

Diagnostic accuracy of procalcitonin, interleukin-6 and interleukin-8 for predicting Gram-negative bacteremia in febrile neutropenia patients with acute lymphoblastic leukemia

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Background. Currently available biomarkers are not specific and sensitive enough for early detection of bacterial infection in patients with cancer who have febrile neutropenia. The objective of this study was to assess diagnostic accuracy of interleukin-6 (IL-6), interleukin-8 (IL-8) and procalcitonin (PCT) in the identification of Gram-negative bacteremia at the beginning of a febrile episode in pediatric oncology patients with acute lymphoblastic leukemia (ALL).

Methods. A total of 40 episodes of febrile neutropenia in 27 childhood cancer patients were enrolled in this study. Serum samples were collected at presentation after confirmation of febrile neutropenia and analyzed according to the recommendations of manufacturers. Patients were classified into Gram-negative bacteremia (GNB) and fever of unknown origin (FUO) groups.

Results. The median concentration of IL-6, IL-8 and PCT were higher in the GNB group compared to the FUO group (65.4 vs. 409.0 pg/ml, $P = 0.0025$; 166.0 vs. 883.0 pg/ml, $P = 0.0002$; 0.27 vs. 0.44 ng/ml, $P = 0.0169$, respectively). The areas under the curves (AUCs) for both IL-6 and IL-8 were 0.94 and 0.93, respectively, indicating that these cytokines discriminated patients with Gram-negative bacteremia with excellent accuracy, whereas PCT had lower diagnostic accuracy (AUC = 0.76).

Conclusions. IL-6 and IL-8 evaluation might be used as an additional diagnostic tool for the prediction of Gram-negative bacteremia in pediatric patients with ALL during febrile neutropenia episodes.

Key words: procalcitonin, interleukin-6, interleukin-8, febrile neutropenia, leukemia, bacteremia

INTRODUCTION

Early diagnosis of bacterial infection in childhood cancer patients with febrile neutropenia (FN) is

challenging due to chemotherapy-related immune system suppression that results in nonspecific laboratory and clinical findings. Since bacterial infections are life-threatening for these patients, empirical treatment with broad-spectrum intravenous antibiotics is started without delay when fever manifests before microbiological evidence of an infection is estimated. It should be noted that morbidity rates are higher among patients with

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Gram-negative bacteremia (GNB) due to GNB association with severe sepsis. The cause of fever remains undetermined in about half of FN episodes, therefore such conventional approach may result in overtreatment for a substantial number of patients (1, 2). Consequently, early biomarkers of bacterial infection at the beginning of an FN episode would be very helpful.

The aim of this study was to evaluate the diagnostic accuracy of interleukin-6 (IL-6), interleukin-8 (IL-8) and procalcitonin (PCT) in the identification of GNB at the beginning of an FN episode among patients with acute lymphoblastic leukemia (ALL).

MATERIALS AND METHODS

This prospective study was performed at the Department of Oncohematology of the Vilnius University Children Hospital. Plasma samples were collected during each febrile neutropenic episode at presentation with FN. An informed consent, after verbal and written information provision, was obtained from all the patients. Permission for this study was provided by the Regional Committee of Bioethics.

Childhood patients who had neutropenia (absolute neutrophil count less than $0.5 \times 10^9/l$) and fever (single body temperature of more than 38.5°C in one measurement) were enrolled.

Bacteremia was defined as a microbial growth in one of the blood culture bottles.

For this study, an individual patient may have had multiple FN episodes.

According to microbiological and clinical findings, the patients with episodes of FN were classified into two groups: 1) the fever of unknown origin (FUO) group consisted of the patients with fever without a recognizable cause, 2) the Gram-negative bacteremia (GNB) group was the patients with positive blood culture for Gram-negative bacteria.

