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Measurement of immature granulocytes (IG) percentage to assess severe bacterial infection in Latvian children: a secondary data analysis

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Abstract

Background: Detection of small proportion of serious bacterial infections (SBI) with potentially life threating course in a large group of children with fever admitted to emergency department (ED) is still complicated. Measurement of immature granulocytes (IG) percentage may be used as a marker of bacterial infections. The aim of the study was to evaluate whether the IG percentage is a useful additional predictive marker of SBI.

Methods: This study included 258 children with febrile infections admitted to the ED. Clinical follow-up, microbiological and radiological tests were used as reference standards for the definition of SBI. Study population was categorized into two groups: (i) infected patients with no suspicion of SBI (n = 75); (ii) patients with suspicion of SBI (n = 183). IG percentage, white blood cell count (WBC) and C-reactive protein (CRP) levels were analyzed from the first routine blood samples at hospital admission.

Results: A statistically significant difference in IG percentage levels was observed in children with SBI and those without - the mean IG percentage was 1.2% for the SBI group, 0.3% for those without SBI. The cutoff level of IG percentage to predict SBI was 0.45 (84% specificity, 66% sensitivity, 90% positive predictive value). We combine variables and evaluate their additive values. The sensitivity of WBC to detected SBI improved from 74% to 85% when IG percentage was added to the prediction models. When CRP, WBC and IG percentage were combined, the sensitivity to predict SBI increased to 93%, the specificity to 86%. (95% CI 77–93%). Receiver operator characteristic analysis to predict SBI showed an area under the curve (AUC) of 0.80 for IG percentage.

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Conclusion: Addition of IG percentage to traditionally used markers of SBI as WBC and CRP may help to identify children with serious bacterial infections. Furthermore IG percentage can be rapidly obtained from the traditional full blood count without any extra sampling and costs.

Keywords: Bacterial infections; sensitivity; specificity; immature granulocytes; Latvia; child; sepsis

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Introduction

Fever is one of the most common causes for admittance of children to the emergency department. Most children suffering from fever have simple self–limiting viral infections, however a small proportion will develop serious bacterial infections that are potentially life-threating. The low prevalence of serious illness and frequent presence of pediatric patients in emergency department at the early stage of the illness, when severity of disease is still not apparent, pose for early and accurate diagnosis of serious infections a challenge [1-4]. To support the decision making in febrile children, a lot of clinical and laboratory prediction models have been investigated, but the interpretation and application process of these models in clinical practice is still complex [2-6].

Nowadays, modern automated haematology analysers are available that can measure additional infection parameters such as immature granulocytes (IG) that may be used as markers of serious bacterial infections [7, 8]. However, despite availability of IG measurement, it has not yet been incorporated in the routine diagnostic tool for patients with infection. New generation analysers can now provide automated, very precisely enumerated actual IG count and percentage in peripheral blood samples [8-10]. Several recent studies have investigated the role of IG percentage measurement as a potential marker to predict severity of an infection [11-14]. However, these studies were mainly focused on critically ill adult patients at intensive care units. Only one study investigated IG in a common outpatient clinical setting covering all age groups including pediatric, obstetrics and geriatric population [15]. Moreover, the performance of IG measurement compared with conventional infection markers such as white blood cell count (WBC), absolute neutrophil count (ANC) and C-reactive protein (CRP) remains unclear [9, 12, 13].

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Thus, the aim of this study was to evaluate whether the measurement of IG percentage is a useful predictive marker of SBI compared with conventional infection markers such as WBC, CRP and ANC in pediatric patients.

Materials and methods

Study population

The population of this retrospective study consisted of patients admitted due to infection to the emergency and observational department of the Children's clinical University hospital in Riga, Latvia during January 2014 and December 2015. The University hospital is the only tertiary level children hospital in the country with around 64 000 pediatric emergency department visits annually.

The inclusion criteria for the study were suspected diagnosis of any type of infection and age between 1 month and 18 years. Exclusion criteria were antibacterial therapy within the last 48 hours, vaccination within 5 days before the start of illness, any immunodeficiency disease, malignancies, congenital metabolic defects and other diseases that may alter the levels of inflammatory markers.

