

## The role of CRP, PCT, IL-6 and presepsin in early diagnosis of bacterial infectious complications in paediatric haemato-oncological patients

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Bacterial infection is the most common complication in paediatric oncological patients during cancer treatment. A suitable tool for early prediction of unfavourable course of infection is still needed. We performed a prospective longitudinal observational study to evaluate the role of serum biomarkers (C-reactive protein, procalcitonin, interleukin-6, presepsin) in the early diagnosis of bacteraemia (gram-negative versus gram-positive) in patients with haematological malignancies. We observed 69 febrile episodes in 33 patients (17 male, 16 female; 1.5-18.9 years, mean 7.31 years, median 5 years). Within this sample, there were 22 cases of positive blood cultures, 16 cases of sepsis, 38 cases of fever with no signs or symptoms of sepsis, and two deaths from infectious complications. All markers tested had good negative predictive value (73% – 93%). CRP was characterized by good specificity for registration bacteraemia (96%, 95% CI: 85% – 99%), but other results were inconclusive. We identified comparably balanced sensitivity (64% – 81%) and specificity (61% – 88%) for interleukin-6 and procalcitonin, and we proved their quality to predict positive blood culture and clinical signs of sepsis as well. Patients with gram-negative bacteraemia had significantly elevated levels of PCT and IL-6 in comparison with a group of patients with gram-positive bacteraemia ( $p = 0.04$  for PCT and  $p = 0.005$  for IL-6). Presepsin was characterized by poor specificity (27%, 95% CI: 15% – 43%) and positive predictive value (24%, 95% CI: 12 – 39%) for predicting bacteraemia, and by better sensitivity (84%, 95% CI: 55% – 98%) and specificity (58%, 95% CI: 42% – 73%) for predicting clinical signs of sepsis.

*Key words: C-reactive protein, procalcitonin, interleukin-6, presepsin, fever, sepsis*

Bacterial infection is the most common treatment-related complication in patients with haematological malignancies [1]. Documented mortality associated with paediatric febrile neutropenia is 2% [2]. The potential for early diagnosis of bacteraemia through serum biomarkers has been the subject to extensive research [3]. In 2012 Phillips et al published large meta-analysis of 25 studies exploring 14 different biomarkers in 3,585 episodes of febrile neutropenia. CRP, PCT and IL-6 were subject to quantitative meta-analysis. The bivariate estimates of diagnostic precision of these biomarkers and outcomes were done. Data were available for meta-analysis for CRP for microbiologically or clinically documented infection (results: cut off > 50mg/dl, sensitivity 65%, speci-

city 73%), for PCT assessing microbiologically or clinically documented infection (results: cut off > 0.2 mg/ml, sensitivity 96%, specificity 85%), for IL-6 reporting microbiologically or clinically documented infection (results: cut off > 235 pg/ml, sensitivity 68%, specificity 94%), and gram-negative bacteraemia (results: cut off > 1000 pg/ml, sensitivity 78%, specificity 96%). Huge inconsistencies and heterogeneity in the studies included in this review were the most important limiting factors [4].

The most widely clinically used markers are C-reactive protein (CRP) and procalcitonin (PCT). Some studies have found correlation between CRP and duration of fever and clinical severity of febrile neutropenic episodes, but these findings

have not been consistently replicated [4-7]. CRP levels also increase slowly relative to cytokines and PCT [3, 4]. Data for the improved predictive value of PCT are quite varied too [8]. Based on some existing data, PCT is not yet recommended for use in routine clinical practice [9, 10]. However, an above-mentioned meta-analysis of 14 different biomarkers, including PCT, indicated that PCT appears promising and should be subject to new primary studies investigating the prediction of significant infectious morbidity [4].

Cytokines are detected in serum in the early stages of infection, and particularly in bacterial infections, they may be higher than CRP in early disease [4, 11, 12, 35]. IL-6, IL-8, and IL-10 have been shown to have good negative predictive value for bacteraemia in adult and paediatric oncology patients [4, 6, 13]. Some of them are perspective for further optimize the risk assessment model, because there is no generally accepted method that can rapidly differentiate between paediatric oncology patients with febrile neutropenia (FN) who are at high risk for developing adverse events (AEs) and those at low risk for AEs [14].

