Neutrophil CD11b Expression and Circulating Interleukin-8 as Diagnostic Markers for Early-Onset Neonatal Sepsis

Irmeli Nupponen, MD*‡; Sture Andersson, MD, PhD*§; Anna-Liisa Järvenpää, MD, PhD*;
Hannu Kautiainen, BA‖; and Heikki Repo, MD, PhD‡¶

ABSTRACT. *Objective.* **To assess neutrophil CD11b and circulating interleukin 8 (IL-8) as markers of early-onset infection in neonates.**

Methods. **The study comprised 39 neonates, with a gestational age of 29 to 41 weeks, suspected of infection within 48 hours of life. Neutrophil surface expression of CD11b was quantified with flow cytometry and plasma IL-8 with an enzyme-linked immunosorbent assay. Both data were available from 35 of 39 neonates. Serum C-reactive protein was determined at initial evaluation and, later, on the basis of the clinical picture. Neonates were allocated retrospectively into 2 groups. In the sepsis group ($N = 22$), 4 had culture-proven sepsis, and 14 had an antenatal risk factor for infection. In the possible-infection group ($N = 13$), each neonate had a noninfective disorder, but co-occurring infection remained a possibility. Twelve healthy term infants served as controls.**

Results. **CD11b expression and IL-8 levels both increased in order of sepsis > possible infection > healthy. Sensitivity and specificity by the CD11b test for sepsis were equal, at 1.00, and those by the IL-8 test 0.91 and 1.00, respectively; 6 (17.1%) of the 35 neonates had CD11b and IL-8 below cutoff levels.**

Conclusions. **Measuring neutrophil CD11b expression and circulating IL-8 provides a means to identify early-onset neonatal sepsis. The findings may be helpful in planning strategies to safely reduce the use of antimicrobials in neonates.** *Pediatrics* **2001;108(1). URL: http://www.pediatrics.org/cgi/content/full/108/1/e12;** *circulating IL-8, C-reactive protein, early-onset sepsis, neutrophil CD11b expression, newborn infant.*

ABBREVIATIONS. IL, interleukin; CRP, C-reactive protein; RFU, relative fluorescence unit; CI, confidence interval; ROC, receiver-operating characteristic curve.

Neonatal sepsis is a life-threatening disease with an incidence of 1 to 10 per 1000 live-births, and a mortality rate of 15% to 50%.[1] The clinical signs are nonspecific and indistinguishable from those caused by a variety of neonatal non-infective disorders, such as aspiration syndrome, maladaptation, and respiratory distress syndrome. It is therefore recommended for all neonates who develop these signs to start empirical antimicrobial therapy.[2,3] This clinical practice, however, renders many neonates unduly susceptible to side effects of antimicrobial agents, increases hospital costs, and promotes the development and spread in hospitals of resistant bacterial strains.[4] Therefore, markers are needed that reliably identify truly infected neonates.

Invading microbes activate the host's innate immune cells,[5] including neutrophils and monocytes. Activated phagocytes produce inflammatory cytokines such as tumor necrosis factor-α, interleukin (IL)-1β, and IL-8. The release of these mediators into the circulation results in the development of systemic inflammation in adults[6] and children.[7] Systemic inflammation is considered to play an important role in the development of organ failure,[8] the major cause of mortality in sepsis. Elevated blood levels of IL-8 predict organ failure in adults with septic shock,[9] occur in patients with unresolving acute respiratory distress syndrome,[10] and serve as a marker of neonatal sepsis.[11,12]

IL-8 is a strong neutrophil-activating agent.[13] On activation, the cell-surface expression of CD11b/CD18 (Mac-1, $\alpha_M\beta_2$, CR3), a β_2-integrin constitutively expressed at low levels on resting neutrophils and monocytes, is enhanced.[14,15] This increase in CD11b/CD18 expression occurs in adults with sepsis,[9,16] and as demonstrated recently, in neonates with early-onset sepsis.[17] IL-8[12] and CD11b expression[17] both proved to be superior to C-reactive protein (CRP), an acute phase reactant, in detection of neonatal sepsis.