A total of 258 patients fulfilled the entry criteria and were selected for data analysis. Patients were classified into two groups: (i) infected patients without suspicion of serious infection (n=75) and (ii) patients with suspicion of SBI (n=183).

Clinical protocol and definitions

Infection severity was defined at the emergency department. Within two till five days two experienced clinicians revised whether infection severity was evaluated correctly at the initial moment of diagnosis to ensure correct classification of the study participants. SBI at emergency department and during the later revision of clinicians

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was defined based on available clinical, imaging, and later also on microbiological data as having either bacteraemia, pneumonia, meningitis, osteomyelitis, intra-abdominal infection, complicated urinary tract infection, skin/soft tissue infection, culture positivity of usually sterile body fluid or diagnosis by radiology (pneumonia, osteomyelitis, intra-abdominal infection) [16]. The group of infected patients with fever but without any suspicion of SBI included in most cases children with upper and lower respiratory tract infections of viral origin and patients with acute gastroenteritis mainly of rotavirus, norovirus or adenovirus.

Data collection and laboratory assays

The study parameters were collected during the first routine blood analysis sampling at the emergency department of patients with suspicion of infection. Blood samples were obtained by venepuncture in EDTA vacutainer tubes. Blood cultures were collected by two separate vein punctures for all patients with suspicion of sepsis.

The IG percentage was calculated with an automated haematology analyser Sysmex XE 2100. The IG measurement that includes promyelocytes, myelocytes and metamyelocytes was performed in the differential channel of the Sysmex XE 2100. A specific lysing reagent causes mature WBC membranes disruption, leaving bare nuclei, but immature myeloid cells remain intact due to low cell membrane lipid content. The increased permeability of leucocytes allows a polymethine dye to enter the cells with high affinity for nuclei acid. The cells are analysed by nucleic acid fluorescence and side scatter.

The IG percentage is defined as the percentage of the total WBC count [10, 12]. CRP levels were measured by the latex method (COBAS INTEGRA; Roche professional Diagnostics), the lowest assay sensitivity being 0.085 mg/L.

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Ethical considerations

This study used secondary, re-identified data and has been approved by the Medical Ethics committee of the Riga Stradins university. All patients included in the study received the standard care according to the hospital guidelines.

Statistical analyses

The data were analysed using IBM SPSS version 22.0. Differences in continuous variables between groups were compared using the Mann-Whitney or independent samples t-test according to type of variable distribution. Correlation analysis was performed using the Spearmen rank, respectively Pearson (normally distributed variables) coefficient. Performance of the selected variables was assessed using receiver-operating characteristic (ROC) curves, sensitivity, and specificity, negative and positive predictive values. The Youden's index was used to determine the best cut-off values for the different indicators to maximize both, sensitivity and specificity (maximum+=sensitivity + specificity – 1). The 95% confidence interval and p value were reported for the area under the curve (AUC) for the best cut-off levels. Furthermore, Cohen's d value was used to calculate effect size. A two-tailed p-value less than 0.05 was considered as statistically significant for all tests.

Results

The baseline characteristics of the study population are presented in Table 1. The mean age of infected patients without serious bacterial infection was 60.3 months, and 76.6 months for the SBI patients, respectively. The prevalence of being male was 42% in patients with and 48% without SBI. The majority of cases with SBI were due to intra-abdominal infections, nephritis or lower respiratory tract infections (69% of all SBI infections). Bacteremia confirmed by two separate

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positive blood cultures was detected in 32 SBI patients (17%). The most common detected bacteria in blood steam were *Staphylococcus aureus* (12 patients), *Escherichia coli* (8 patients), *Streptococcus pneumoniae* (4 patients). *Neisseria meningitidis* was identified both from blood culture and cerebrospinal fluid in three patients. The upper and lower respiratory tract viral infections, and Rotavirus and Norovirus infections were the main causes of infection in patients without SBI.

IG percentage, CRP, ANC and WBC all were significantly higher in SBI patients compared with those without severe infection.

The area under the curve (AUC) of IG percentage to predict SBI using a cut-off value of 0.45 was 0.80 (95% confidence interval (CI) 0.74-0.85) (Figure 1). The corresponding AUC for CRP was 0.94 (95% CI 0.91-0.96), for ANC 0.78 (95% CI 0.72-0.84) and for WBC 0.79 (95% CI 0.73-0.84).