Presepsin (soluble sCD14 subtype, sCD14-ST) is a circulating molecule fragment derived from sCD14 and serves as mediator of lipopolysaccharid response against infectious agents. More recent studies have presented presepsin as a valuable potential biomarker for early diagnosis of sepsis, risk stratification and evaluation of prognosis in adult patients in the emergency department. Presepsin was superior to IL-6, CRP and PCT with regard to discriminating between survivors and non-survivors, and between low-grade sepsis versus severe sepsis or septic shock [15, 16]. Presepsin has seldom been studied in paediatric patients and results thus far are inconclusive [17 – 21].

Only few studies have concentrated on use of routinely available biomarkers (CRP, PCT) for optimizing antibiotic therapy. Several studies tested the utility of CRP measurements for shortening the duration of anti-infective therapy. Use of CRP may be limited in children to help discontinuing therapy, but evidence is poor [22 – 24]. Procalcitonin can be also a useful tool to shorten the duration of antibiotic therapy. According to rare paediatric studies, inclusion of PCT measurements within decision algorithms of antibiotic management for specific infections may be appropriate in neonates and young children under 36 months [25, 26].

Characteristics of the most frequently investigated markers are summarized in Table 1.

### Patients and methods

We performed a prospective longitudinal observational study to evaluate the role of serum biomarkers (CRP, PCT, IL-6, presepsin) in early diagnosis of bacteraemia or its signs in patients suffering from haematological malignancies. We established the main characteristics for each marker (cut-off level, sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV]) following three study questions:

- Which of markers are the best for prediction of positive blood culture?
- Which of markers are the best for prediction of signs of sepsis?
- Which of markers are the best correlates with specific type of bacteraemia (gram positive versus gram negative)?

**Patients.** Children aged 0 to 18.9 years, with a primary diagnosis of a haematological malignancy, who were admitted

**Table 1. Main characteristic of biomarkers**

Marker	Type	Time of increasing	Peak level	Half time + cut off	Advantage	Disadvantage
CRP	APP	4-6 h	36 – 48 h	19 h 10 mg/l*	Availability Routinely used	Increase of the level is influenced by cancer treatment
PCT	Pro-hormone	3-4 h	8 – 24 h	48 h 0.5 µg/l*	Predictor of mortality Possible tool for shorten ATB therapy	Limitations in neonates and transplanted patients (using ATG)
IL-6	Cytokine	1 h	4 h	8 h 7.0 ng/l*	Good NPV Predictor of neonatal sepsis Very early top level!	Increase during autoimmune diseases, burns, traumas as well
Presepsin	sCD14**	2 h	3 h	4-5h Mild sepsis: 338 – 950 ng/l* Severe sepsis/Septic shock: >950 ng/l*	Predictor of mortality Ability distinguish sepsis from SIRS	Little data in paediatrics

CRP: C-reactive protein, PCT: Procalcitonin, IL-6: Interleukin-6, APP: acute-phase protein, NPV: negative predictive value, ATB: antibiotics, SIRS: Systemic Inflammatory Response Syndrome, ATG: Anti-thymocyte globulin

Sources: [3, 4, 6, 7, 10, 11, 15, 16, 25 – 30, 35]

\*cut off levels established by standards of cooperating laboratories

\*\* CD14 is glycoprotein expressed on the membrane surface of monocytes/macrophages and its soluble fragment called presepsin

to the inpatient paediatric oncology service from November 2013 to May 2015 were eligible to enroll. Written informed consent was obtained from parents or legal guardians of all enrolled children. Study was specified by protocol. The primary inclusion criterion was the presence of fever, hypothermia, chills, or another sign of possible sepsis. We excluded patients with febrile episodes thought to be an adverse effect of chemotherapy, patients who did not have blood cultures drawn (for example, due to central venous catheter malfunction) and patients with proven non-bacterial infection. The study was approved by the ethical committee of *Children's Teaching Hospital* with Policlinic in Bratislava, Slovakia.

**Procedure sampling and analysis.** Blood samples for detection of biomarkers were drawn concomitantly with blood culture, prior to administration of antibiotics. All samples (markers and blood culture) were collected during the initial episode; repeated sampling was performed in clinically unstable patients. Laboratory standard cut-off values for abnormal levels of considered markers were: 10 mg/l for CRP; 0.5 µg/l for PCT; 7.0 ng/l for IL-6; and 338 ng/l for presepsin. Investigation of serum CRP was performed by using analyser VITROS Fusion 5.1 (Ortho-Clinical Diagnostics, Rochester, USA). Determination of CRP was based on an enzymatic heterogeneous, sandwich immunoassay format. Analyses of serum PCT; and also serum IL-6, were determined on cobas E-411 analyser (Roche, Basel, Switzerland) by the electrochemiluminescence immunoassay "ECLIA". Analysis of Presepsin (CD14) was based on non-competitive chemiluminiscent enzyme immunoassay (CLEIA) combined with \*MAGITRATION® technology using analyser Pathfast Mitsubishi.

