Evaluation of IL-8-Concentrations in Plasma and Lysed EDTA-Blood in Healthy Neonates and Those with Suspected Early Onset Bacterial Infection

THORSTEN W. ORLIKOWSKY, FELIX NEUNHOEFFER, RANGMAR GOELZ, MARTIN EICHNER, CHRISTINE HENKEL, MANFRED ZWIRNER, AND CHRISTIAN F. POETS

Department of Neonatology [T.W.O., F.N., R.G., C.F.P.], University Children's Hospital, 72076 Tübingen, Germany; Department of Medical Biometry [M.E.], 72070 Tübingen, Germany; and University Hospital of Obstetrics and Gynecology [C.H., M.Z.], 72076 Tübingen, Germany

ABSTRACT

Plasma IL-8 is a diagnostic parameter of early-onset bacterial infection (EOBI) in neonates but has a short half-life. The detergent-lysed whole-blood (DLWB) IL-8 consists of both extracellular and cell-bound IL-8. The objective of this study was to investigate kinetics of plasma and DLWB IL-8 in healthy newborns and those with suspected EOBI and to test the hypothesis that determination of DLWB IL-8 results in higher sensitivity for EOBI detection. Sixty-one neonates with clinical and serologic signs of EOBI composed the study group; 188 neonates with risk factors but without EOBI served as control subjects. IL-8 concentrations were determined in plasma and DLWB. In the control group, DLWB IL-8 concentrations were 280-fold higher (9599 pg/mL; SD 4433) up to 24 h post partum than corresponding plasma levels (34.2 pg/mL; SD 18.1). The sensitivity of DLWB *versus* plasma IL-8 for EOBI was 0.97 *versus* 0.71 after 6 h and 0.70 *versus* 0.32 after 24 h. Corresponding

values for specificity were 0.95 *versus* 0.90 after 6 h and 0.92 *versus* 0.99 after 24 h. After 24 h, the negative predictive value for DLWB *versus* plasma IL-8 was 0.80 *versus* 0.66. DLWB IL-8 showed a higher sensitivity for EOBI within 6 h after first clinical suspicion than plasma IL-8. It also remained elevated longer. Our results suggest that DLWB IL-8 results in a higher sensitivity for EOBI. (*Pediatr Res* 56: 804–809, 2004)

Abbreviations

CRP, C-reactive protein
DLWB, detergent-lysed whole blood
EOBI, early-onset bacterial infection
I/T ratio, immature to total neutrophil ratio
NPV, negative predictive value
PPV, positive predictive value
ROC, receiver operator characteristics

Neonates are susceptible to infections (1). The differential diagnosis of early-onset bacterial infection (EOBI) therefore must always be present for the neonatologist, regardless of how minor, unexpected, or discrete the clinical symptoms. EOBI is usually defined as occurring up to 72 h after birth (2) and is associated with a high morbidity and mortality risk (1). The nonspecific clinical signs as well as the currently established biochemical and hematologic parameters have their diagnostic limitations (3,4) [reviewed in (5,6)].

Plasma IL-8 is an appreciated, highly predictive, and easily accessible chemokine to detect EOBI (7). IL-8 secretion is not limited to infections (8–10), yet it occurs within 1–3 h of endotoxin challenge (11). As with most cytokines, its plasma

half-life is short (<4 h) (12,13). Circulating IL-8, which can be detected in plasma or serum *via* immunoassay, is immediately bound to two distinct high-affinity IL-8 receptors that are abundantly present on neutrophils before internalization and degradation (14,15). Therefore, plasma IL-8 reflects only a small portion of the total IL-8 blood pool, because the majority is cell associated (16–18).

Cell association is enhanced by chemokine binding, non-IL-8-specific receptors. These have been identified on various cell types (19), including the Duffy antigen-related chemokine receptors (16), presented on erythrocytes. Duffy antigen-related chemokine-ligated IL-8 is biologically inactive to neutrophils but has the ability to recirculate, because the receptor may be ligated by other cytokines (16,17) or pathogens (20,21).

