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ORIGINAL ARTICLE

CD15s is a potential biomarker of serious bacterial infection in infants admitted to hospital

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Abstract Early recognition of serious bacterial infection (SBI) in children is essential for better treatment outcome. Flow cytometry analysis of neutrophil surface molecules has been more frequently utilized as a tool for diagnosis of infection. The infants (n=105) under 6 months of age presenting to the pediatric emergency department with fever without apparent source who were hospitalized with suspicion of having SBI were enrolled in this prospective study. Sixty-nine infants were included into the training pool and were classified into bacterial or viral infection group. Validation pool consisted of 36 infants. The values of white blood cells counts, absolute neutrophil count (ANC), C-reactive protein (CRP), procalcitonin (PCT), neutrophil CD11b, CD15s and CD64 expression, and the percentage (%CD15s+) and absolute count (AC-CD15s+) of CD15s+ neutrophils were determined. In infants with SBI, %CD15s+ was 10.5 times more likely to be higher than the cut-off value. ANC, CRP, PCT, CD64, and AC-CD15s+ were also found as useful biomarkers for differentiation between bacterial and viral infection. The best fit multivariate logistic regression model included CRP, PCT, and %CD15s+ as strong predictors of SBI. The model's sensitivity (87 %) and specificity (83 %) indicated high model's accuracy. After validation on independent dataset, model's accuracy maintained high: 86 % sensitivity and 93 % specificity, confirming its reliability and supporting CRP, PCT, and %CD15s+ as real predictors. The findings of this study support assumption made in the literature on significance of CD15s in inflammation processes. Also, this study demonstrated for the first time that CD15s is potentially valuable biomarker of SBI in infants.

Keywords Infant · Infection · Biomarkers · Antigens · CD15s · CD11b · CD64

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Introduction

Up to 23.6 % of infants under 3 months of age with fever without apparent source (FWAS) will develop serious bacterial infection (SBI) [19] whose early recognition is essential for better outcome [20]. White blood cells count (WBC), absolute neutrophil count (ANC), C-reactive protein (CRP), procalcitonin (PCT), and microbiologic cultures are usually used when evaluating patients with suspected SBI [12]. However, the value of CRP in an emergency department (ED) setting is limited [22]. PCT has been reported as a better prognostic indicator, but its value has also been challenged [26].

The inability to accurately distinguish patients with SBI from those with viral illness using routine laboratory tests has led to the investigation of new biomarkers [8, 9, 28]. Flow cytometry analysis of various neutrophil cell surface

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molecules has been increasingly investigated, and among them, CD11b and CD64 have shown potential as early biomarkers of infection [3, 18, 29]. It is well known that, in response to infection, circulating leukocytes tether to the vessel wall and then roll in response to hydrodynamic drag forces. Only certain adhesion molecules, including selectins, and some integrins have been found to support rolling [25]. Selectins bind to carbohydrate ligands in which the sialyl Lewis^x (sLe^x, CD15s) moiety is of key importance [2]. In 2007, it was shown that CD11b/CD18 is a major membrane protein whose both subunits are decorated with CD15s [30]. However, despite the important role of CD15s during infection, the change in CD15s expression during the infection has not been previously evaluated. The present study has compared the diagnostic performance of neutrophil CD11b, CD15s, and CD64 expression with CRP and PCT in early detection of SBI in infants presenting to the pediatric ED for FWAS who were hospitalized with suspicion of having SBI.

Materials and methods

This prospective observational study was conducted at the Pediatric ED of the University Hospital of Split, Croatia during a period of 6 months (July–December 2011). The Ethics Committee of the University Hospital of Split approved the study protocol. The parents/guardians of the enrolled infants gave a written informed consent.

Subjects

The consecutive infants under 6 months of age presenting with FWAS who were hospitalized with suspicion of having SBI were eligible for inclusion. Other inclusion criteria were gestational age ≥37 weeks and rectal temperature ≥38 °C lasting at least 24 h as reported by parents/guardians. Exclusion criteria were antibiotic treatment during the previous 48 h, known immunodeficiency, vaccination during previous 5 days, and presentation to ED during the weekend. All diagnostic procedures were performed at the time of admission. Laboratory, microbiology, and radiology personnel were blinded to the clinical information. Based on the final diagnosis set on the hospital discharge day by two expert pediatricians who had access to all data except to the flow cytometry results, the infants were classified into two groups: (1) patients with SBI or (2) patients with viral infection (VI).