**Statistical analysis.** The receiver operating characteristic (ROC) curve was used for determination of the cut-off level for the particular markers; we choose cut-off value that maximizes the sum of sensitivity and specificity. The result of combination of biomarkers was considered positive if at least one of biomarker result was positive. The quality of biomarker was

assessed using the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The difference between gram positive and gram negative bacteria in investigated markers was analysed using the unpaired T-test. The results were considered statistically significant when  $p < 0.05$ . Statistical analyses were performed using the StatsDirect version 2.8.0 statistical software StatsDirect Ltd. <http://www.statsdirect.com>.

**Definitions of basic terms.** Fever was defined as an axillary body temperature greater than 38.0°C in one measurement lasting more than one hour, or a temperature greater than 38.5°C measured at a single time. Neutropenia was defined as an absolute neutrophil count (ANC) less than  $0.5 \times 10^9/l$  or an ANC  $\leq 1.0 \times 10^9/l$  expected to decrease to an ANC less than  $0.5 \times 10^9/l$ . Bacteraemia was defined as a positive blood culture, independent of clinical signs of sepsis. Sepsis was defined as a set of clinical symptoms: temperature greater than 38 °C or less than 36 °C, tachycardia, tachypnea, other clinical symptoms, signs or test results associated with infection (e.g., radiographically-confirmed pneumonia, CSF-confirmed meningitis, petechiae, purpura, etc.), and/or signs of multiple organ dysfunction or failure (e.g., alteration of mental status, hypotension, oliguria, increase in transaminases, bilirubin, disseminated intravascular coagulation etc.) [13]. Fever of unknown origin (FUO) was defined as a febrile episode with confirmed negative blood culture, absence of sepsis, and clinically or microbiologically documented local infection.

## Results

The study group (Table 2) was characterized by heterogeneous clinical symptomatology and presentation of risk factors (severity of neutropenia) what is resumed in Table 3. Of the 22 positive blood cultures, only seven of these patients developed sepsis. In another nine cases of clinical sepsis, blood cultures were negative.

**Table 2. Patient characteristic**

Parameter	Result
Boys/Girls	17:16
The age range*	1.5 – 18.9 years
Average age*	7.31 years
The median*	5 years
Number of febrile episodes	69 febrile episodes in 33 patients
Number of febrile episodes with manifested signs of sepsis	16 episodes in 12 patients
Number of positive blood culture	22 episodes in 12 patients
Number of neutropenic episodes	58 (84.05%): 91.53% ratio of $ANC \leq 0.5 \times 10^9/l$
Average time from the beginning of neutropenia to the first signs of potential infection	11.43 days (the median 6 days)
Spectrum of diagnosis	24 cases of ALL, 6 cases of AML, 3 cases of NHL
Deaths	Two patients due to infectious complication and two patients because of their disease progression.

\* Each age parameter was registered at the time of the event (signs of potential infection with taking the blood culture sample)

ALL: acute lymphoblastic leukaemia, AML: acute myeloid leukaemia, NHL: Non-Hodgkin lymphoma