Table 2 shows the sensitivity, specificity and predictive values for different markers of serious bacterial infections in the study population. The sensitivity for the best cut-off level for IG percentage (66%) was lower compared to CRP, ANC or WBC. However, in regard specificity and positive predictive value IG percentage performed better than ANC or WBC. CRP, when using an optimal cut-off level of 56.5 showed the best diagnostic test performance. We combine variables and evaluate their additive values. The sensitivity of WBC to detected SBI improved from 74% to 85% when IG percentage was added to the prediction models and to 92% (95% CI 88-96%) when CRP and WBC were measured. Finally, when CRP, WBC and IG percentage were combined, the sensitivity to predict SBI increased to 93% (95% CI 88-96%) and the specificity to 86% (95% CI 77-93%).

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Table 3 presents the odds ratio of the different biomarkers to predict SBI in children. Increase in WBC was associated with a 1.31 fold increased risk (95% CI 1.14-1.49) of SBI and an increase in CRP resulted in a 1.08 fold increased risk of SBI (95% CI 1.05-1.11) in children. Also IG percentage was statistically significantly associated with an increased probability of SBI (2.24 fold increased risk (95% CI (1.20-3.38)).

Discussion

Our study found that IG percentage is a useful marker to predict the severity of infection and it adds information to the conventional infection markers WBC, ANC and CRP for early identification of pediatric patients with SBI. Specifically, when IG percentage was used together with CRP and WBC nine out of 10 children with SBI were correctly identified.

The prevalence of SBI in the children population is still high and the fast identification of patients with potentially serious and life threating conditions is important in the clinical setting. Elevated count of IG in the peripheral blood indicates increased bone marrow activity as the response to bacterial infection [17]. Adult patients with clinically apparent bacterial infection showed significantly higher numbers of myeloid progenitor cells compared with healthy controls [17].

Whereas most of studies investigating IG as a marker of SBI were performed on seriously ill adult patients or neonatal patients [10, 12, 13, 17, 18], only a few studies included not seriously ill patients [9-11, 15]. Furthermore, information about IG percentage in children population is scant [9, 15]. Our study added valuable information on the performance of IG percentage in children with different grades of infections directly of all age-group. Our results are in line with those of previous

studies revealing an association between a higher IG percentage in patients and positive blood bacterial culture [9, 10, 12]. Moreover, patients with suspected septicemia and positive blood culture had higher level of IG percentage than those with negative blood culture [10, 12]. Furthermore, it was found that IG percentage in blood culture positive children patients were significantly higher than in culture negative patients [9].

In the study of Nierhaus *et al* IG percentage showed the highest discriminative value for infection in the first 48 hours in surgical intensive care patients. Interestingly, in contrast to CRP, only IG percentage was a significant predictor of severe infection [13]. Current evidence suggests that IG percentage may perform better in early diagnosis of severe infection than for instance CRP or WBC [12, 19].

To increase the accuracy of prediction of severe infections the combination of IG with other markers may be used [9, 10, 12, 20-23]. Past research, in agreement with our results, have revealed that the predictive value of IG percentage in critically ill adult patients improves for each day when IG percentage is added to WBC and CRP [12]. Moreover, the IG percentage adds to WBC and CRP in the early exclusion of infection and can be obtained routinely without extra blood sampling or costs [12]. Considering that CRP usually starts to increase after 24 hours of infection start, measuring WBC and IG percentage may help to identify children with SBI at an very early stage helping clinical decision makers to start treatment accordingly.

In our study the best sensitivity and specificity was obtained using a threshold of 0.45 for IG percentage in children. Previous research have revealed optimal cut-off levels for IG percentage varying between 0.4-0.5 with sensitivities varying between 58-96% and specificity ranging from 76-80% [9, 10, 12, 15]. Thus, which cut-off level to be used within that particular range may depend on the local circumstances.

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Pooling data of existing research projects may offer a better estimate of the ideal threshold to be used in children to diagnose SBI.