Because the clinical signs of neonatal sepsis are nonspecific, we reasoned that, as in adults,[18] an evaluation of systemic inflammatory status in neonates by using humoral and cellular markers of systemic inflammation might be helpful in deciding whether the clinical signs are the result of infection or a noninfective insult. To address this question, we studied prospectively neutrophil CD11b expression and plasma IL-8 concentration in neonates suspected of infection during their first 48 hours of life.

METHODS

Participants

The study was conducted between January 1998 and June 1999, at the Hospital for Children and Adolescents and the Department of Obstetrics and Gynecology, University of Helsinki, Finland. The

study protocol was approved by the institutional review boards, and informed consent was obtained from the parents. The series consisted of 39 neonates, with a gestational age of 29 to 41 weeks, who were treated in the neonatal unit for suspected infection. Inclusion criteria were the presence of at least 1 clinical sign suggesting infection at the age of 0 to 48 hours, and a blood sample for bacterial culture having been requested by the clinician. Clinical signs were divided into 5 categories: 1) temperature instability (hypothermia, hyperthermia); 2) respiratory distress (grunting, intercostal retractions, apnea, cyanosis); 3) cardiovascular (tachycardia, bradycardia, poor perfusion, shock); 4) neurologic (hypotonia, lethargy); and 5) gastrointestinal (feeding intolerance, abdominal distension). The symptoms were recorded by the nursing staff members in the neonatal unit. Blood leukocyte count, platelet count, and serum CRP concentration were determined at the request of the clinicians at the initial evaluation, and also during the subsequent 3 days, on the basis of the clinical picture.

The neonates were assigned retrospectively to 2 groups, a sepsis group and a possible-infection group (Table 1), with special emphasis placed on the presence of noninfective disorders such as aspiration syndrome, respiratory distress syndrome, or maladaptation, which might have accounted for both the clinical symptoms and increases in CRP levels even in the absence of infection.

The sepsis group (N = 22) comprised 4 neonates with positive blood culture and 18 with negative blood culture. Of these 18, 12 had maternal risk factor(s) for infection, and 14 had increased peak CRP concentration (>10 mg/L). Of note, all infants with clinical symptoms and maternal risk factor(s) did not necessarily have septic infection. In clinical medicine, however, such infants are initially considered to have sepsis. To develop criteria that are relevant to and simple enough for clinical practice, we assigned infants with maternal risk factor(s) into the sepsis group.

The possible-infection group comprised 13 neonates, each of whom had a noninfective disorder. Their peak CRP level was <10 mg/L in 4 neonates and elevated (range: 10–44) in 9 neonates. In clinical medicine, for such neonates it is difficult or impossible to exclude with certainty the presence of co-occurring infections.

Finally, 12 healthy term neonates who were born after uncomplicated pregnancy and delivery, with physiologic hyperbilirubinemia at the age of 24 to 120 hours, but who did not require phototherapy and who had normal CRP levels, served as controls (Table 1).

Blood Samples

From each neonate with suspected infection, 1 blood sample of 1000 μL to 1500 μL was taken by venipuncture for bacterial culture and for CD11b and IL-8 determinations. In 32 of 39 neonates, the sample was collected within the first 24 hours and in 7 neonates between 24 and 48 hours of life. All of these samples were drawn before the first doses of antimicrobials. A similar blood sample was also obtained from each of 12 healthy term neonates concurrently with the clinical sample for serum bilirubin measurement. Each blood sample was placed into a pyrogen-free tube containing citrate phosphate dextrose (Baxter Health Care Ltd, Norfolk, England; 0.14 mL/mL blood) and cooled immediately to 0°C in an ice-water bath to minimize neutrophil activation ex vivo.[19]