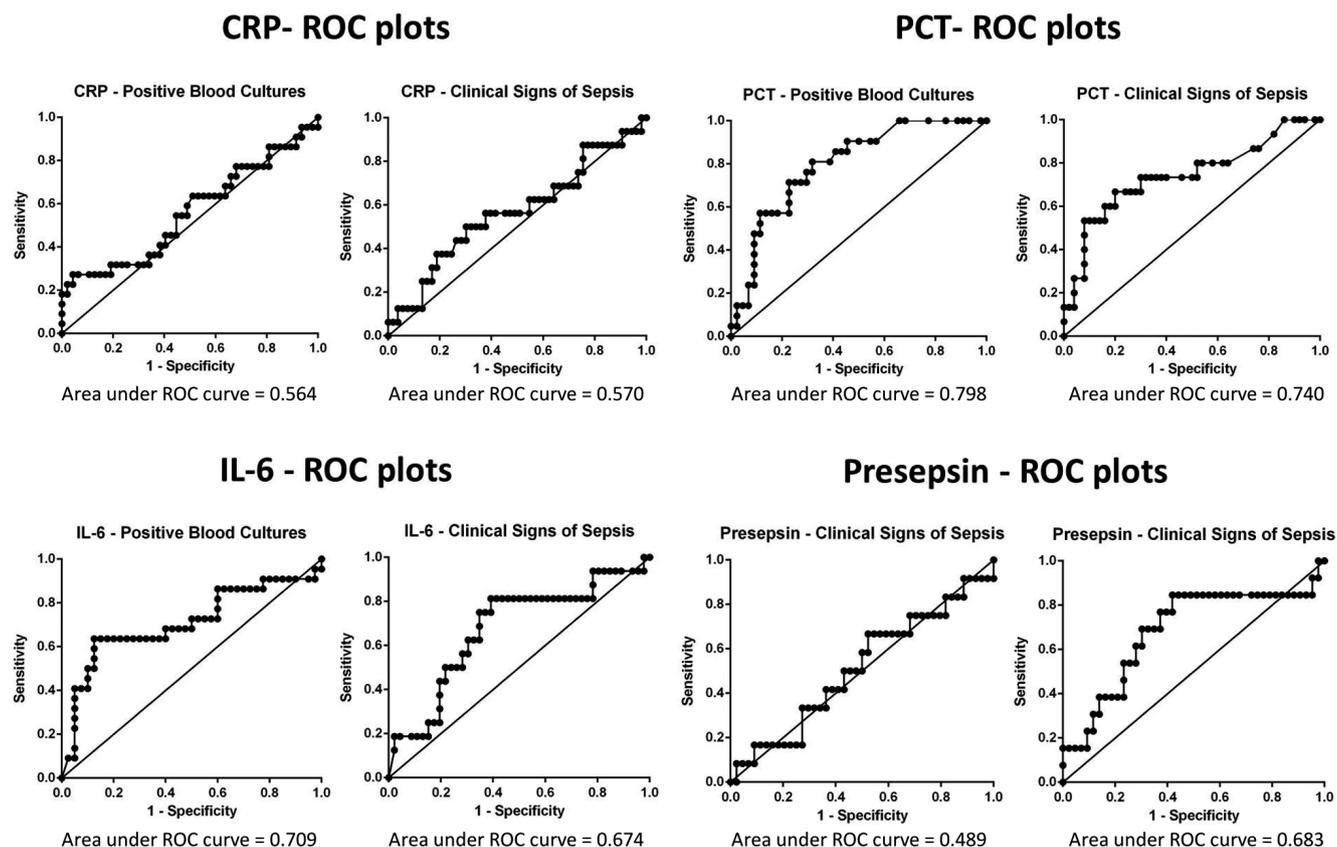


Figure 1. ROC analysis of the biomarker performance

Our data showed various outcomes for each marker in different situations (Results are summarized in Table 4 and Table 5 and relevant ROC curves are shown in Figure 1). CRP analysis revealed high specificity and higher cut-off level for predicting bacteraemia compared to sepsis (76.3 mg/l versus 49.4 mg/l), while sensitivity, PPV and NPV were higher in case of manifested sepsis.

PCT analysis showed better sensitivity for diagnostic bacteraemia and better specificity for registration signs of sepsis. PPV and NPV are nearly identical for both situations, but NPV riches better results. Cut off value of PCT is higher in septic patients (0.68 µg/l versus 0.25 µg/l). IL-6 examination is presented by preferable specificity for detection of positive blood culture and good sensitivity for early sepsis identification. We noted high NPV in both clinical circumstances; PPV achieves better result in diagnosis of bacteraemia. Calculated cut off level of IL-6 is higher during the episodes with proven positive blood culture (196.6 ng/l versus 106.1 ng/l). We confirmed good NPV in both of these markers (PCT, IL-6) and found out small differences in specificity and sensitivity ratio for predicting two different clinical situations.

Presepsin predicted with 12 cases of positive blood culture (of 22 cases total) and 13 cases of documented sepsis (of 16 cases total). We found out quite good sensitivity (83% and

84%) and NPV (86% and 93%) in predicting bacteraemia and also septic status in patients. We concluded PPV and specificity of this biomarker as poor ones. Specificity reached a better result in ability to predict clinical signs of sepsis (58% versus 27%). Calculated cut off levels are lower than officially laboratory used thresholds values (240 ng/l and 299 ng/l versus 338 ng/l).