Cell-associated IL-8 can be determined by lysing red blood cells with a detergent. Data from adult patients with sepsis

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Correspondence: Thorsten W. Orlikowsky, M.D., Univ.-Kinderklinik Tübingen, Calwerstrasse 7, 72076 Tübingen, Germany; e-mail: thorsten.orlikowsky@med.uni-tuebingen.de

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suggested that detergent-lysed whole blood (DLWB) IL-8; *i.e.* cell-bound and extracellular IL-8, obtained by cell lysis, may provide additional information for the assessment of bacterial infection (21,22). Therefore, the objective of this study was to evaluate IL-8 kinetics in newborns with and without EOBI in DLWB and plasma. We speculated that DLWB IL-8 may have a higher sensitivity and specificity for EOBI detection than plasma IL-8.

METHODS

Patients. Neonates who were admitted consecutively and met inclusion criteria for the study were enrolled with institutional ethics committee approval and parental consent. For determination of the reference range of DLWB IL-8, blood was collected from neonates who had risk factors for EOBI but no clinical signs or subsequent laboratory changes and underwent blood testing. To exclude EOBI, blood was drawn at 6, 24, and 48–60 h post partum in addition to close observation by experienced neonatologists at least three times per day. In addition, blood was collected from neonates with EOBI as defined below. IL-8 blood samples were obtained before and after antibiotic treatment.

All blood samples were processed and analyzed within 2 h. A prerequisite was a nonhemolytic sample, which mostly was obtained by venipuncture. Blood specimens that were macroscopically hemolytic or processed after >2 h were excluded.

Definition of bacterial infection. A diagnosis of EOBI was based on the presence of at least two of the following criteria within the first 72 h of life (23): one or more clinical signs compatible with EOBI, plus a consecutive elevation of CRP >10 mg/L within 24 h after first clinical suspicion, or positive blood culture results. On the basis of previous studies (23), clinical signs were defined as follows: fever ($\geq 37.8^{\circ}\text{C}$ rectal), hypothermia ($\leq 36.5^{\circ}\text{C}$), temperature instability ($\geq 1.5^{\circ}\text{C}$), pallor, grayish skin color, poor perfusion (capillary refill >2 s), tachypnea (>60 respirations per minute at rest), dyspnea (grunting, nasal flaring, retractions), respiratory insufficiency, apnea, rising fraction of inspired oxygen in previously stable neonate, arterial hypotension (mean arterial blood pressure <37 mm Hg), muscular hypotonia, irritability, hyperexcitability, neck stiffness, and lethargy.

The CRP cutoff has been used for clinical purposes in our institution for many years according to previous investigations (24). Neonates who did not meet criteria for culture-proven or clinical infection were considered noninfected.

Workup program for suspected EOBI. Indications for close clinical observation and our blood screening program were one or a combination of the following criteria: history of amniotic infection, maternal leukocytosis ($>12,000$ granulocytes/ mm^3), and/or maternal CRP elevation to >10 mg/L after exclusion of infectious foci unrelated to the fetus (gastrointestinal or urinary tract infections); fetal tachycardia (>160 beats/min); prolonged rupture of membranes (≥ 12 h) in the absence of labor; maternal fever (rectal temperature $\geq 38.0^{\circ}\text{C}$); and foul-smelling amniotic fluid. Growth of group B streptococci in vaginal smear was routinely screened in case of prolonged rupture of membrane.

Sample processing and detection of IL-8. Plasma IL-8 concentrations were detected in lithium-heparin-coated tubes. Results were available after 50 min. Twenty-five microliters of plasma was diluted with 100 μL (1:5) sample diluent (I8; DPC Biermann, Bad Nauheim, Germany). The detergent concentration had previously been optimized to lyse blood cells without affecting the binding of the analyte to the assay antibody (17) (Immulite; DPC Biermann). The detection limit of plasma IL-8 was 2 pg/mL (standardized in accordance with the National Institute for Biologic Standards and Controls Reference Preparation 89/520) and was calibrated to 7500 pg/mL. Inter- and intra-assay variations for plasma and DLWB IL-8 were <5% at 100 pg/mL and 15,000 pg/mL, respectively. According to an analysis using receiver operator characteristics (ROC) curves, the threshold for IL-8 plasma concentration was set to 60 pg/mL.