The definitions used for classification of patients in the SBI group were [19] (a) sepsis/bacteremia microbiologically confirmed by positive blood culture or bacterial meningitis diagnosed by positive cerebrospinal fluid culture; (b) urinary tract infection (UTI) confirmed by positive urine culture (>10^5 colony forming units per milliliter of a single microorganism in a sample collected by sterile method); (c) chest radiographic features consistent with pneumonia (lobar consolidation or an infiltrate) confirmed by pediatric radiologist; (d) bacterial gastroenteritis confirmed by positive stool culture; or (e) cellulitis with an appropriate physical examination. The patients were classified in the VI group if they had (a) positive viral antigen detection, (b) characteristic evolution of viral disease (i.e., exanthema subitum), or (c) spontaneous recovery without antibiotics within 48 h following admission.

None of the infants had focal (non-serious) bacterial or fungal infection or had received vaccine against *Streptococcus pneumonia* or meningococcal conjugate vaccine since they are not included in the immunization program. Healthy infants, without any suspicion of infection or chronic medical conditions, whose blood was taken for various reasons in the University Hospital of Split outpatient clinic, were included as controls.

Determination of CRP and PCT levels

The 2 ml of the whole blood had been collected in the Vacutainer tube without anticoagulant (Becton Dickinson, Plymouth, UK) and transferred to the Department of the Medical Laboratory Diagnostics for determination of CRP and PCT levels. The CRP was measured with a Multigent CRP Vario immunoassay (Sentinel, Milan, Italy) by the automated ARCHITECT ci8200 instrument (Abbot, Wiesbaden, Germany), while PCT was measured using Vidas B-R-A-H-M-S PCT test (Biomerieux, Lyon, France) by the mini-VIDAS instrument (Biomerieux, Lyon, France) according to the manufacturer's instructions.

Determination of neutrophil CD11b, CD15s, and CD64 expression

The unused amount of peripheral blood sample collected to perform the complete blood count had been stored refrigerated (4 °C) until the flow cytometry measurement was done in the Department of Biochemistry at the School of Medicine Split. Whole blood had been pre-treated with an Fc-receptor-blocking reagent and was incubated with primary anti-CD15s antibody (Pharmingen, San Diego, CA, USA). After two washes in PBS, cells were incubated with secondary fluorescein isothiocyanate (FITC)-conjugated, affinity chromatography-purified rabbit anti-mouse antibody (Pharmingen, San Diego, CA, USA). For double leucocytes labeling, whole blood had been pretreated with a FcR-blocking reagent and incubated with phycoerythrin (PE)-conjugated antibody reactive to human CD11b (IQ Test, Beckman Coulter, Marseille, France) and phycoerythrin-cyanine 5 (Pc5)-conjugated antibody reactive to human CD64 (IQ Test, Beckman Coulter, Marseille, France). After erythrocyte lysis with red blood cell lysis solution, 10^5 events were recorded on a Coulter Epics XL flow cytometer (Beckman Coulter Corporation, Miami, FL, USA).

Fluorochrome and isotype-matched controls as well as unstained cell samples were measured and processed as negative controls. Non-specific binding of a secondary antibody was prevented using cells labeled only with secondary FITC-conjugated antibody. Analysis of samples was done by FloJo analysis software (Tree Star Inc., Ashland, OR, USA). The neutrophils were gated on the basis of their side and forward scatter characteristics. The results were expressed as geometric mean fluorescence intensity (GMI) of cells showing expression of the assessed adhesion molecules. Furthermore, the percentage of neutrophils expressing CD15s was determined (%CD15s$^+$). The absolute count of CD15s$^+$ neutrophils (AC-CD15s$^+$) was calculated by multiplying the %CD15s$^+$ with ANC.