Table 3. Clinical characteristic

69 febrile episodes in 33 patients	
22 episodes of positive blood culture (31.89%) in 12 patients (36.36%)	
n	%
7 times signs of sepsis	31.8%
15 times fever without signs of sepsis	68.2%
20 times neutropenia: (17 times ANC ≤ 0.5 x 10 <sup>9</sup> /l)	90.1% (85.0%)
47 episodes of negative blood culture (68.11%) in 21 patients (63.64%)	
n	%
9 times signs of sepsis	19.2%
38 times fever without signs of sepsis	80.9%
38 times neutropenia: (35 times ANC ≤ 0.5 x 10 <sup>9</sup> /l)	80.9% (92.1%)

We also performed an analysis of combinations of markers using our calculated cut-off values. The summary is presented in Table 6 and Table 7.

Our results, visualised in Table 8 and Figure 2, showed significant difference for marker PCT ( $p = 0.040$ ) and IL6 ( $p = 0.005$ ) between gram positive and negative bacteraemia.

## Discussion

Many studies examined role of biomarkers in detecting initial bacteraemia or another microbiologically confirmed infection. Same reviews even excluded patients with fever of unknown origin. On the other hand, there are also papers assessing value

**Table 4. Biomarkers analysis – relation to positive blood culture**

	n*	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
CRP	69	76.3 mg/l	0.27 (0.11 – 0.50)	0.96 (0.85 – 0.99)	0.75 (0.35 – 0.97)	0.73 (0.60 – 0.84)
PCT	65	0.25 µg/l	0.81 (0.58 – 0.94)	0.68 (0.52 – 0.81)	0.54 (0.36 – 0.73)	0.88 (0.73 – 0.97)
IL-6	62	196.6 ng/l	0.64 (0.41 – 0.83)	0.88 (0.73 – 0.96)	0.74 (0.48 – 0.91)	0.81 (0.67 – 0.92)
Presepsin	55	240.0 ng/l	0.83 (0.52 – 0.98)	0.27 (0.15 – 0.43)	0.24 (0.12 – 0.39)	0.86 (0.58 – 0.98)

\*Number of investigations

CI: confidence interval, PPH: positive predictive value, NPH: negative predictive value

**Table 5. Biomarkers analysis – relation to signs of sepsis**

	n*	Cut off	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
CRP	69	49.4 mg/l	0.50 (0.25 – 0.75)	0.71 (0.57 – 0.82)	0.35 (0.16 – 0.57)	0.82 (0.68 – 0.92)
PCT	65	0.68 µg/l	0.67 (0.38 – 0.88)	0.80 (0.66 – 0.90)	0.50 (0.27 – 0.73)	0.89 (0.76 – 0.96)
IL-6	62	106 ng/l	0.81 (0.54 – 0.96)	0.61 (0.45 – 0.75)	0.42 (0.25 – 0.61)	0.90 (0.74 – 0.98)
Presepsin	55	299 ng/l	0.84 (0.55 – 0.98)	0.58 (0.42 – 0.73)	0.37 (0.21 – 0.58)	0.93 (0.76 – 0.99)

\*Number of investigations

CI: confidence interval, PPH: positive predictive value, NPH: negative predictive value

**Table 6. Analysis of biomarker's combinations – relation to positive blood culture**

Combinations of biomarkers	N*	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
CRP + PCT	65	0.81 (0.58 – 0.94)	0.63 (0.47 – 0.78)	0.52 (0.34 – 0.69)	0.87 (0.71 – 0.96)
CRP + IL-6	62	0.68 (0.45 – 0.86)	0.83 (0.67 – 0.92)	0.68 (0.45 – 0.86)	0.83 (0.67 – 0.92)
PCT + IL-6	59	0.54 (0.36 – 0.72)	0.88 (0.70 – 0.98)	0.86 (0.64 – 0.97)	0.61 (0.43 – 0.76)
Presepsin + CRP	55	0.75 (0.43 – 0.95)	0.25 (0.13 – 0.40)	0.21 (0.10 – 0.37)	0.78 (0.49 – 0.95)
Presepsin + PCT	53	1.00 (0.73 – 1.00)	0.17 (0.07 – 0.32)	0.26 (0.14 – 0.41)	1.00 (0.59 – 1.00)
Presepsin + IL-6	49	0.75 (0.43 – 0.95)	0.27 (0.14 – 0.44)	0.25 (0.12 – 0.42)	0.77 (0.46 – 0.95)

