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Diagnostic accuracy of G-CSF, IL-8, and IL-1ra in critically ill children with suspected infection

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Abstract *Objective:* To elucidate the diagnostic accuracy of granulocyte colony-stimulating factor (G-CSF), interleukin-8 (IL-8), and interleukin-1 receptor antagonist (IL-1ra) in identifying patients with sepsis among critically ill pediatric patients with suspected infection. *Design and setting:* Nested case-control study in a multidisciplinary neonatal and pediatric intensive care unit (PICU). *Patients:* PICU patients during a 12-month period with suspected infection, and plasma available from the time of clinical suspicion (254 episodes, 190 patients). *Measurements and results:* Plasma levels of G-CSF, IL-8, and IL-1ra. Episodes classified on the basis of clinical and bacteriological findings into: culture-confirmed sepsis, probable sepsis, localized infection, viral infection, and no infection. Plasma levels were significantly higher in episodes of culture-confirmed sepsis than in episodes with ruled-out infection. The area under the receiver operating characteristic curve was higher for IL-8 and G-CSF than for IL-1ra. Combining IL-8 and G-CSF improved the diagnostic performance, particularly as to the detection of Gram-negative sepsis. Sensitivity was low (<50%) in detecting *Staphylococcus epidermidis* bacteremia or localized infections. *Conclusions:* In this heterogeneous population of critically ill children with suspected infection, a model combining plasma levels of IL-8 and G-CSF identified patients with sepsis. Negative results do not rule out *S. epidermidis* bacteremia or locally confined infectious processes. The model requires validation in an independent data-set.

Keywords Intensive care, newborns · Granulocyte colony-stimulating factor · Interleukin-8 · Interleukin-1 receptor antagonist · Logistic regression models

Introduction

Premature infants, term newborns, and critically ill children are at increased risk of sepsis [1, 2, 3]. Early diagnosis is impeded because noninfectious processes, such as low cardiac output syndrome, trauma, major surgery, and neonatal respiratory distress can mimic the onset of sepsis [4, 5, 6]. Microbiological results are usually not available at the time of initial assessment. Extensive use of empirical antimicrobial therapy is common [7].

Several biological markers with variable sensitivity and specificity have been evaluated to improve the early diagnosis of sepsis [8, 9, 10]. Among the most promising parameters for increasing diagnostic accuracy are granu-

locyte colony-stimulating factor (G-CSF), interleukin-8 (IL-8), and interleukin-1 receptor antagonist (IL-1ra) [11, 12, 13]. It has been claimed that measurement of IL-1ra even allows diagnosis to be advanced by up to 48 h [11]. However, many of the encouraging data have been obtained from selected populations of infants [11] or after excluding surgical cases [12].

It remains unknown whether these favorable findings can be extended to the more heterogeneous population encountered in pediatric intensive care, which includes surgical and trauma patients. Therefore we compared the diagnostic properties of IL-8, G-CSF, and IL-1ra determinations in identifying patients with sepsis among a heterogeneous group of pediatric intensive care patients who were clinically suspected to have infection.

Methods

Patient population and setting

The population comprised all infants and children admitted during a 12-month period to the level III multidisciplinary neonatal and pediatric intensive care unit of a tertiary referral center. The unit serves a population base of approximately 3 million and provides postoperative care after major pediatric or neonatal surgery including cardiac surgery, treatment for children with severe trauma or medical conditions, and for outborn neonates with critical illness.

Study design

We prospectively followed a cohort of critically ill patients to enroll all episodes of illness in which clinicians suspected infection. During these episodes we performed nested case-control analyses using a stringent and a broadened definition of sepsis. For the primary analysis we used plasma samples obtained simultaneously with a diagnostic sepsis-workup. In a secondary analysis we evaluated plasma samples collected 1 day prior to clinical suspicion of sepsis to test whether cytokine measurement allows advancement of diagnosis, as suggested by Kuster and coworkers [11].

The study was approved by the institutional review board, who permitted the additional diagnostic assays on plasma obtained for routine laboratory tests.