Data analysis

Data were analyzed with statistical package SPSS 19.0 (IBM Corp., Armonk, NY, USA). Quantitative data were described by median and interquartile range. Median values between the three groups of patients were compared with Kruskal–Wallis statistical test, whereas Mann–Whitney *U* test was utilized in comparisons of viral to bacterial median value. For categorical data, χ^2 test with Yates correction was used. p values ≤ 0.05 were considered statistically significant. The potential of a single biomarker in discriminating viral from bacterial infection was investigated in several steps. Firstly, the receiver operating characteristic curve (ROC) analysis was applied on each biomarker, and the area under curve (AUC) was calculated. The AUC's confidence interval and the significance were estimated by DeLong nonparametric method, while the biomarker's optimal cut-off value was determined by Youden's index. Secondly, sensitivity, specificity, and positive and negative likelihood ratios were determined for the optimal cut-off point. The biomarkers that were marked as promising by ROC analysis were further combined and included as predictors into different multiple logistic regression (MLR) models for predicting SBI/VI presence. Stepwise model-building procedure was used. Significance of each independent predictor was estimated by Wald chi-square test. Before entering the model, continuous variables were either dichotomized at the Youden's index and entered as categorical or were entered as continuous predictors. Nagelkerke R^2 fit-parameter and model's classification error were utilized to find the best fit among several competing MLR models. The final model was validated on independent dataset. Post hoc power analysis was performed for all biomarkers used in ROC analysis (PASS 12, NCSS, LLC, Kaysville, UT, USA). The number of infants in the training pool (39 in SBI group; 30 in VI group) was sufficient to achieve power >80 % for detecting the difference between AUC of 0.50 for null hypothesis and AUC of minimally 0.71 for alternate hypothesis, using two-sided *z* test with significance level of 0.05. The only exemption is %CD15s+ for which the power was 75 %.

Results

During the study period, 6,720 children were presented to the ED, and 726 among them (10.8 %) were infants under 6 months of age. Of those, 149 (20.5 %) were hospitalized with suspicion of having SBI. The infants who met the inclusion criteria during the first 5 months of the study period were assigned to the training pool (n=69), and the ones during the last month were assigned to the validation pool (n=36). Based on the final diagnosis, the infants from the training pool were later classified into two groups: infants with SBI (n=39) and infants with VI (n=30). Healthy controls formed the third group (n=38). Study groups were comparable, except for the mean weight (Table 1).

The most common diagnosis in the SBI group was UTI (n=22). The responsible organisms included *Escherichia coli* (18 cases), *Klebsiella pneumoniae* (3), and *Enterococcus* (1). Pneumonia was diagnosed in 13 infants, sepsis (*E. coli* and group B *Streptococcus*) in 2, bacterial gastroenteritis (*Salmonella* group D) in 1, and cellulitis in 1 infant. In VI group, antigen detection was positive in 18 infants (Respiratory syncytial virus in 13 and Rotavirus in 5). Ten infants spontaneously recovered without antibiotics, and in two, exanthema subitum was diagnosed. Table 2 presents the laboratory findings of the enrolled infants.

Compared to the control group, WBC was significantly higher in the SBI and the VI group ($p<0.05$). Regarding neutrophil CD antigens, the expression of CD11b was significantly higher in the SBI group as compared to controls ($p<0.05$), but showed no significant difference between the SBI and VI groups. Expression of CD15s did not significantly differ between the groups. All other evaluated biomarkers (ANC, CRP, PCT, CD64, %CD15s$^+$, and AC-CD15s$^+$) were significantly different between all the groups.

Based on these results, potentially useful biomarkers for differentiation between bacterial and viral infection were selected and evaluated by the AUC parameter (Table 3). The closer the value of AUC was to 1, the biomarker was more powerful discriminator. ROC analysis confirmed that considered biomarkers were useful for differentiation (AUC >0.6 and $p<0.05$ for all). Table 3 also shows the sensitivity, specificity, and likelihood ratios for the differentiation test that utilized the optimal cut-off value of the considered biomarker for classification.

Potential biomarkers were further combined and entered as predictors into different MLR regression models for prediction of SBI/VI presence. Variables that were identified in various models as significant independent predictors included CRP, PCT, ANC, CD64, as well as AC-CD15s$^+$ and

Table 1 Demographic characteristics of the study subjects

	SBI (n=39)	VI (n=30)	Control (n=38)	Statistics[a]	Post hocs[b]
	Median (interquartile range)				
Males	26	18	17	p=0.140	
Age (days)	133 (61–164)	81 (33–160)	108 (36–150)	p=0.218	
Weight (g)	6,400 (5,440–7,450)	5,050 (3,975–6,925)	6,000 (4,275–6,925)	p=0.032	(SBI, VI)
Gestational age (weeks)	39 (38–40)	39 (36–40)	40 (38–40)	p=0.318	
Birth weight (g)	3,400 (3,070–3,700)	3,305 (2,525–3,800)	3,555 (3,112–3,832)	p=0.260	
Birth length (cm)	50 (48–51)	49.5 (45–52)	50 (48–51)	p=0.663	

