

## Significantly higher procalcitonin levels could differentiate Gram-negative sepsis from Gram-positive and fungal sepsis

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**Abstract** Procalcitonin (PCT) levels can distinguish between infectious and non-infectious systemic inflammatory response. However, there are some differences between Gram-negative (G<sup>-</sup>), Gram-positive (G<sup>+</sup>), and fungal bloodstream infections, particularly in different cytokine profiles, severity and mortality. The aim of current study was to examine whether PCT levels can serve as a distinguishing mark between G<sup>+</sup>, G<sup>-</sup>, and fungal sepsis as well. One hundred and sixty-six septic patients with positive blood cultures were examined on C-reactive protein (CRP) and PCT on the same date of blood culture evaluation. The median (interquartile range, IQR) of CRP and PCT in G<sup>+</sup>, G<sup>-</sup>, and fungal cohorts and comparison of measured values between groups were made using the Kruskal–Wallis test with subsequent Bonferroni's corrections, with  $p < 0.05$ . In 83/166 (50 %) of blood cultures, G<sup>+</sup> microbes, 78/166 (47 %) G<sup>-</sup> rods, and 5/166 (3 %) fungi were detected. PCT concentrations (ng/ml) were significantly higher in G<sup>-</sup> compared to other cohorts: 8.90 (1.88; 32.60) in G<sup>-</sup>, 0.73 (0.22; 3.40) in G<sup>+</sup>, and 0.58 (0.35; 0.73) in fungi ( $p < 0.00001$ ). CRP concentrations did not differ significantly in groups. Significantly higher PCT levels could differentiate G<sup>-</sup> sepsis from G<sup>+</sup> and

fungemia. In contrast to CRP, PCT is a good discriminative biomarker in different bloodstream infections.

**Keywords** Sepsis · Procalcitonin · C-reactive protein · Bacteremia · Fungemia

### Introduction

Sepsis, a systemic inflammatory response to noxious infection, is a relatively frequent and serious complication in critically ill patients. Mean sepsis incidence of around 30–40 % in intensive care units (ICU) patients is commonly reported: for instance, the SOAP study (Sepsis Occurrence in Acutely Ill Patients), which evaluated data from 3147 ICU patients from 198 centers, reported sepsis during hospitalization in 38 % of patients, whereas mortality of these septic patients was 27 % compared to 14 % in non-septic ICU cohort [1]. Early and accurate diagnostics of sepsis with rapid initiation of adequate therapy is crucial for patients' surviving. However, microbiological testing of sepsis and accurate identification of etiological agents are time-consuming; moreover, failure of capture agents in blood cultures could occur even in apparently septic individuals, and clinical signs could not be always clear promptly. Diagnostic criteria for sepsis [2, 3], therefore, include (1) clinical symptoms such as changes in body temperature, breathing, and heart rate, and (2) laboratory markers of inflammation. To identify sepsis and to determine septic patients' prognosis, more than one hundred different biomarkers were reported until today. However, a biomarker with 100 % sensitivity and specificity for sepsis was not described yet [4]. The combination of commercially well-available markers C-reactive protein (CRP), procalcitonin (PCT), and leukocyte count is used for

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evaluation in most cases. CRP is less sensitive (75 and 88 %, respectively) and less specific (67 and 81 %, respectively) than PCT for differentiating bacterial from non-infective causes of inflammation [5]. The leukocyte count also has significant limitations, especially in immunosuppressed patients. PCT has the highest sensitivity and specificity for predicting systemic bacterial inflammation; moreover, high PCT concentrations have a positive predictive value for severe sepsis and septic shock, and correlate with the severity of inflammation [6–8] and distinguish between viral and bacterial infections. [5, 6] Plasma PCT levels can be influenced by multiple factors such as individual genetically determined immune alert as well as the degree and type of microbial aggression or the extent of inflammation.

In our current study, we have evaluated PCT and CRP levels in septic patients with positive blood cultures. The main goal of the work was to examine whether PCT and CRP levels could serve as a distinguishing marker between Gram-negative (G–), Gram-positive (G+), and fungal sepsis.