Inclusion and exclusion criteria

Patients with suspected bacterial infection were eligible. Clinically suspected bacterial infection was defined as the presence of an explicit statement to that effect in the patient's records plus the initiation of a standard diagnostic workup to rule out infection (including two sets of blood cultures), and/or the initiation of empirical antibiotic therapy. Patients with long-term hospitalization could contribute more than one episode, provided each occasion of clinical suspicion (onset of episode) was at least 10 days apart and antibiotic treatment was discontinued for at least 72 h before the onset of the next episode. Episodes were excluded from the analysis when waste plasma obtained at diagnostic workup for suspected infection yielded less than 85 µl. For the primary analysis only results from plasma samples obtained in parallel to blood cultures drawn at the time of clinical suspicion of infection or within 2 h were used. We also excluded episodes of patients admitted with a proven localized viral infection (e.g., Respiratory syncitial virus).

Table 1 Patient characteristics of 254 episodes of suspected infection

Category	
Episodes/patients	254/190
Female gender: episodes	91 (36%)
Age: mean (years)	2.8±4.3
Age: median/interquartile range (years)	0.69/0.03–3.6
Length of stay: median/interquartile range (days)	7/4–16
Admission diagnosis	
Postoperative care after cardiac surgery	68 (27%)
Postoperative care after other major surgery	37 (15%)
Trauma	18 (7%)
Suspected infection	30 (12%)
Respiratory failure	48 (19%)
Circulatory failure	28 (11%)
Neurological disorders	13 (5%)
Monitoring[a]	10 (4%)
Others	2 (1%)
Outcomes	
Discharged alive: episodes/patients	232/170
Death: episodes/patients	22/20

[a] Patients admitted for cardiac catheterization, decannulation from tracheostomy, or adjustment of home ventilation

Patient population

During the study period 775 patients were admitted to the unit. In 347 patients (46%) clinicians suspected 461 episodes of bacterial infection. Sufficient plasma from the sample obtained at suspicion of infection was available in 254 episodes (56%), which were contributed by 190 patients. An average of 2.9±1.1 samples were measured per patient. Twenty-seven episodes (11%) were classified as *sepsis with positive blood culture* and served as cases when the stringent case definition was applied. A further 27 episodes were classified as *probable sepsis* (negative blood cultures). Of the 25 episodes considered as localized infection without major systemic manifestation 15 were due to ventilator-associated pneumonia. The 130 episodes classified as *no infection* (50%) included 58 (45% of 130) episodes in which patients developed fever of unknown origin after surgery. Among the 45 episodes deemed to be unclassifiable, there were 8 episodes with a Gram-positive isolate for *Staphylococcus epidermidis*, *S. sanguis*, or *S. hominis* in which only one set of blood cultures was drawn from indwelling lines. Table 1 summarized the characteristics of the study population. Systemic antibiotic therapy was initiated at suspicion of infection in 215 episodes (85%) and within 24 h thereafter in a further 29 episodes (11%).

Specimen collection and cytokine determination

All plasma samples were refrigerated at –80°C within 6 h of collection. Samples were thawed once for cytokine determination and all cytokines were determined within 8 h after thawing (parallel assays). Cytokines were measured using commercially available enzyme-linked immunosorbent assays (ELISA, R&D Systems, Abington, UK). Blood cultures were processed on automated analyzers (Bactec 9240, Becton Dickenson, Fullerton, Calif., USA). C-reactive protein was determined using a turbidimetric assay (Boehringer-Mannheim, Mannheim, Germany) on a random access analyzer (Beckman Synchron CX5, Beckman Instruments, Fullerton, Calif., USA).

Classification of episodes and definitions

Two investigators (J.F., A.B.) independently assessed all episodes of suspected infection from all clinical, laboratory, and microbiological data available prior to cytokine measurement. Disagreements were settled by consensus with a third investigator (S.F.).