SBI serious bacterial infection, *VI* viral infection, *C* control group

[a] Different statistical tests were used in accordance with the data type and distribution: Pearson chi square (gender) and Kruskal–Wallis (all other variables)

[b] Post hocs: only pairwise comparisons significant at 0.05 level are reported in brackets

%CD15s$^+$ neutrophils. The best fit model, with categorical variables CRP, PCT, and %CD15s$^+$ as significant predictors, was chosen among competing models (Table 4). Compared to the second-rated successful model (CRP and PCT as predictors), inclusion of %CD15s$^+$ increased R^2 by 5 % and improved overall classification accuracy by 4.3 %. In the final model, the highest odds for SBI are found with %CD15s$^+$. The performance of the final model was very good with 85.5 % of cases correctly classified as SBI or VI. The model's sensitivity was 87 %, specificity was 83 %, while positive and negative likelihood ratios were 5.11 and 0.16, respectively.

In order to validate the model, it was tested on the 36 infants. These infants were comparable to the training pool on most demographic characteristics (Table 5) with exceptions of weight in SBI groups. In SBI group, UTI was diagnosed in ten infants. The responsible organism in all of them was *E. coli*. Pneumonia was diagnosed also in ten infants, and sepsis (caused by *E. coli*) was diagnosed in one infant. In VI group, antigen detection was positive in ten infants (*Respiratory syncytial virus* in eight and *Rotavirus* in two). Five infants spontaneously recovered without antibiotics.

The accuracy of the model was even higher, with 89 % of infants correctly classified. The sensitivity was 86 %, specificity was 93 %, while positive and negative likelihood ratios were 12.86 and 0.15, respectively.

Model for the prediction of a diagnosis is available at URL address: http://genom.mefst.hr/~ajeronci/SBI/Predict_SBI_or_VI.xls.

Discussion

Infants with FWAS require comprehensive physical and laboratory examination. In this study, diagnostic value and

Table 2 Laboratory findings of the study subjects

	SBI (n=39)	VI (n=30)	Control (n=38)	Statistics[a]	Post hocs[b]
	Median (interquartile range)				
WBC (×10^9/l)	14.6 (11.2–18.3)	13.5 (9.2–15.5)	9.0 (7.4–11.5)	p<0.001	(SBI,C), (VI,C)
ANC (×10^9/l)	7.1 (4.8–10.5)	4.1 (1.8–6.7)	2.1 (1.5–3.1)	p<0.001	All
CRP (mg/l)	48.6 (21.6–86.3)	4.5 (0.8–19.8)	0.3 (0.1–0.4)	p<0.001	All
PCT (ng/ml)	0.71 (0.26–2.12)	0.11 (0.07–0.24)	0.04 (0.03–0.08)	p<0.001	All
CD11b (GMI)	1.81 (1.67–2.24)	1.74 (1.35–2.00)	1.44 (1.22–1.95)	p=0.007	(SBI,C)
CD64 (GMI)	2.70 (2.26–3.62)	2.31 (1.91–2.49)	1.74 (1.41–2.12)	p<0.001	All
CD15s (GMI)	2.99 (2.26–4.22)	3.17 (2.4–4.48)	2.87 (1.89–4.06)	p=0.514	
%CD15s$^+$	93.5 (89.2–97.7)	87.0 (80.4–95.9)	77.1 (69.1–87.2)	p<0.001	All
AC-CD15s$^+$	6.28 (4.49–9.77)	3.59 (1.56–5.55)	1.71 (1.24–2.33)	p<0.001	All

SBI serious bacterial infection, *VI* viral infection, *C* control group, *WBC* white blood cell count, *ANC* absolute neutrophils count, *CRP* C-reactive protein, *PCT* procalcitonin, *GMI* geometric mean fluorescence intensity, *%CD15s*$^+$ percentage of CD15s$^+$ neutrophils, *AC-CD15s*$^+$ absolute count of CD15s$^+$ neutrophils

[a] In accordance with the data type and distribution, Kruskal–Wallis test was used

[b] Post hocs: only pairwise comparisons significant at 0.05 level are reported in brackets; *all*, all pairs significantly differ