Determination of CD11b Expression by Flow Cytometry

Neutrophil CD11b expression was assessed as described previously.[19,20] The blood sample, kept at 0°C maximally for 24 hours, was processed for flow cytometry by one of the authors (I.N.) who knew neither clinical nor laboratory findings of the neonate. Neutrophils in 25-μL aliquots of whole blood were labeled by use of saturating concentrations of the R-phycoerythrin conjugate of mouse anti-CD11b monoclonal antibody (IgG1, clone 2LPM19c) or the corresponding control antibody (IgG1-RPE, clone DAK-GO1), both purchased from Dako (Glostrup, Denmark). After labeling, 2 mL of a 1/10 diluted ice-cold FACS lysing solution (Becton Dickinson, San Jose, CA) were added to each tube to remove most of the unbound antibody. After a 3-minute incubation at 0°C, the cells were collected by centrifugation at 4°C. Because most red cells remain unlysed in the cold, the cell pellet was resuspended in 2 mL of FACS lysing solution and further incubated for 5 minutes, all at room temperature. After centrifugation, leukocytes were resuspended in 1% formalin at 0°C. A FACScan flow cytometer and CellQuest analysis software (both from Becton Dickinson) were used for the acquisition and analysis of the data. Neutrophils were identified on the basis of their light-scattering properties. For each sample, 10 000 events were recorded. CD11b expression is reported in relative fluorescence units (RFU), ie, as the mean channel of the positive fluorescing cell population.

Determination of IL-8

Plasma was separated by centrifugation and then stored in aliquots at −70°C until analysis. IL-8 enzyme-linked immunosorbent assay kit (Quantikine, R&D Systems, Minneapolis, MN) was used with the plasma samples in a blinded fashion. The detection limit of the assay, as indicated by the manufacturer, was 10 pg/mL. All samples were run in duplicate.

CRP Determination

Plasma CRP concentrations were measured immunoturbidimetrically (detection limit: 5 mg/L). Any levels greater than 10 mg/L were defined as abnormal.

Data Analysis

Four neonates were excluded from the data analysis because either their plasma IL-8 concentration or neutrophil CD11b expression value was missing. Statistical comparison between the groups (sepsis group, possible-infection group, and neonate control group) was performed with the Jonckheere-Terpstra test for ordered alternatives;[21] P values were calculated by the Monte-Carlo method. Sensitivity and specificity, and their 95% confidence interval (CI) values were calculated for CD11b expression, IL8, and CRP. Receiver-operating characteristic (ROC) curves were used for the determination of thresholds for the sepsis group versus healthy neonate group. The relationship between CD11b and IL-8 was determined with the Spearman rank correlation test. The correlation coefficient (r) and its 95% CI are presented.

RESULTS

The clinical characteristics of the patients are presented in Table 2. The sepsis group comprised 22

TABLE 1. Criteria for Classification of Neonates

Sepsis Group		Possible-Infection Group	Healthy Term Neonates
Group A	Group B		
Symptoms* present Blood-culture positive	Symptoms* present Blood-culture negative Peak CRP ≥10 mg/L or antenatal risk factor(s)† for infection No noninfective disorder‡	Symptoms* present Blood culture negative Noninfective disorder‡	Physiologic hyperbilirubinemia No antenatal risk factors† for infection

* Symptom categories: body temperature (hypo- or hyperthermia), respiratory (grunting, intercostal retractions, apnea, cyanosis), cardiovascular (tachycardia, bradycardia, poor perfusion, shock), neurologic (hypotonia, lethargy), gastrointestinal (feeding intolerance, distension).
† Antenatal risk factors for infection: preterm (>24 hours) rupture of membranes, early onset of labor, maternal fever.
‡ Noninfective disorders: respiratory distress syndrome, tachypnea transitoria neonatorum, aspiration syndrome, anemia, autoimmune thrombocytopenia, interrupted aortic arch, large for gestation with maladaptation, pulmonary hypoplasia.