The main outcome categories were *culture-confirmed sepsis*, *probable sepsis*, *localized infection* (without systemic signs of sepsis), *no infection* (defined as controls), and *unclassifiable episodes*. The adjudication criteria have been published in detail elsewhere [6, 7]. In principle adjudication was based on the presence of microbiological evidence (blood cultures, cultures from local sites) on clinical evidence and on laboratory evidence. Adjudication criteria were comparable to previous studies in newborns [11] and considered recommended definitions of inflammatory responses in critically ill children [6, 15, 16].

To define cases we used two different definitions of sepsis - accounting for the difficulty to adjudicate on the presence of sepsis in the absence of an undisputed gold standard. The first (stringent) case definition required clinical, laboratory and microbiological confirmation (positive bacterial blood cultures) of sepsis. The second (broadened) case definition included all episodes with *culture-confirmed sepsis* plus episodes deemed to be *probable sepsis* (negative blood cultures). This broadened definition follows the American College of Chest Physicians/Society of Critical Care Medicine definition of sepsis, which does not require positive blood cultures as criterion of sepsis [6, 15]. A control was defined as an episode of suspected infection in which the subsequent clinical course, laboratory data, and microbiological results excluded an infection, and an alternative diagnosis could later be established.

Microbiological evidence of sepsis was assumed if cultures grew a pathogen other than *S. epidermidis*, or cultures obtained from two independent sites grew *S. epidermidis*. We considered objective clinical evidence of sepsis to be present if two or more of the following criteria were met: thermal instability or fever (<36.0°C, >38.5°C); tachypnea/dyspnea (e.g., >60/min in newborns, >50 in infants, >40 in toddlers) or increased ventilation requirements (increase in mean airway pressure × frequency by 30% or more, increase in average oxygen requirement by 20% or more); otherwise unexplained new infiltrate on chest radiographs persisting for more than 48 h [16, 17, 18]; increased frequency of apnea, desaturation, or bradycardia (neonates, more than one spell per hour); pallor; cold limbs or delayed capillary refill time; arterial hypotension (inotropes required to maintain mean arterial blood pressure above age appropriate lower limit) [6]; enteral feeding intolerance (gastric residual >1.5 ml/kg per feeding); seizures or other alteration in mental state; and acidosis (arterial pH <7.25 and/or a base excess of more than –10 mmol/l).

Laboratory evidence of sepsis required two or more of the following criteria at clinical suspicion of infection: white blood cell count less than 4000/ mm^3, immature to total neutrophil ratio exceeding 0.3 [19, 20], otherwise unexplained thrombocytopenia of less than 100,000 per mm^3, or the plasma level of C-reactive protein higher than 20 mg/l [21].

Statistical analysis

Raw cytokine levels were compared across outcome categories by the Kruskal-Wallis test. The diagnostic performance of each parameter or of combinations was assessed by logistic regression analysis. The logit of the predicted probability served as the dependent variable and the cytokine value determined at suspicion of infection as the independent variable. Candidate variables were entered using a stepwise selection procedure [22, 23]. Because plasma levels showed a highly skewed distribution, we used the log-transformed values of G-CSF, IL-8, and IL-1ra, which approximated normal distribution. In the first analysis we entered episodes of *culture-confirmed sepsis* as cases and episodes classified as *no infection* as controls. This analysis is comparable to several studies in the field (e.g., the report on IL-1ra [11]), but artificially omits those patients with *probable sepsis* but negative cultures. In a second analysis we entered episodes of *culture-confirmed sepsis* plus episodes of *probable sepsis* as cases. If not explicitly stated, we present the data from the first analysis. Finally, we subjected the samples available from 24 h prior to the diagnosis of sepsis to the same analyses to determine whether cytokine determinations allow advancing of diagnosis.

