of the results. Also, even though all sampling was performed prior to antibiotic administration prior use of antibiotics by the patient before admission to the hospital may have affected our results. In addition, even though necessary measures were taken to avert this, there is always the risk of improper sterilization during blood sample collection resulting in contamination and there is the risk of inadequate blood collection which could result in negative results. Our main advantage and novelty are that many studies in this field were retrospective, but we carried out a prospective observational study relying on first-hour biomarker blood levels to predict the presence and development of bacteremia.

Conclusions

Out of the biomarkers analyzed, first-hour blood levels of only PCT and LAC were found to be independently associated with having bacteremia in a state of sepsis. PCT demonstrated the highest predictive power of bacteremia among all the non-composite markers. This was followed closely by LAC.

Combinations of biomarkers improved the diagnosis of verified bacterial sepsis. Both combined 1 and combined 2 outperformed PCT as a single test in the prediction of the bacteremia in adult patients in the setting of ED care. The composite biomarker combined 2 demonstrated the best predictive performance for bacteremia in septic patients. By integrating biomarkers into levels to algorithm for diagnosing and assessing severity of sepsis, specific patient groups with bacteremic sepsis could be spotted earlier to direct physicians to more aggressive treatments to avert mortality or cut back unnecessary antibiotic usage. In our next research we aspire to use biomarkers to distinguish Gram +ve and Gram –ve etiologies so that a more appropriate fine-tuned treatment could be utilized before culture results are available.

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Annex – Supplementary Items

Supplementary Table 1a. Detailed overview of organisms isolated from blood samples of bacteremic sepsis patients.

Isolated organisms:	Times isolated
Klebsiella pneumonia	5
Staphylococcus epidermidis multi resistant strain	5
Escherichia coli	3
Enterobacter cloaca	3
Acinetobacter baumanii	3
Staph. hominis multi resistant strain	2
Methicillin-resistants staphylococcus aureus	1
Enterococcus fecalis	1
Enterecoccus feacium	1

Supplementary Table 1b. Broad overview of organisms isolated from blood samples of bacteremic sepsis patients.

Gram staining chracteristics of the isolated bactreia	Times isolated
Gram (-)	14
Gram (+)	10
Gram (+) and Gram (-)*	4

4 patients had both Gram +ve and Gram –ve bacteria in their bloodstream*.

Supplementary Table 1c. Detailed overview of organisms isolated from non bacteremic septic patients.

Isolated organisms	**Times isolated**
Acinetobacter baumanii	4
Klebsiella pneumonia	4
Candida albicans	2
Non-albicans *Candida*	2
Corynebacterium striatum	2
Enterococcus feacium	2
Methicillin-resistants staphylococcus aureus	1
Klebsiella aerogenes	1
Escherichia coli	1
Pseudomonas auriginosa	1
Stenotrophomonas maltophilia	1
Morganella morganii	1
Enterobacter cloacae	1
Streptococcus pneumonia	1

Supplementary Table 1d. Broad overview of the organisms isolated from non bacteremic septic patients.

Isolated organism type	Times isolated
Gram (-) bacili	14
Gram (+) coccus	4
Fungi	4
Gram (+) cocobacili	2

Supplementary Table 1e. Overview of the tissue origins of isolated organisms from non bacteremic septic patients.

Types of cultures which organisms were isolated.	Times isolated
Respiratory culture	13
Urinary culture	2
CNS culture	2
Pleural Culture	1
Abscess (Pus)	1