TABLE 2. Clinical Characteristics of Participant Groups

	Sepsis	Possible Infection	Healthy Neonates
Number of participants	22	13	12
Gestational age, wk, mean (SD)	37.6 (3.8)	37.4 (3.0)	37.7 (1.8)
Birth weight, g, mean (SD)	2979 (1007)	3150 (874)	2990 (558)

SD indicates standard deviation.

neonates, the possible-infection group 13 neonates, and the control group 12 healthy term neonates. Four neonates were blood-culture positive, 2 for group B streptococci, 1 for *Escherichia coli*, and 1 for coagulase-negative staphylococci. There were no significant differences in mean gestational age or birth weight between the groups. All the infants with suspected infection received ampicillin 200 mg/kg/d and nethilmicin 6 mg/kg/d for 3 to 8 days. One of the 4 infants excluded from the data analysis died of *Streptococcus agalactiae* sepsis at the age of 72 hours. The noninfective diagnoses of the infants in the possible-infection group were respiratory distress syndrome, maladaptation, anemia, interrupted aortic arch, and aspiration syndrome. The clinical symptoms of these infants resolved consistently within 3 days.

The relationship between neutrophil CD11b expression and plasma IL-8 is presented in Fig 1. CD11b expression levels of neutrophils correlated positively with plasma concentrations of IL-8 (Fig 1, $r = 0.82$, [95% CI 0.70–0.90]). There were no associations between birth weight or gestational age and the levels of IL-8, CD11b expression, or CRP. Visual analysis of the data indicated that the values of 12 healthy control neonates fell in the lower left quadrant, whereas the values of 19 of the 22 neonates with sepsis fell in the upper right quadrant. Of the 3 septic neonates outside the upper right quadrant, the first

had a high CD11b expression level (246 RFU), low IL-8 level (17 pg/mL), and high CRP level (84 mg/L); with no risk factors for infection. The second had marginally elevated levels of CD11b (176 RFU) and IL-8 (51 pg/mL); the mother had been treated with antimicrobial chemotherapy for clinical chorioamnionitis. The third had a CD11b expression of 178 RFU and IL-8 concentration of 24 pg/mL; this mother had fever and symptoms of acute respiratory infection at delivery.

The majority of the values for the possible-infection group fell in the lower left quadrant and none in the right upper quadrant. The highest level of CD11b expression (204 RFU) and IL-8 (92 pg/mL) in this group occurred in neonates whose mothers had clinical chorioamnionitis and were treated with antimicrobials. The 3 neonates with the lowest CD11b expression levels (86, 93, and 97 RFU) had diagnoses of anemia, maladaptation, and transitory tachypnea, respectively, but had no risk factors for infection. Their clinical signs resolved within 24 hours.

In the sepsis group, 4 neonates had on admission high CD11b expression levels (211, 255, 303, and 365 RFU, respectively); increased IL-8 levels (126, 200, 62, and 107 pg/mL, respectively); and low CRP levels, which did not increase during the follow-up of 3 to 4 days (range of the peak levels: 5–8 mg/L). Their clinical signs persisted for over 24 hours, and antimicrobial treatment was continued for 7 days or more.

The results in Table 3 show that CD11b expression levels, plasma IL-8 concentrations, and peak CRP concentrations all increased in the order: sepsis group > possible-infection group > healthy neonates (P for monotonic trend <0.001). Peak CRP correlated with CD11b expression ($r = 0.62$, [95% CI: 0.38–0.78]) and with IL-8 ($r = 0.58$, [95% CI: 0.33–0.76]).