The diagnostic accuracy of G-CSF, IL-8, and IL-1ra plasma levels was expressed as the area under the receiver operating characteristic curve (AUC) [24]. The AUC is an overall measure of a test's discriminative ability. An AUC of 1 indicates a perfect test, clinically useful tests yield an AUC larger than 0.75. The AUC of a nondiscriminative test (e.g., tossing a coin) has an AUC of 0.5 [25]. Multilevel likelihood ratios were calculated for strata derived from the distribution of results (low=0–49.9th percentile, moderately elevated=50th–69.9th percentile, high=70th–89.9th percentile, very high=90th percentile and above). Likelihood ratios indicate by how much a given cytokine result raises or lowers the pretest probability of sepsis. Likelihood ratios greater than 10 or less than 0.1 usually indicate clinically conclusive evidence, likelihood ratios of 1:2 or 0.5:1 change the pretest probability only to an insignificant degree [26, 27]. Statistical analyses were performed using SAS (version 6.12, SAS, Cary, N.C., USA).

Results

Cytokine results

All three parameters were markedly elevated in cases of culture-positive or probable sepsis, whereas the magnitude of response was minimal in localized infections or episodes without infection (Fig. 1). The traditional measures of test accuracy and multilevel likelihood ratios for IL-8, G-CSF, and IL-1ra are presented in Table 2. The listed values for sensitivity, specificity, and negative and positive predictive values correspond to the point closest to the upper left-hand corner on the receiver operating characteristic curve. Figure 2 shows the relationship between sensitivity, specificity, and plasma levels for all three parameters. The true negative rate (specificity) remained similar for G-CSF and IL-8 over a wide range of possible cutoff levels and was higher than for IL-1ra. The sensitivity was higher for G-CSF and IL-8 than for IL-1ra, but all parameters showed a relevant rate of false negative results. The latter was attributable to the low sensitivity in episodes of culture-confirmed sepsis with *S. epidermidis* isolates. The highest value of each cytokine obtained during the clinical course from episodes with a positive blood culture result is presented in Table 3. For some patients the level was obtained prior to clinical suspicion. Post-hoc analysis revealed lower G-CSF levels for episodes with Gram-positive pathogens than with Gram-negative isolates (Wilcoxon test, p=0.04). In the case of *S. epidermidis* bacteremia cytokine levels of all parameters were lower than with other causative organisms (all p<0.002).

The best parameters for discriminating between the presence or absence of infection were IL-8 (AUC=0.81)