\*Number of investigations

CI: confidence interval, PPH: positive predictive value, NPH: negative predictive value

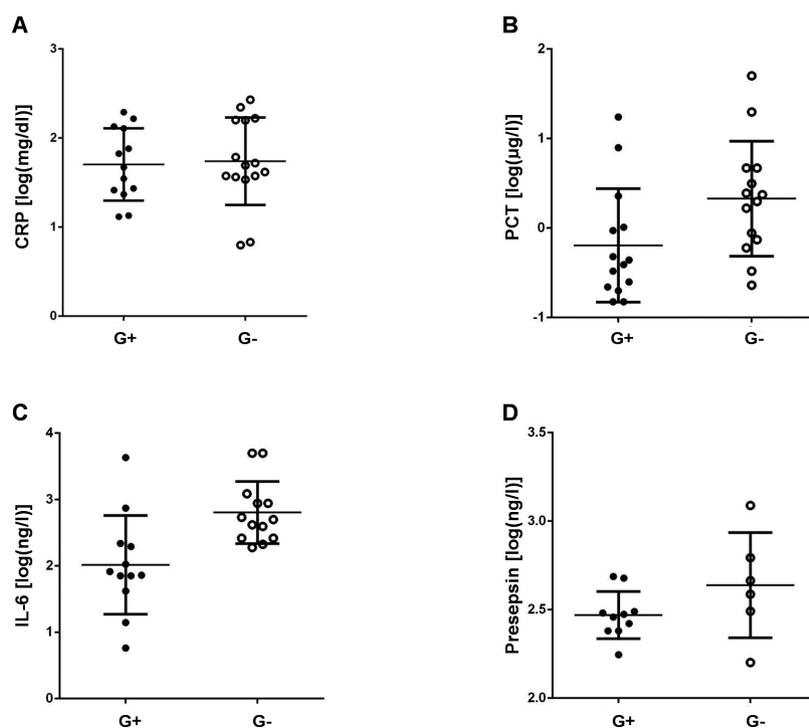


Figure 2. Scatter plots – differences in biomarker levels between gram positive and gram negative bacteraemia

of markers in context of predicting clinically manifested potential infectious complication independently of microbiologically documented etiological agents [4]. We consider clinical signs of infection/sepsis are more relevant for patient's prognosis than assessment based predominantly on microbiologically proven positive blood culture. This opinion is also supported by recent studies and by our empirical experience as well [14, 31].

Identification of laboratory markers to efficiently and accurately stratify for patients based on risk of serious infectious

complication is badly needed in paediatric oncology [14, 32]. Our results for PCT and IL-6 are presented by slightly smaller values of sensitivity and specificity than results of above-mentioned meta-analysis published by Philips et al. or other single studies [4, 6, 33]. Despite this fact we concluded that PCT and IL-6 were the most promising markers for further analysis.

According Sklavou et al. the change rate of CRP over time is offered as a prognostic index of bacterial infection

Table 7. Analysis of biomarker's combinations – relation to signs of sepsis

Combinations of biomarkers	N*	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
CRP + PCT	65	0.67 (0.38 – 0.88)	0.54 (0.39 – 0.68)	0.30 (0.16 – 0.49)	0.84 (0.67 – 0.95)
CRP + IL-6	62	0.88 (0.62 – 0.98)	0.39 (0.25 – 0.55)	0.33 (0.20 – 0.50)	0.90 (0.68 – 0.99)
PCT + IL-6	59	0.87 (0.60 – 0.98)	0.50 (0.35 – 0.65)	0.37 (0.21 – 0.55)	0.92 (0.73 – 0.99)
Presepsin + CRP	55	0.77 (0.46 – 0.95)	0.42 (0.27 – 0.58)	0.28 (0.15 – 0.46)	0.86 (0.64 – 0.97)
Presepsin + PCT	53	0.85 (0.55 – 0.98)	0.5 (0.33 – 0.66)	0.35 (0.19 – 0.55)	0.91 (0.71 – 0.99)
Presepsin + IL-6	49	0.92 (0.63 – 1.00)	0.44 (0.28 – 0.62)	0.38 (0.21 – 0.56)	0.94 (0.71 – 1.00)