Venous blood samples were taken on admission (day 1) before commencing antimicrobial treatment. Concentrations of interleukins were estimated on a fully automated analyzer Immulite 1000 (Siemens Healthcare Diagnostics) using solid phase, chemiluminescent immunometric assay. Concentrations of PCT were evaluated by enzyme-linked fluorescent immunoassay (Vidas Brahms PCT, Biomerieux).

Venous blood samples for one set of blood cultures were aseptically collected into separate vials, which were incubated in a Bactec 9240 incubator (Becton Dickinson). 30–60 minutes later, another set of blood culture samples was aseptically obtained and processed in the same way as previous samples. Identification of microorganisms was performed by standard microbiological methods. All samples for the microbiological assessment were taken before the antimicrobial treatment started.

Summary statistics were expressed as median. A comparison between the groups of patients was performed for all FN episodes using the Mann-Whitney non-parametric test. Associations were estimated as statistically significant when p value <0.05 . The diagnostic properties of biomarkers were evaluated by receiver-operating characteristic (ROC) analysis. Statistical analysis was performed on the data from FN episodes. The MedCalc statistical software was used for calculations (version 11.4.2.0, Mariakerke, Belgium).

RESULTS AND DISCUSSION

A total of 40 FN episodes in 27 patients with acute lymphoblastic leukemia were enrolled in this study. 12 patients were enrolled more than once. There were 13 females and 14 males with a median age of 6.5 years (range 1–17 years).

The GNB group consisted of 10 patients who had 10 FN episodes. *Escherichia coli* was detected in five patients, *Klebsiella pneumoniae* was identified in two patients and the rest of patients had *Enterobacter asburiae*, *Pseudomonas aeruginosa*, *Bacteroides urealyticus* Gram-negative bacteria.

The median concentration of IL-6, IL-8 and PCT were significantly higher in the GNB group compared to the FUO group (65.4 vs. 409.0 pg/ml, $P = 0.0025$; 166.0 vs. 883.0 pg/ml, $P = 0.0002$; 0.27 vs. 0.44 ng/ml, $P = 0.0169$, respectively).

The diagnostic accuracy of all evaluated biomarkers for GNB identification in febrile neutropenic patients with ALL was assessed according to AUC; sensitivity, specificity, negative and positive predictive values (NPV, PPV) were determined based on the Youden Index (Table). The areas under the curves (AUCs) for both IL-6 and IL-8 were above 0.90 suggesting that these cytokines discriminated patients with Gram-negative bacteremia

with excellent accuracy, whereas PCT had lower diagnostic accuracy ($AUC_{PCT} = 0.76$).

In this study we assessed the value of pro-inflammatory cytokines (IL-6, IL-8) and PCT as additional diagnostic tools for Gram-negative bacteremia determination at the beginning of FN in the pediatric patients with ALL. Also, we determined critical cut-off values according to the Youden Index that determines the maximum potential efficiency of each biomarker by giving representative significance to specificity, sensitivity, PPV and NPV (3).

Previous studies presented limited data for IL-6, IL-8 and PCT usage as predictors of GNB. In the previous study we showed that IL-8 could be used as a screening tool for the prediction of Gram-negative infection in childhood cancer patients (6). Fleischhack and colleagues (4) suggested that PCT might be a more useful diagnostic biomarker than IL-6 and IL-8 for GNB evaluation in pediatric cancer patients. Engel and colleagues (5) showed that IL-8 was superior to CRP and potentially useful for predicting Gram-negative bacteremia with NPV and specificity more than 90%, while Kern et al. (2) demonstrated that IL-8 could be used as a predictor of GNB in childhood cancer patients.

The findings of the present study extended previous observations and showed that IL-6 and IL-8 had high diagnostic accuracy ($AUC_{IL-6} = 0.94$, $AUC_{IL-8} = 0.93$) and were superior to PCT ($AUC_{PCT} = 0.76$) in contrast to the Fleischhack and colleagues study. High sensitivity and NPV is preferred to diagnose such serious conditions as GNB in FN patients even if the false positive rate is higher because giving more false negative results is more dangerous. In our study the most sensitive biomarkers for predicting GNB were IL-6 and IL-8 (sensitivity 100%, NPV 100%, cut-off was 115 pg/ml; sensitivity 89%, NPV 96%, cut-off was 631 pg/ml, respectively).