## Materials and methods

### Samples

We have performed a retrospective study of plasma PCT and CRP levels in ICU patients with positive blood cultures during the six-month period. One hundred and sixty-six (166) patients with positive blood cultures were assessed. The clinical status of patients was classified according to the Sepsis Definition Conference criteria [3]. The majority of patients from our cohort were from hematological ICUs, and as there is no routine Sequential Organ Failure Assessment (SOFA) performed on these patients, this score was not included in our study. Plasma PCT and CRP values were analyzed from the day of positive blood culture realization. The proven hemoculture-positive individuals were divided into three groups according to detected infectious agents: (1) G– bacteremia, (2) G+ bacteremia, and (3) fungal infection groups.

### Laboratory examinations

Serum PCT concentrations were measured by sandwich electrochemiluminescence immunoassay (Cobas ECLIA, Japan). Analyses were performed according to manufacturer's recommendations. The concentration of 0.5 ng/mL was chosen as the cutoff value. CRP was measured by a turbidimetry Modular SWA (Roche Diagnostic, Switzerland). The cutoff value of greater than 5.0 mg/l was adopted. One set of blood cultures (aerobic, anaerobic) was

taken by sterile venipuncture and processed using the Bactec 9420 system (Becton–Dickinson, Heidelberg, Germany). Bacteremia was defined by microbial growth in one blood culture bottle, and only for coagulase-negative Staphylococcus species were two positive blood culture bottles from different venipunctures required; otherwise, the results were assessed as contamination of blood during venipuncture.

### Statistical analysis

Statistical analysis was performed using the software Statistica CZ 9.0 (StatSoft Inc, USA). Different groups were compared using the two-sided Kruskal–Wallis non-parametric test. Follow-up tests were conducted by using the Bonferroni approach. The threshold for significance was set at  $p < 0.05$ .

## Results

Basic demographic characteristics of analyzed cohorts are included in Table 1.

In our cohorts, 75 cases of G+ cocci were found in blood cultures, G+ rods in 8 individuals, G– cocci in 2 patients, G– rods in 76 patients, and in 5 patients fungal infection was found. One hundred and forty-seven (89 %) positive blood cultures were aerobic and 19 (11 %) anaerobic.

Median (interquartile range, IQR) of CRP and PCT in different cohorts and comparison of measured values are included in Table 2.

PCT concentrations were significantly higher in G– compared to other cohorts, see Fig. 1.

Moreover, significantly higher PCT levels were found in patients with *Escherichia*, *Klebsiella*, and *Pseudomonas* in

**Table 1** Basic demographic characteristics of analyzed cohorts

	<i>n</i> (%)
Gender	
Females	93 (58 %)
Males	73 (42 %)
Age, median (interquartile range)	64.5 (55; 76)
Main diagnosis (ICU department)	
Internal/hematological	45 (27 %)
Internal/metabolic	33 (20 %)
Internal/cardiologic	10 (6 %)
Internal/hemodialysis	20 (12 %)
Surgical	18 (11 %)
Anesthesiology and intensive care	10 (6 %)
Other	30 (18 %)

**Table 2** CRP and PCT concentrations in different cohorts according to blood culture results

Factor	CRP, mg/L		PCT, ng/mL	
	Median (IQR)	<i>p</i> value	Median (IQR)	<i>p</i> value
<b>Gender</b>				
Females ( <i>n</i> = 93)	113 (95.40; 185.40)	0.93; NS	2.16 (0.29; 5.98)	0.28; NS
Males ( <i>n</i> = 73)	121.30 (61.20; 219)		3.02 (1.49; 9.95)	
<b>Blood culture</b>				
G+ bacteremia ( <i>n</i> = 83)	123.10 (65.80; 211.50)	0.93; NS	0.73 (0.22; 3.40)	<0.0001
G– bacteremia ( <i>n</i> = 78)	129.90 (87; 210)		8.90 (1.88; 32.60)	
Fungal infection ( <i>n</i> = 5)	149 (128; 152)		0.58 (0.35; 0.73)	
<b>Bacterial species</b>				
<i>Acinetobacter</i> ( <i>n</i> = 4)	147 (67; 199)	0.77; NS	24.70 (5.95; 65.67)	<0.00001
<i>Candida</i> ( <i>n</i> = 5)	149 (128; 152)		0.58 (0.35; 0.73)	
<i>Corynebacterium</i> ( <i>n</i> = 3)	126.50 (106.50; 240)		0.58 (0.51; 10.90)	
<i>Enterobacter</i> ( <i>n</i> = 6)	100.50 (87; 177)		7.02 (2.73; 20.90)	
<i>Enterococcus</i> ( <i>n</i> = 12)	158.70 (93.85; 282.15)		1.77 (0.48; 3.24)	
<i>Escherichia</i> ( <i>n</i> = 26)	249.70 (200; 326)		15.30 (2.70; 38.0)	
<i>Klebsiella</i> ( <i>n</i> = 23)	129.90 (83; 196.20)		15.23 (5.45; 34.61)	
<i>Pseudomonas</i> ( <i>n</i> = 8)	146 (102; 211)		17.00 (1.64; 28.76)	
<i>Staphylococcus</i> ( <i>n</i> = 54)	105 (61; 213)		0.98 (0.36; 3.20)	
<i>Streptococcus</i> ( <i>n</i> = 7)	103.60 (59.30; 124)		1.47 (0.41; 3.85)	
<b>Microbial environment</b>				
Aerobic ( <i>n</i> = 147)	129.9 (78; 211)	0.95; NS	2.65 (0.58; 17)	0.62; NS
Anaerobic ( <i>n</i> = 19)	105 (60; 283.60)		2.15 (0.57; 6.25)	