Naturally, our study had some limitations. For instance, we did not include a control group consisting of healthy children in our study that may give valuable insight into the pattern of inflammatory markers in children without SBI. Furthermore, our study focused on early laboratory diagnosis of patients with SBI and was not able to include information on any genetic influences or cellular abnormalities as potential triggers for the infection progress to a life threatening state.

Conclusions

Our findings suggest that SBI in children is associated with an increase in IG percentage. The IG percentage differs between patients with non-SBI and those with SBI. Furthermore, IG percentage adds to WBC and CRP in early detection of serious bacterial infections in pediatric population and provides the additional diagnostic tool for physicians in identifying of a small proportion of high risk children in very intensive flow of patients at emergency department. IG percentage can be rapidly obtained from the traditional full blood count without any extra sampling and costs.

List of abbreviation

ANC	Absolute neutrophil count
AUC	Area under the curve
CRP	C-reactive protein
IG	Immature granulocytes
SBI	Serious bacterial infections
WBC	White blood cell count

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Declarations

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of Riga Stradins University.

Consent for publication

Not applicable.

Availability of data and material

The datasets analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JP planned the study, collected and analysed data, wrote the report. IG and DG were responsible for study planning and data analysis, they reviewed the manuscript. JP was involved in the data analysis and performed the statistics. All authors have read and approved the final version of the manuscript.

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Table 1. Baseline characteristics, infection focus and markers of the study population according to severity of infection.

	Patients without SBI[1] **(n=75)**	**SBI patients** **(n=183)**
Baseline characteristics		
Boys, % (n)	48% (36)	42% (78)
Age (months)	60.3±56.6[2]	76.6±65.7
Length of hospitalization (days)	1.7±0.8[2]	10.5±5.0
Infection focus	% (n)	% (n)
Intra-abdominal	0 (0)	24 (44)
Nephritis/pyelonephritis	0 (0)	23 (42)
Lower respiratory tract	16 (12)	20 (36)
Osteomyelitis	0 (0)	11 (21)
Meningitis	0 (0)	7 (11)
Occult bacteremia	0 (0)	5 (10)
Skin/soft tissue infection	0 (0)	5 (10)
Upper respiratory tract	15 (11)	3 (5)
Toxic shock syndrome	0 (0)	1 (2)
Pericarditis	0 (0)	0.5 (1)
Gastroenteritis	69 (52)	0.5 (1)
Infection markers		
IG percentage[3]	0.3±0.1[2]	1.2±1.6

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CRP[4]	12.0 ±14.0	130.0 ±94.8
ANC[5]	7.2±3.6	13.8 ±7.3
WBC[6]	10.3 ±3.5	18.1 ±8.6

[1]Serious bacterial infection; [2] Mean (standard deviation); [3]Immature granulocytes; [4]C-reactive protein; [5]Absolute neutrophil count; [6]White blood cell count;

Table 2. Sensitivity, specificity and predictive values for different markers and their combinations of serious bacterial infections in children.

Variable	Cutoff value	Sensitivity % (95% CI)[3]	Specificity % (95% CI)	PPV[1] % (95% CI)	NPV[2] % (95% CI)
IG[4] percentage	0.45	66 (59-73)	84 (73-91)	90 (84-95)	51 (42-60)
CRP[5]	56.5	75 (68-81)	100 (95-100)	100 (97-100)	62 (53-71)
ANC[6]	8.6	73 (65-79)	73 (61-82)	86 (80-91)	52 (42-62)
WBC[7]	11.75	74 (67-80)	73 (61-82)	54 (80-91)	87 (44-64)
WBC and IG percentage		85 (79-90)	64 (52-74)	85 (78-90)	64 (52-75)
WBC and CRP		92 (88-96)	85 (74-92)	92 (86-94)	83 (73-90)
WBC, CRP and IG percentage		93 (88-96)	86 (77-93)	94 (89-97)	84 (74-91)

[1]Positive predictive value; [2]Negative predictive value; [3]Confidence interval; [4]Immature granulocytes; [5]C-reactive protein; [6]Absolute neutrophil count; [7]White blood cell count

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Table 3. Logistic regression coefficients of different biomarkers to predict serious bacterial infections in children.