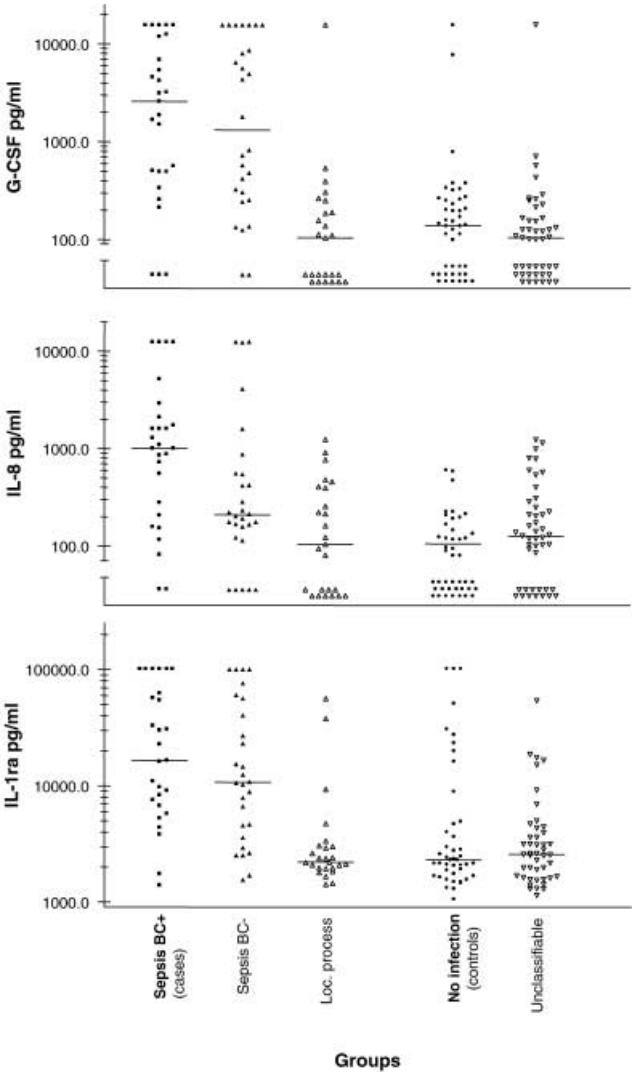

Fig. 1 Raw values of cytokines obtained at suspicion of infection stratified by outcome category. The groups are abbreviated as following: *sepsis BC+* sepsis with positive blood culture; *sepsis BC–* probable sepsis according to clinical and laboratory data, negative blood cultures; *loc process* localized infection without systemic involvement; *viral inf* viral infection; *no infection* unclassifiable

and G-CSF (AUC=0.80). G-CSF and IL-8 also provided the largest likelihood ratios for positive and negative results (Table 2). Conversely, IL-1ra levels yielded lower discriminative ability (AUC=0.73). Post-hoc analysis attributed most false positive IL-1ra results to newborns with respiratory distress and patients developing noninfectious fever following surgery. Combining G-CSF and IL-8 improved the prediction (AUC=0.84; Table 2, Fig. 3). Post-hoc analysis showed that the combined model would not have missed any case of Gram-negative sepsis. The equation of the model was:

logit=-8.657+0.565×ln (G-CSF)+0.996×ln (IL-8)

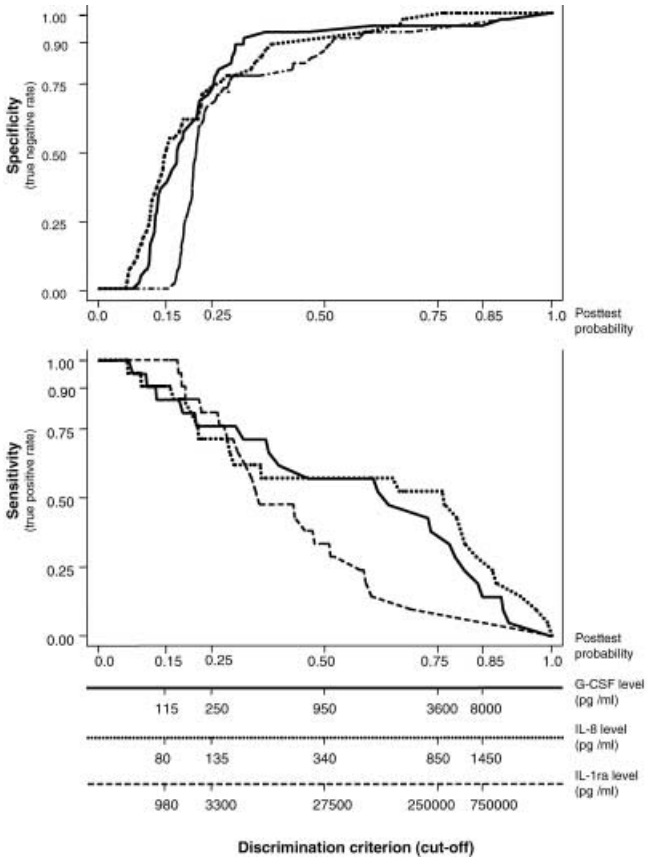

Fig. 2 Sensitivity and specificity of G-CSF, IL-8, and IL-1ra plotted against the predicted probability of sepsis obtained from the logistic regression model. The additional horizontal axes represent the corresponding plasma levels of G-CSF, IL-8 and IL-1ra

Table 2 Diagnostic characteristics of G-CSF, IL-8, and IL-1ra in the case of infection. Sensitivity, specificity, and predictive values are calculated for the cutoff values which represented the best discriminative power as derived from the receiver operating characteristic curves