PCT procalcitonin, CRP C-reactive protein, G, Gram-positive microbe, G– Gram-negative microbe, IQR interquartile range, NS non-significant

blood cultures. *Candida*, *Streptococcus*, and *Staphylococcus* were linked with the mild PCT levels elevation.

The ROC plots and respective AUCs for PCT and CRP in the diagnosis of G– bacteremia are shown in Fig. 2. Sensitivities of 75.10 and 61.20 and specificities of 87.80 and 54.30 % for the G– bacteremia were achieved with a PCT cutoff value of 15 pg/mL and a CRP cutoff value of 86.2 mg/L. The AUC for PCT was 0.871, which was significantly higher than that for CRP (0.705; *p* = 0.002).

For cases with G– bacteremia, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of different PCT concentrations were calculated, see Table 3.

In summary, significantly higher PCT levels were observed in G– bacteremia compared to G + bacteremia and fungal infection. No such significant differences were found for CRP.

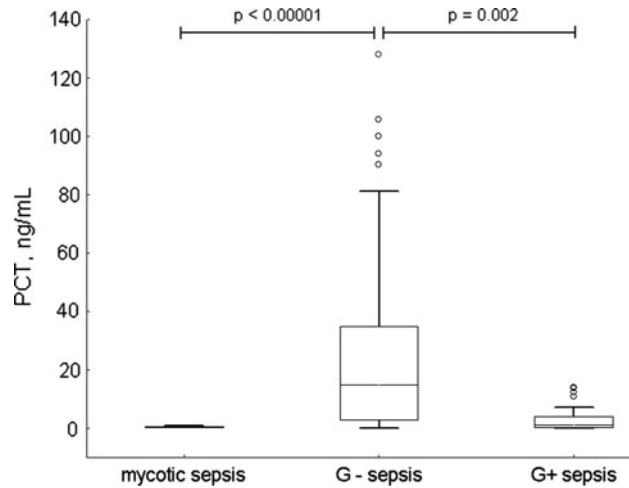
## Discussion

In the current study, we have focused on the rapid diagnosis of sepsis. We have assessed PCT and CRP as most commonly used markers and have looked for possible

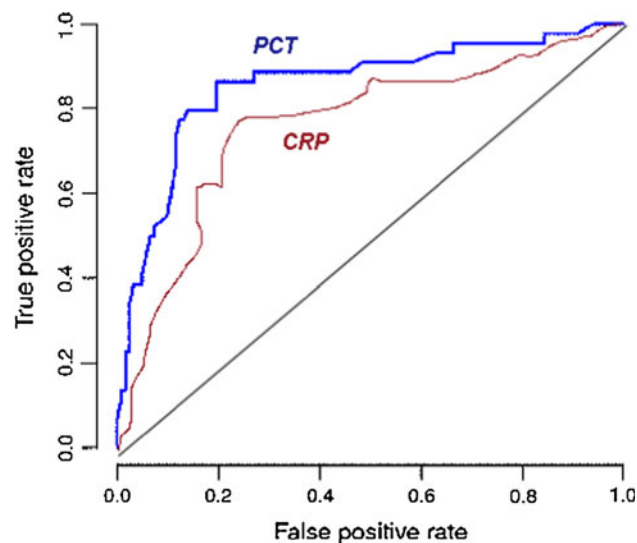
relationships with the nature of a causal microbial agent. PCT levels in our G– septic cohort were significantly higher than those of G+ and fungal sepsis. The difference in PCT levels between G+ and fungal agents was not statistically significant. Plasma CRP levels between different types of microbial agents do not differ statistically as well.