Variable	Regression coefficient	P value	Odds ratio (95% CI)[1]
WBC[2]	0.27	<0.001	1.31 (1.14-1.49)
IG[3] percentage	0.80	0.041	2.24 (1.20-3.38)
CRP[4]	0.08	<0.001	1.08 (1.05-1.11)

[1]Confidence interval; [2]White blood cell count; [3]Immature granulocytes; [4]C-reactive protein

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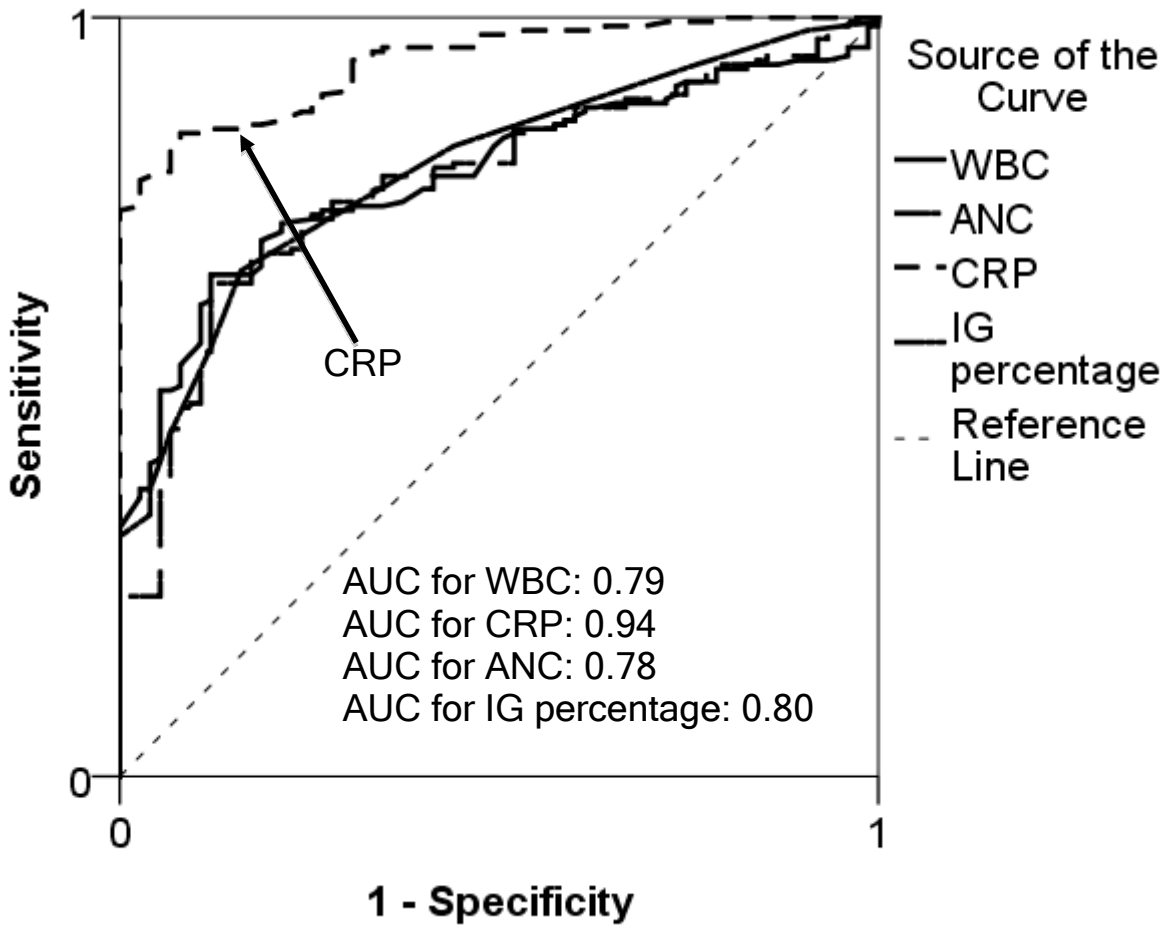

Figure 1. Receiver operating curves for immature granulocyte (IG) percentage, white blood count (WBC), absolute neutrophil count (ANC) and C-reactive protein (CRP) in predicting serious bacterial infections in children.