	G-CSF	IL-8	IL-1ra	Combined model[a]
Sensitivity (%)	57	57	33	67
Specificity (%)	95	93	89	93
Positive predictive value (%)	86	80	58	82
Negative predictive value (%)	82	82	74	85
Area under the receiver operating curve	0.80	0.81	0.73	0.84
Likelihood ratios				
Very high levels[b]	9.4	>10	3.0	>10
High levels[c]	6.2	2.1	1.9	7.5
Moderately elevated levels[d]	0.57	0.86	0.80	0.45
Low levels[e]	0.33	0.17	0.45	0.11

[a] G-CSF + IL-8
[b] G-CSF, IL-8, IL-1ra: >3,000, 900, >40,000 pg/ml
[c] G-CSF, IL-8, IL-1ra: 750–3,000, 250–900, 12,000–40,000 pg/ml
[d] G-CSF, IL-8, IL-1ra: 120–750, 80–250, 2,000–12,000 pg/ml
[e] G-CSF, IL-8, IL-1ra: <120, <80, <2,000 pg/ml

Table 3 Peak cytokine levels (pg/ml) from episodes with positive blood cultures (*OA* outcome adjudication, *S* classified as sepsis with positive blood culture, *U* unclassifiable episode, *Severity* severity of illness according to American College of Chest Physicians/Society of Critical Care Medicine criteria, *MOF* multiple-organ failure, *Day* day of highest measured level, *day –1* day prior to clinical suspicion, *day 0* day of clinical suspicion of infection, *day 1* day after clinical suspicion)

	OA	Severity	G–CSF		IL–8		IL–1ra	
Isolated pathogen			Peak	Day	Peak	Day	Peak	Day
Staphylococcus epidermidis	U	Shock[a]	<98	0	119	0	1,969	0
Staphylococcus epidermidis	U	Severe sepsis[a]	126	0	109	1	3,102	0
Staphylococcus epidermidis	S	MOF[a]	<98	0	799	0	1,406	0
Staphylococcus epidermidis	S	Shock	489	0	83	0	16,585	0
Staphylococcus epidermidis	U	Shock[a]	<98	0	85	0	2,499	0
Staphylococcus epidermidis	U	Shock[a]	104	0	<78	0	1,391	0
Staphylococcus epidermidis	S	Shock[a]	214	0	400	0	9,281	–1
Staphylococcus epidermidis	U	Severe sepsis	190	0	369	–1	2,465	0
Staphylococcus epidermidis	S	Sepsis	1,895	0	875	1	9,061	1
Staphylococcus epidermidis	S	Sepsis	>15,625	0	421	0	7,877	0
Staphylococcus epidermidis	S	Sepsis	684	1	209	0	17,420	0
Staphylococcus epidermidis	U	Sepsis	145	0	169	–1	2,415	–1
Staphylococcus haemolyticus	U	Severe sepsis	101	0	138	0	1,529	0
Staphylococcus aureus	S	Shock	7,020	1	2,816	1	54,420	0
Staphylococcus aureus	S	Shock	<98	0	1,614	0	6,746	0
Staphylococcus hominis	S	Sepsis[a]	615	0	276	0	31,062	0
Staphylococcus warneri	S	Severe sepsis	>15,625	1	>12,500	1	>100,000	0
Streptococcus sanguis	U	Shock	<98	0	229	–1	2,711	–1
Streptococcus pneumoniae	S	Sepsis	1,468	1	<78	1	32,820	0
Streptococcus pneumoniae	S	MOF[a]	1,763	1	1,144	1	>100,000	1
Enterococcus fecalis	S	Sepsis	366	–1	1,591	0	10,820	0
Enterococcus fecalis	S	Shock	>15,625	0	>12,500	0	>100,000	0
Unidentified Gram-positive anaerobe bacilli	S	Shock	5,998	–1	119	–1	5,441	–1
Enterobacter cloacae	S	Severe sepsis	14,869	–1	1,478	–1	14,885	–1
Enterobacter cloacae	S	Severe sepsis	>15,625	–1	2,399	–1	>100,000	–1
Fusobacterium nucleatum	S	Sepsis	4,640	0	1,276	0	31,200	0
Haemophilus influenzae	S	Sepsis	12,650	0	1,008	0	22,530	0
Klebsiella oxytoca	S	Sepsis	3,101	0	3,918	1	3,783	0
Klebsiella pneumoniae	S	Sepsis	>15,625	1	1,214	1	9,700	2
Neisseria meningitidis	S	Shock	6,933	0	5,131	0	62,390	0
Neisseria meningitidis	S	Sepsis	5,425	0	155	0	56,400	0
Serratia marcescens	S	MOF[a]	>15,625	0	>12,500	–1	>100,000	0
Serratia marcescens	S	Severe sepsis	259	0	1,493	–1	9,148	0
Serratia marcescens	S	Severe sepsis	288	–1	1,030	–1	15,040	0
Unidentified Gram-negative pathogens	S	Shock	>15,625	1	>12,500	1	>100,000	1