G+ bloodstream infections slightly dominated in our cohort (G+ in 50 % cases and G– in 46 % cases). Unambiguous fungal infection was identified in only 3 % of individuals, which was in accordance with other observations [4] of formidable detection of fungal bloodstream infections.

*Staphylococci* were identified as the most frequent causal pathogens in our cohort (33 %), followed by *E. coli* (16 %) and *Klebsiella* (14 %). In general, *Staphylococci* (i.e., *Staphylococcus aureus*) and Gram-negative rods from family Enterobacteriaceae are the most common causative agents of sepsis. On the other hand, isolation of coagulase-negative staphylococci is frequently a consequence of contamination of blood samples. In patients with *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp. in blood cultures, the highest PCT values were found; on the other side, *Candida* spp, *Streptococcus* spp., and *Staphylococcus*



**Fig. 1** PCT concentrations in different cohorts according to hemoculture results. PCT procalcitonin, G+ Gram-positive, G- Gram-negative. The box plot represents the lower and upper quartiles, the horizontal line represents the median, and the whiskers represent the sample minimum and maximum. Bonferroni adjustment was used in multiple comparison procedures



**Fig. 2** The ROC curves of PCT (AUC 0.871) and CRP (AUC 0.705) for the diagnosis of G- bacteremia. PCT for cutoff 15 pg/mL. % sensitivity: 75.10 %. % specificity: 87.80. CRP for cutoff 86.2 mg/L. % sensitivity: 61.20 %. % specificity: 54.30 %. PCT procalcitonin, CRP C-reactive protein, AUC area under the curve

spp. were associated mainly with the mild elevation of PCT. In CRP, we did not find any significant differences from this point of view.

Inflammatory cascades in septicemia are complex processes. In vitro studies have clearly described the functional differences of G-, G+, and fungal agents invading the human host organism. These differences reflect the different pathways of activation and initiation of inflammatory cascades. At the beginning of these defensive cascades, the detection of the pathogen or its parts (pathogen-associated molecular patterns, PAMPs) through Toll-like receptors (TLRs) takes part. Superficial TLR1, TLR12, TLR14, TLR15, TLR16, and TLR110 are primarily intended for recognizing bacterial products, while intracellular TLR3, TLR7, TLR8, and TLR9 recognize nucleic acids that are produced during the replication of viruses. There is evidence that TLR4 recognizes lipopolysaccharide (LPS) patterns of G- bacteria, while TLR2 identifies lipoteichoic acid of G+. After the TLRs activation, different inflammatory cascades are triggered, in particular, via nuclear factor  $\kappa$ B and other mediators leading to the synthesis of proinflammatory cytokines and acute-phase proteins [9]. G- infections probably increase the production of TNF-alpha more compared to G+ microbes [10], and differences were found also in plasma levels of IL-1, IL-6, IL-10, and IL-8 [11, 12]. Interestingly, similar differences have been recently described also for PCT [13]. Mohamed et al. [12] have demonstrated significantly lower in vitro production of IL-6, IL-8, TNF-alpha, and IL-1b in staphylococcal infections compared to the production of these proinflammatory cytokines in *E. coli*. Bjerre et al. [11] reported higher TNF-alpha, IL-10, and IFN-gamma production in G- sepsis, and in particular, Gram-negative meningococcal septicemia. The differences in the levels of IL-6 and IL-18 as well as PCT between G+ and G- infections were observed also by Feezor et al. [10]. Abe et al. [14] affirmed that G- bacteremia induces greater magnitude of inflammatory response than G+ bacteremia. And this may be the answer for a higher elevation levels of PCT in G- bacteremia, as described by Feezor and Charles [10, 13]. Charles also concluded that G- bacteremia could be associated with higher PCT values than those found in G+ bacteremia, regardless of the severity of the disease [13]. But why CRP is not higher at the same time in G- is hard to say. To clarify this clearly, more studies have to be