Table 3 Area under the receiver operator characteristics curve (AUC) for differentiation between bacterial and viral infection, and performance measures of the accompanying differentiation test using biomarker's optimal cut-off point

Biomarker	AUC	95 % CI	*p*	Optimal cut-off	Sensitivity (%)	Specificity (%)	Positive likelihood ratio	Negative likelihood ratio
CRP (mg/l)	0.88	0.806–0.959	<0.001	>11.3	90	73	3.37	0.14
PCT (ng/ml)	0.86	0.781–0.947	<0.001	>0.37	67	93	10.00	0.36
AC-CD15s$^+$ ($\times 10^9$/l)	0.75	0.633–0.848	<0.001	>5.47	67	77	2.86	0.43
ANC ($\times 10^9$/l)	0.73	0.611–0.852	<0.001	≥5.69	69	73	3.03	0.4
CD64 (GMI)	0.71	0.590–0.831	0.003	>2.43	64	77	2.75	0.47
%CD15s$^+$	0.68	0.551–0.814	0.01	>84.6	90	47	1.68	0.22

CRP C-reactive protein, *PCT* procalcitonin, *AC-CD15s*$^+$ absolute count of CD15s$^+$ neutrophils, *ANC* absolute neutrophil count, *GMI* geometric mean fluorescence intensity, *%CD15s*$^+$ percentage of CD15s$^+$ neutrophils

predictive power of several SBI/VI biomarkers were evaluated. Optimal model for SBI prediction with CRP, PCT, and %CD15s$^+$ as strong predictors and with 85.5 % of correctly classified cases was presented with sensitivity and specificity of 87 % and 83 %, respectively. The model validation on the independent dataset showed that high performance of the model, with almost 90 % correctly classified cases, was retained. Percentage of CD15s$^+$ neutrophils, but not the expression per se, was the significant predictor in the final model and had the highest odds for SBI. In the infant with SBI, %CD15s$^+$ was found almost 11 times more likely to be higher than the cut-off value, which was even higher than for widely used CRP and PCT. Therefore, the concern about not finding the real difference due to somewhat lower power of 75 % for %CD15s+ was not justified. Since there are no data in the literature about AUC for %CD15s+ in the context of bacterial infection prediction, we have decided to make post hoc testing of power for this variable.

The results in this study confirmed that CRP and PCT are good indicators of the infection with similar performance in differentiating bacterial from viral infection, but also supported the need for more reliable and accurate biomarkers.

The primary function of neutrophils is elimination of the invading microorganisms. In response to stimuli, the active migration of neutrophils is initiated [2]. CD15s and related moieties expressed on neutrophil cell surface play an important role in the initial step of leukocyte extravasation into inflamed tissues by serving as the ligands for selectins [10, 14]. Their significance is additionally confirmed by the fact that their ligation by a specific antibody gravely influences neutrophil functions including induction of neutrophil aggregation, induction of secondary granule release, and leads to neutrophil transmigration impairment [30]. The same study also showed that, following *N*-formyl-methionine-leucine-phenylalanine (fMLP) stimulation, the expression of CD15s was increased [30]. The increased expression, upon neutrophil activation, is observed for CD11b and CD64, as well [6, 11, 17, 23]. Thus, detection of expression of these changes by flow cytometry might be a rapid diagnostic method requiring a minimal amount of blood.

Taking into consideration the findings which point to the increased expression of CD11b during infection [18, 29] and upregulation of CD15s following fMLP stimulation, the hypothesis was that the CD15s should also be substantially increased during infection. However, this study demonstrated statistically significant difference in %CD15s+ and AC-CD15s+ among the SBI, VI, and control group ($p<0.001$), but the difference was not found for fluorescence intensity of CD15s. Both AC-CD15s+ and %CD15s+ proved to be valuable biomarkers for differentiation between bacterial and viral infection with AUC of 0.75 and 0.68, respectively. This finding supports the fact that the role of CD15s moieties in inflammation processes is critical. One of them, %CD15s+, was even retained as a significant predictor of SBI after an optimal model for prediction had been made. The accuracy of the proposed model proved that CD15s may be a valuable biomarker in detection of SBI and in differentiation between bacterial and viral infection.