[a] Shock preexistent (e.g., compromised circulation after cardiac surgery)

All analyses were repeated using the broadened case definition (culture-confirmed sepsis plus probable sepsis vs. no infection). Figure 3 presents the corresponding receiver operating characteristic curve (combined model IL-8 and G-CSF AUC=0.86). In all analyses IL-8 and G-CSF outperformed IL-1ra (data not shown).

Early diagnosis

An important clinical question is an early diagnosis up to 24 h prior to the documented clinical suspicion [11]. Samples from the calendar day preceding the day of suspicion of infection were available in 82 episodes. Of these, 15 later progressed to *culture-positive sepsis*. G-CSF levels were elevated (>950 pg/ml) in 7 episodes (47%, 95% CI 21–73%), and IL-8 and IL-1ra levels (>12000 pg/ml [11]) were elevated (>340 pg/ml) in 6 episodes (40%, 95% CI 16–68%). In 41 control episodes G-CSF remained lower than 950 pg/ml in 40 (97%); and IL-8 remained lower than 340 pg/ml in 39 (95%) and IL-1ra lower than 12000 pg/ml in 37 episodes (93%).

Discussion

The primary aim of this study was to elucidate the diagnostic accuracy of G-GSF, IL-8, and IL-1ra in distinguishing critically ill infants and children with sepsis from those who are suspected to have bacterial infection. Unlike other studies with selected patient populations, we enrolled a heterogeneous cohort of medical and surgical pediatric intensive care patients. In our dataset including 254 episodes of suspected bacterial infection,

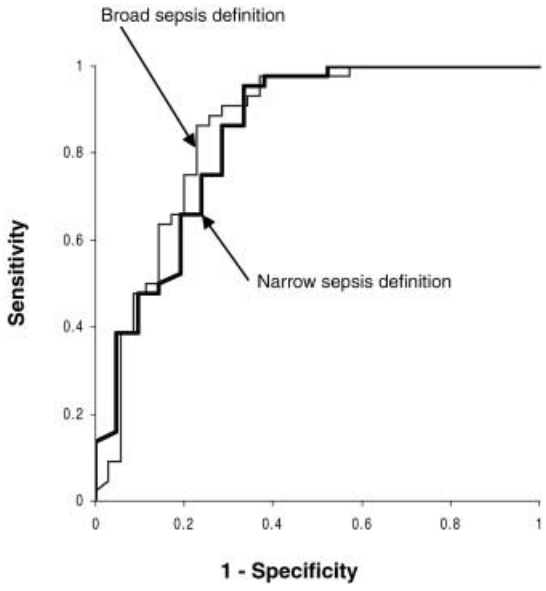

Fig. 3 Receiver operating characteristic curve for the model combining results from G-CSF and IL-8. *Broad line* Curve for culture-confirmed sepsis compared to controls (narrow sepsis definition, area under the curve AUC=0.84); *thin line* curve using the broadened sepsis definition (culture-confirmed sepsis plus probable sepsis, AUC=0.86)