Fig 1. Correlation between neutrophil CD11b expression level and IL-8 concentration. Dotted lines denote median values for the whole study population. RFU indicates relative fluorescence unit.
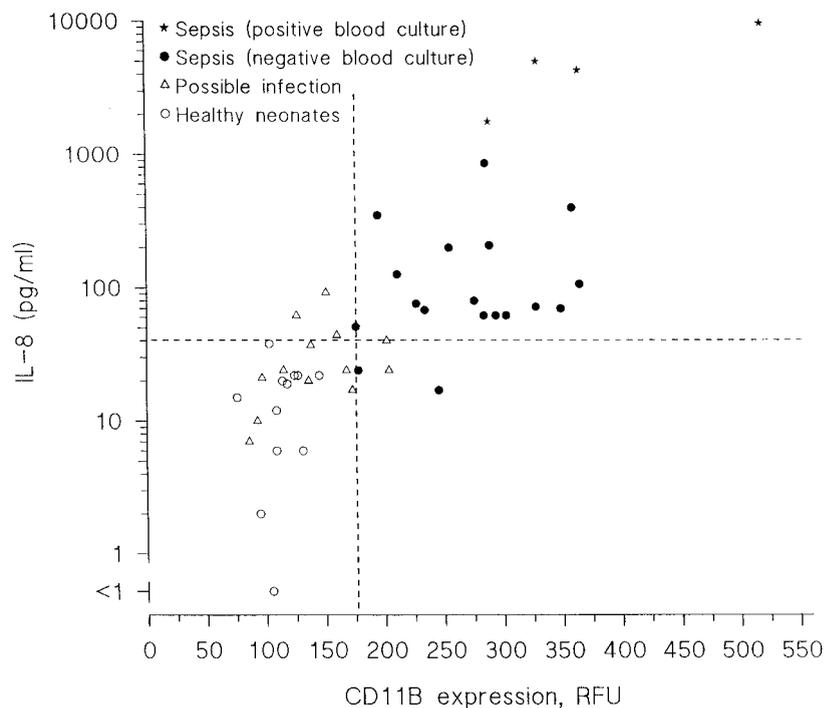
TABLE 3. Neutrophil CD11b Expression Levels, Plasma IL-8 Concentrations, and Serum CRP Concentrations

Marker	Sepsis N = 22 Median (Range)	Possible Infection N = 13 Median (Range)	Healthy Neonates N = 12 Median (Range)	P* Value Between Groups
CD11b (RFU)	288 (176–519)	138 (86–204)	112 (76–145)	<.001
IL-8 (pg/mL)	94 (17–9576)	24 (7–200)	17 (0.4–38)	<.001
Peak CRP (mg/L)	48 (5–188)	11 (5–44)	6 (5–8)	.002

* Jonckheere-Terpstra test for ordered alternatives.

ROC analysis served for the determination of the best threshold for sepsis group versus healthy neonates. CD11b expression, IL-8 concentration, and the peak CRP concentration had sensitivities of 1.00, 0.91, and 0.82, respectively, and each marker had a specificity of 1.00 (Table 4).

DISCUSSION

The results show that in newborn infants, peripheral blood neutrophil CD11b expression and circulating levels of IL-8 can serve as promising markers of early-onset sepsis. Our findings confirm the results of Berner et al[11] and Weirich et al,[17] reported while the present study was in progress, and extend them to show that the markers provide a means to distinguish infected infants with systemic inflammation from the symptomatic infants who have a noninfective disorder without systemic inflammation. Although Weirich et al[17] excluded from their study infants with noninfective disorders, we focused on such infants because discrimination between sepsis and a noninfective disorder, both result in identical clinical signs, is crucial in clinical decision-making.

The important question is whether neutrophil CD11b expression and circulating IL-8 serve as markers of systemic inflammation in neonates. The neonate's innate immune system is known to be immature.[22] The total cellular content of CD11b/CD18 complexes is lower in neonates than in adults.[23,24] In resting neutrophils from adults, 95% of the total cell content of CD11b/CD18 complexes occurs as membrane components of specific granules and secretory vesicles, ie, in intracellular storage granules, with only 5% of the complexes expressed on the cell surface.[25] Secretory vesicles are formed at a late stage of neutrophil maturation.[26] Their exocytosis occurs readily ex vivo and in vivo and is controlled by multiple intracellular signaling mechanisms.[27] When stimulated in vitro with FMLP, a neutrophil agonist, neutrophils from neonates show a reduced ability to enhance CD11b expression.[28–32] The results in the present study and the previous study[17] show, on the first day of life, a two- to fourfold increase in neutrophil CD11b expression in infants with blood-culture positive sepsis. This increase is similar to that demonstrated in neutrophils from adults with blood-culture positive sepsis.[9] Moreover, production of the cytokines like IL-6 by neonate monocytes is depressed in vitro,[33] denoting functional immaturity of the innate immune system. Yet, high circulating levels of IL-6[22,34] and IL-8[11,12] do occur in septic neonates. In concert, these findings support the view that CD11b and IL-8 serve as markers of systemic inflammation also in neonates.