With regard to CD64, significantly higher values of CD64 were found in the SBI group compared to the VI group. However, the performance of CD64 was worse when compared to PCT and CRP. These results are similar to the Rudensky et al. [21]. Like in the present study, the majority of viral infections were caused by respiratory syncytial virus

Table 4 Significant categorical predictors in the final model

Predictor (cut-off point)	Odds ratio for SBI (95 % CI)[a]	*p*
CRP (≤11.3 mg/l)	9.4 (1.83–48.54)	0.007
PCT (≤0.37 ng/l)	9.3 (1.29–67.62)	0.027
%CD15s$^+$ (≤84.6 %)	10.5 (1.72–64.53)	0.011

CRP C-reactive protein, *PCT* procalcitonin, *%CD15s*$^+$ percentage of CD15s$^+$ neutrophils, *SBI* serious bacterial infection

[a] Category with values below the ROC cut-off point are given as reference

Table 5 Demographic and laboratory characteristics of the study subjects in validation group

	SBI (n=21)	VI (n=15)	Statistics[a]
	Median (interquartile range)		
Males	7	11	p=0.735
Age (days)	64 (52–110)	108 (44–152)	p=0.470
Weight (g)	5,010 (4,650–6,000)	6,000 (5,075–7,425)	p=0.054
Gestational age (weeks)	39 (38–40)	40 (39–40)	p=0.404
Birth weight (g)	3,400 (2,930–3,630)	3,500 (3,335–3,870)	p=0.304
Birth length (cm)	49 (47–51)	51 (49–52)	p=0.125
CRP (mg/l)	94.0 (35.3–170.5)	2.8 (1.6–8.6)	p<0.001
PCT (ng/ml)	0.8 (0.32–4.16)	0.11 (0.07–0.23)	p<0.001
%CD15s+	92.1 (87.5–94.1)	84.6 (80.7–94.2)	p=0.153

SBI serious bacterial infection, *VI* viral infection, *CRP* C-reactive protein, *PCT* procalcitonin, *%CD15s+* percentage of $CD15s^+$ neutrophils

[a]Different statistical tests were used in accordance with the data type and distribution: Pearson chi square (gender) and Mann–Whitney test (all other variables)

which seems to cause upregulation of CD64 expression to a degree similar to that seen in bacterial infection [21]. CD11b did not prove itself as a valuable biomarker of infection. So far, its expression has been reported increased [18, 29], decreased [15], and normal [1, 13] so CD11b remains controversial and requires further examination.

When interpreting the results, the following limitations have to be taken into consideration. One is a relatively small number of infants included in the study, but similar to others [24]. The other is the fact that majority of pneumonias in infants are viral, and it is hard to distinguish them from bacterial pneumonias. Since the final diagnosis was set on by two expert pediatricians taking into consideration physical examination, laboratory results, radiograph, and the RSV antigen testing performed in all infants with suspicion of respiratory tract infection, it is unlikely these infants were misclassified. The third limitation is that the analysis of cell surface markers expression is suggested immediately after obtaining blood samples to avoid cell apoptosis [16]. However, other authors claim that the CD64 expression is stable for more than 30 h at room temperature [4, 5, 7]. Contrary to this, labile expression of CD11b was reported [4, 5]. Due to the inability to perform flow cytometry during the weekend and to avoid longer storage of the samples, only the patients admitted on working days were included in the study, and all analysis was performed within 16 h from admission following the protocol in which method for determination of CD11b expression was reported stable [27].

The last limitation refers to the significantly higher body weight of the study subjects in SBI group of the training pool, but since the weight of all infants included in this study was >10th percentile for his/her age, we do not think that this finding had any influence on the results.

In conclusion, $\%CD15s^+$, CRP, and PCT combined together were shown to be strong predictors and the useful biomarkers to predict SBI in infants under 6 months of age with FWAS. This model has potential to help clinicians in early and reliable diagnosis of SBI and in treatment decision making which will lead to early initiation of appropriate antibiotic therapy. Also, early viral infection detection will reduce antibiotic use, will have positive effect on antibiotic resistance prevention, and will decrease healthcare system costs. Flow cytometry method is easily performed, and the results are available in only 1 h. Since the flow cytometers are becoming more and more available in the clinical laboratories of the hospitals, especially the tertiary level hospitals, the clinical use of these results should definitely be taken into consideration.

To our knowledge, the results demonstrated for the first time that the CD15s is potentially a valuable biomarker for detection of SBI in infants and that determination of CD15s expression changes might be of extreme importance for early detection of infection, but further studies are warranted.

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Conflicts of interest The authors declare that they have no conflict of interest.

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