The strength of this study was homogeneity of the patients' group and prospective study design. Concerning study limitations, the amount of pa-

tients was small, and the exact time of blood sampling and link with the beginning of an FN episode have not been taken into account.

In conclusion, the data from this study indicate that PCT is less accurate than IL-6 and IL-8 for predicting GNB in FN childhood patients with ALL on admission. However, due to a limited number of patients, larger prospective studies with multiple measurements of the same biomarker through defined periods of time are required to confirm these findings.

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Table. Diagnostic accuracy of IL-6, IL-8 and PCT for identifying Gram-negative bacteremia positive patients according to receiver operating characteristic (ROC) analysis

	AUC (95% CI)	Cut-off	Sensitivity, %	Specificity, %	PPV, %	NPV, %	p-value	Youden Index
IL-6	0.94 (0.78–1.00)	115 pg/ml	100	86	70	100	<0.0001	0.86
IL-8	0.93 (0.79–0.99)	631 pg/ml	89	96	86	96	<0.0001	0.85
PCT	0.76 (0.59–0.88)	0.39 ng/ml	80	70	47	91	0.0071	0.53

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**PROKALCITONINO, INTERLEUKINO-6 IR
INTERLEUKINO-8 DIAGNOSTINIS TIKSLUMAS
VERTINANT GRAM-NEIGIAMĄ BAKTERIEMIJĄ
FEBRILINĖS NEUTROPENIJOS METU
PACIENTAMS, SERGANTIEMS ŪMIA
LIMFOBLASTINE LEUKEMIJA**

Santrauka

Įvadas. Šiuo metu klinikinėje praktikoje naudojami biožymenys nėra pakankamai jautrūs ir specifiniai

diagnozuojant ankstyvą bakterinę infekciją pacientams, sergantiems onkologinėmis ligomis febrilinės neutropenijos (FN) epizodo metu. Šio tyrimo tikslas – įvertinti interleukino-6 (IL-6), interleukino-8 (IL-8) ir procalcitonino (PCT) diagnostinį tikslumą nustatant Gram-neigiamą bakteriemiją vaikams, sergantiems ūmia limfoblastine leukemija (ALL) febrilinės neutropenijos epizodo metu.

Metodai. Analizuoti 27 onkologiniai pacientai, kuriems taikomo gydymo eigoje išsivystė 40 FN epizodų. Serumo mėginiai buvo imami patvirtinus FN ir tiriami pagal pateiktas gamintojų rekomendacijas. Pacientai buvo suskirstyti į dvi tiriamąsias grupes – Gram-neigiamos bakteriemijos (GNB) ir neaiškios kilmės karščiavimo (FUO).

Rezultatai. IL-6, IL-8 ir PCT koncentracijų mediana buvo didesnė GNB grupėje, palyginti su FUO grupe (65,4 ir 409,0 pg/ml, $P = 0,0025$; 166,0 ir 883,0 pg/ml, $P = 0,0002$; 0,27 ir 0,44 ng/ml, $P = 0,0169$). IL-6 ir IL-8 plotas po kreive (AUC) atitinkamai 0,94 ir 0,93, o PCT žymens diagnostinis tikslumas buvo mažesnis (AUC = 0,76).

Išvados. IL-6 ir IL-8 nustatymas gali būti naudojamas kaip papildoma diagnostinė priemonė vertinant Gram-neigiamą bakteriemiją pacientams, sergantiems ūmia limfoblastine leukemija febrilinės neutropenijos epizodo metu.

Raktažodžiai: procalcitoninas, interleukinas-6, interleukinas-8, febrilinė neutropenija, leukemija, bakteriemija