**Table 3** Reliability of PCT in G- bacteremia

	PCT > 0.5 pg/mL	PCT > 5 pg/mL	PCT > 15 pg/mL
PPV %	55.00 (95 % CI 46.40–63.40)	73.50 (95 % CI 62.00–82.60)	83.20 (95 % CI 66.50–92.00)
NPV %	86.50 (95 % CI 72.00–94.10)	75.50 (95 % CI 64.00–81.20)	74.90 (95 % CI 60.90–87.60)

undertaken. However, our results are in line with greater magnitude of plasma PCT level in G– sepsis in comparison with G+ sepsis as in above-mentioned studies. But we did not compare SOFA in our cohort, so we could not confirm the dependence of the severity of disease.

To clarify exactly the reason for the different elevation of PCT and its independence on severity of disease, more studies should be conducted.

Martini et al. [15] have suggested setting the PCT boundary value at 2 ng/mL, whereas values under this value had negative predictive value of 94 % for the bacterial septicemia, in comparing bacteremia versus fungemia. In our study, we clearly demonstrated better diagnostic value for G- sepsis of PCT by using ROC. The ROC plots and respective AUCs for PCT and CRP in the diagnosis of G– bacteremia shown sensitivities of 75.10 and 61.20 %, and specificities of 87.80 and 54.30 % for the G– bacteremia were achieved with a PCT cutoff value of 15 pg/mL and a CRP cutoff value of 86.2 mg/L. The AUC for PCT was 0.871, which was significantly higher than that for CRP (0.705;  $p = 0.002$ ). Similar conclusions were reported by Charles et al. [13], no significant differences in CRP but definite divergence in PCT levels (mean PCT concentrations of 39.0 ng/mL in G– and 5.42 ng/mL in G+,  $p = 0.003$ ); however, this study does not include subjects with fungal sepsis. Significantly higher PCT levels in G– septicemia compared to G + and mycotic sepsis ( $p = 0.0001$ ) were noticeably expressed in our patients with hematological malignancies and patients after bone marrow transplantation. Hence, PCT levels could serve as a simple utility for confirmation or exclusion of G– bacterial septicemia in these groups of patients. Our findings are in line with observations of Montagna et al. [16] In their study of neutropenic septic patients, the low PCT levels often showed fungal infection (values around 1.0 ng/mL for *Candida* spp. and 1.91 ng/mL for *Aspergillus* spp.); however, a small number of patients limited this study's results. CRP levels did not differ significantly in our three cohorts. It affirms the role of CRP as a highly sensitive but poorly specific marker of infection, which is not able to distinguish the type of infection. The finding the combination of high plasmatic level of CRP (more than 100) and low level of PCT (less than 2.0) is highly suspect to mycotic affection.

In contrast to CRP, PCT could distinguish the severity of clinical condition (sepsis, severe sepsis, septic shock, multi-organ dysfunction syndrome/MODS/degrees) as well as inform us about the type of microbial agent [6, 17, 18]. Generally, PCT is more specific marker for infection than CRP (ROC analysis), and CRP has good sensitivity but less specificity [6, 13, 16–18]. Also the physiological role of both markers is not the same. We actually do not know how does it work exactly. Each marker is slightly different.

We use to combine both markers together with other markers, such as WBC and IL-6, and with clinical signs. Thus, we improve the diagnostic effect.

In conclusion, plasma PCT level is an additional marker to both clinical and microbiological criteria of sepsis, providing the possibility to estimate the type of microbe as well as the seriousness and severity of infection and consequently to consider first-choice antibiotic treatment. We definitely do not want to reduce the role of microbiological diagnosis with the identification of microbe and assessment of sensitivity to different antibiotics. This part of laboratory examination is an absolutely unexceptionable part of sepsis management. However, the time needed for this testing in manifold is larger compared to the few minutes required for PCT measurements. PCT could be considered as a good discriminative biomarker in different bloodstream infections.

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**Conflict of interest** Authors certify that they have no affiliation with or financial involvement with any organization or entity with a direct financial or any other interest in the subject matter or materials discussed in the manuscript.

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