\*Number of investigations

CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value

**Table 8. Biomarkers analysis – differences between patients with gram positive and gram negative bacteraemia**

	Gram positive bacteraemia (mean, SD)	Gram negative bacteraemia (mean, SD)	p-value
CRP	1.70 (0.41)	1.74 (0.49)	0.830
PCT	-0.19 (0.63)	0.33 (0.64)	0.040
IL-6	2.01 (0.74)	2.81 (0.47)	0.005
Presepsin	2.47 (0.13)	2.64 (0.29)	0.237

and a marker of the total duration of fever in febrile neutropenic children [5]. However, because of late peak levels of CRP, this marker cannot be used in urgent diagnostics of severe infections. Moreover, we found out poor sensitivity for ability to identify bacteraemia, moderate sensitivity and low PPV for discrimination of sepsis symptomatology. CRP level is also elevated during non-infectious inflammatory states including patients with malignancy, during oncology treatment or after surgery [3, 11]. This is another documented limitation.

We enrolled presepsin in this study as the last one. Insufficient data probably distorted the outcomes. We determined low cut off levels and weak specificity and PPV in contrast to good sensitivity and high NPV. There are only few existing paediatric studies focused on analysis of presepsin's diagnostic usefulness, mainly in newborns [18, 21]. Papers considered febrile episodes in childhood oncology patients reported also inconclusive results [17, 20]. Olad et al. demonstrated that in the absence of other clinically detectable sources of inflammation, presepsin level was significantly higher in blood culture positives, but there was no significant difference between positive and negative blood culture group or between febrile or afebrile group [17]. Urbonas et al. proved that there is no significant difference in presepsin levels between patients with bacteraemia/sepsis and fever of unknown origin [20].

The analysis of a combination of markers showed variations which are suitable in the diagnosis of bacteraemia. The most useful results for blood culture predictions are presented by the combination of CRP with PCT and CRP with IL-6. The combination of PCT with IL-6 is usable too. Each pair with presepsin has very poor specificity and PPV. We noticed slightly higher specificity of combined pairs with presepsin in septic patients compared to a group of patients with proven bacteraemia.

Initially elevated values of PCT and IL-6, are significantly associated with infectious complication causing by gram-negative microorganisms. This finding correlates with known efficiency of cytokines and PCT in predicting gram-negative bacteraemia [6, 34]. We did not confirm this thesis in presepsin because of insufficient data. The difference between mean presepsin in gram-positives and gram-negatives was not statistically significant even in study published by Olad et al [17].

## Conclusion

In summary, we cannot recommend investigation of CRP in fast diagnostics of bacteraemia/sepsis in paediatric oncology patients because of inconclusive results and its known limitations. The best markers for prediction of bacteraemia are PCT and IL-6. While PCT has better specificity and NPV, IL-6 has better sensitivity and PPV. The most favourable markers for prediction of clinical signs of sepsis are also PCT and IL-6, but in this situation we noticed better sensitivity for IL-6, comparable specificity for PCT and solid NPV for both of these markers. All studied markers are characterized by good NPV. Especially presepsin had a particular strong NPV; and reports slightly better results for prediction of clinical signs of sepsis; however, it is necessary to collect more data for better understanding of the role of presepsin. Therefore we plan to continue enrolling patients on this study.

Highly elevated IL-6 and PCT at presentation should be an indicator for classification as high risk. IL-6 is also useful tool for monitoring early states of infectious disease because of its fast serum peak and following decline [4, 6, 11, 12, 35]. In our clinic we start antibiotic therapy in accordance with international 2012 JCO Guideline for management of paediatric febrile neutropenia. We evaluate elements informative for risk stratification included patient-specific factors (including age, malignancy type, and disease status); treatment-specific factors (type and timing of chemotherapy), and episode-specific factors (including height of fever, hypotension, mucositis, blood counts, and available biomarkers – CRP and PCT) [32]. The main criterion for initiating anti-infective therapy remains clinical presentation. Routine usage of interleukin-6, which is characterized by rapid kinetics, can lead to an earlier initiation of antibiotic therapy; potentially before clinical presentation of the infection, and reduce frequency of adverse events. This ability was confirmed in a prospective study of preterm infants [36], but not yet in paediatric oncology patients.

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