An additional question of importance is whether CD11b expression and IL-8 are related to severity of systemic inflammation. Enhanced CD11b expression serves as an activation marker of neutrophils,[35–37] occurs in adult patients with infection[9,16,38] or noninfective disorders,[39–41] is related to severity of the systemic inflammatory response syndrome in acutely ill adults,[18] and may predict the development of organ failure in patients with cirrhosis of the liver[42] and septic shock.[9] In the present study, the infant with the highest level of CD11b expression (570 RFU), who was excluded from the analyses because of missing IL-8 data, died of streptococcal sepsis at the age of 3 days. In adults with sepsis, plasma levels of IL-8 are higher in nonsurvivors than in survivors,[9] and in the study of Franz et al,[43] a very premature infant who succumbed to a blood culture-proven sepsis had extremely high levels of circulating IL-8, greater than 10 000 pg/mL. In concert, the findings above suggest that CD11b and IL-8 are related to severity of systemic inflammation.

Of the 18 infants assigned to the sepsis group, 4 had low peak CRP levels in serial measurements, whereas the levels of neutrophil CD11b expression and circulating IL-8 were high. We have no explanation for the discrepancy. Each of the 4 had risk factor(s) for infection, clinical symptom(s), and received antimicrobials for at least 1 week. Serial serum CRP levels are highly sensitive for sepsis,[44,45] suggesting that the infants did not have sepsis. Although it is possible that they had some noninfective disorder that promoted phagocyte activation, no such disorder was found. Consequently, the relationship between CRP and the novel markers CD11b and IL-8 in neonates with clinical symptoms needs to be studied further.

Our neonates in the possible-infection group had marginally elevated levels of neutrophil CD11b expression or of circulating IL-8, or both, indicating that they had mild systemic inflammation. Several noninfective events can trigger mild systemic inflammation, as determined by enhanced neutrophil CD11b expression.[18] The mild systemic inflammatory response observed may have had its origin in the noninfective insult, cryptic infection at its early

TABLE 4. Cutoff Points, Sensitivity, and Specificity of the Markers in Diagnosis of Sepsis

Marker	Cutoff Point	Sensitivity (95% CI)	Specificity (95% CI)
CD11b (RFU)	≥150	1.00 (0.87 to 1.00)	1.00 (CI 0.78 to 1.00)
IL-8 (pg/mL)	≥50	0.91 (0.73 to 0.98)	1.00 (0.78 to 1.00)
Peak CRP (mg/L)	>10	0.82 (CI 0.62 to 0.94)	1.00 (CI 0.78 to 1.00)

stage, or in both. We hypothesize that in such infants it is safe to stop antimicrobials. Subsequently, the infants should be observed by watching their clinical signs and their systemic inflammatory status, ie, levels of CD11b expression and IL-8. In addition, such infants should be examined extremely carefully for the presence of noninfective disorders.

Here, neutrophil CD11b expression level correlated positively with IL-8 concentration, and the sensitivity and specificity of the 2 assays were comparable. The CD11b assay requires small sample volumes—an advantage in the study of preterm neonates, can be used as a routine clinical test,[20,46] and provides results within 30 to 60 minutes after sampling. IL-8 and CD11b are both superior to CRP in the detection of systemic inflammation at its early stage, as shown by the results in the present study and multiple previous studies.[11,12,17,43] CRP may, however, serve as a criterion for discontinuation of antibiotic therapy, and when used as the guide to treatment, can decrease antimicrobial consumption, at least in some clinical settings.[47] Recently, IL-8 was found to reduce in a cost-effective manner unnecessary antibiotic therapy for nosocomial bacterial infection in newborn infants by 73%.[43] In the present study, 6/35 (17.1%) neonates with clinical symptoms had no systemic inflammation, and several other neonates in the possible-infection group might have avoided antimicrobial therapy had there been monitoring of the course of their systemic inflammation.

CONCLUSION

Measuring neutrophil CD11b expression and circulating IL-8, markers of systemic inflammation, provides the means to identify early-onset neonatal sepsis. The findings may be helpful in planning strategies safely to diminish the use of antimicrobials in neonates with clinical signs of early-onset sepsis.

ACKNOWLEDGMENTS

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