plasma levels of IL-8 and G-CSF yielded high discriminative accuracy, which is similar to the data reported on newborns [12]. Second, in contrast to the favorable results reported for IL-1ra on premature infants [11], the diagnostic performance of IL-1ra measurements was low. This was attributable to a low specificity particularly in patients after surgery or during the first postnatal days. Third, combining IL-8 and G-CSF relevantly increased the predictive accuracy, particularly with regard to not missing patients with Gram-negative sepsis. Fourth, the model discriminated well between patients with sepsis and patients with localized infection or a noninfectious inflammatory response. Fifth, we confirmed earlier findings [11] that cytokine measurement allowed advancing the diagnosis of sepsis in a considerable proportion of patients. Finally, we showed that the parameters are unsuited to diagnosing bacteremia from *S. epidermidis*.

Our study differs from others in the field in several ways. First, we used only patients as controls in whom clinicians suspected bacterial infection. This reduces the possible bias arising from overestimating test accuracy by selection of patients as controls [28] who were a priori distinguishable from cases. The finding that clinicians prescribed antibiotics in almost all of our control patients underscores the physicians' uncertainty to rule in or rule out bacterial infection from currently available diagnostic tools. Moreover we performed two independent analyses using a narrow and a broad definition of sepsis cases. The two analyses yielded similar results.

Synthesizing the data from this study, the following practical conclusions may be drawn: If a clinician obtains elevated plasma levels for IL-8 (>340 pg/ml) or G-CSF (>950 pg/ml) in a patient with minimal symptoms otherwise not warranting immediate initiation of antibiotic treatment, the patient is highly likely to progress to sepsis within the next 24 h. However, negative results do not rule out infection. If diagnostic testing is employed at a later stage during clinical course, elevated levels of either of the cytokines are suggestive of sepsis and warrant initiation of sepsis directed therapy. When negative results are obtained, localized infections without generalized inflammatory response (e.g., ventilator associated pneumonia or abscess) are neither confirmed nor ruled out, a finding consistent with the literature [29, 30]. Negative results do also not rule out bacteremia with *S. epidermidis*. Thus, if both IL-8 and G-CSF are low and localized processes are unlikely, adopting a watchful approach awaiting further evidence (e.g., results from cultures) may be justified [31]. Unlike IL-1ra levels, plasma levels of IL-8 and G-CSF remained negative in surgical patients with postoperative fever who did not have infection. This suggests a propensity for IL-8 and G-CSF to differentiate between sepsis and other causes of symptoms in the postoperative care of sick patients.

A limitation of the study was the small amounts of plasma available, which hindered additional determination of IL-6 [32] or procalcitonin [28]. Further potential caveats arise from the absence of an undisputed and externally quantifiable gold standard for the diagnosis of sepsis in all pediatric and neonatal patients. The case-control design using patients with *culture-confirmed sepsis* as cases may overestimate the tests' accuracy [33]. The broadened sepsis definition including episodes of *probable sepsis* was based on outcome adjudication and, therefore, prone to misclassification error. This error may have affected the second analysis towards underestimating test accuracy. However, it is unlikely that the potential bias affected the parameters differentially. Therefore the comparison of the three tests remains valid even though residual bias cannot be ruled out. The third limitation is that we were unable to identify patients with viral sepsis. For this reason we excluded viral episodes in the analysis. While IL-8 and G-CSF levels identify more patients with sepsis than blood cultures alone, caution should also be exerted when basing treatment decisions on negative test results. The main application of this diagnostic tool is for sepsis workup during the early course of disease. Clinicians should bear in mind that none of these tests provide perfect information. They assist, but do not replace clinical reasoning. Finally, it should be borne in mind that we did not test the clinical performance of our model in a data-set independent of the derivation data-set or in another study population. Thus our algorithm should be regarded as "hypothesis generating." It needs to be validated by independent studies.

In summary, we observed that measuring plasma levels of IL-8 and G-CSF but not IL-1ra is helpful for the diagnosis of bacterial sepsis in a heterogeneous cohort of critically ill newborns and children. A combined prediction model using IL-8 and G-CSF levels improved the diagnostic accuracy over the determination of a single parameter.

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