Native EDTA-blood, obtained for routine hematologic workup, served as a source for DLWB IL-8. For a single determination, a total volume of 50 μL of whole blood was required. A blood cell lysate was immediately prepared from each EDTA-blood sample: a 0.05-mL aliquot of EDTA-blood was well mixed with 0.05 mL of lysis solution (buffered solution with detergent; Milenia Biotec, Bad Nauheim, Germany) and incubated in stoppered 1.5-mL polypropylene tubes for 5 min at room temperature, as previously described (17). The resulting lysate was used for IL-8 measurements without further centrifugation *via* chemiluminescence immunoassay (Immulite test code I8; DPC Biermann), performed on a random-access fully automated assay suitable for 24 h. Results were usually available within 50 min after blood sampling.

Biochemical and hematologic determinations. White blood cell count (determined in EDTA-blood) was performed on a fully automated cell counter (Coulter Counter T660, Krefeld, Germany). The immature neutrophils:total neutrophils ratio (I/T ratio) served as a neutrophil index, differentiated with microscopy by experienced technicians, and was calculated as the sum of the nonsegmented neutrophil granulocytes divided by the sum of all granulocytes. A segmented neutrophil granulocyte was defined by at least one indentation of the nucleus to less than one third of the maximal nuclear diameter. An I/T ratio ≥ 0.2 was considered elevated. CRP was measured by enzyme sandwich immunoassay (Vitros 250; Ortho Diagnostics, Rochester, NY). Intra- and interassay variations were <5%. The threshold was set to 10 mg/L (7).

Statistical analysis. For IL-8 in DLWB and plasma, CRP and I/T ratio, specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV), as well as the appropriate 95% confidence intervals, were calculated. ROC curves were constructed to describe the relationship between the sensitivity and the false-positive rate ($1 - \text{specificity}$) for different parameters (25). Choosing optimal cut-off points from ROC curves would allow for the detection of almost all cases with true-positive findings (high sensitivity) with a minimum number of false-positive results (high specificity).

Cytokine concentrations are shown as Box-Whisker plots. Data were grouped; results obtained between 0 and 6 h are depicted as 6 h; results obtained between 6 and 12 h are depicted as 12 h; those obtained between 24 and 36 h are

depicted as 36 h, etc. For the comparison of healthy neonates and those with EOBI within the same interval, the *t* test was applied, using the decadic logarithm of the measurements. A *p* < 0.05 was defined as statistically significant. All charts were created with the help of software (SigmaPlot 2000; SPSS, Chicago, IL).

RESULTS

Patients. Serologic and clinical follow-up data were complete for 249 neonates with pre-, peri-, or postnatal risk factors and/or symptoms of EOBI. Twenty-four additional neonates had to be excluded because of incomplete records (*n* = 13) or simultaneous presence of other diseases potentially causing elevated IL-8 levels, such as chromosomal abnormalities or measurements after surgery (*n* = 11). The groups comprised 188 neonates without EOBI and 61 who met criteria for EOBI. Latter patients were discharged without the diagnosis of other inflammatory diseases. Positive blood cultures were found only in two of these (*Streptococcus* group B and *Staphylococcus aureus*). Four blood cultures were positive for *Staphylococcus epidermidis*, which were considered contaminated.

Patient characteristics are shown in Table 1. We found 67% male neonates in the EOBI group, compared with 49% in the reference group (*p* < 0.05). Prenatal risk factors such as maternal fever (3.5 versus 0%), pathologic cardiotocograph (CTG) (21 versus. 9%), and fetal tachycardia (12 versus. 4%) were seen more frequently in the EOBI group. A total of 24.5% (15 of 61) neonates with EOBI were positive for group B streptococci in ear and/or throat. All patients (61 of 61) in the EOBI group received antibiotics according to our institutional standards (mean duration: 8 d; SD 2.8), compared with 4.8% (9 of 188) of reference group infants. Because the suspicion of EOBI did not hold (CRP <10 mg/L, negative blood culture), treatment was discontinued after 1.6 d (SD 0.5) in the latter group; these neonates were regarded as noninfected.

IL-8 kinetics in noninfected neonates. Plasma IL-8 concentrations were <60 pg/mL throughout and independent of postnatal age, with a mean of 34.2 pg/mL (SD 18.1) in the first 6 h and 30.5 pg/mL (SD 13.3) after 60 h. DLWB IL-8 concentrations followed postnatal age-dependent kinetics. Compared with plasma, they were elevated 200- to 300-fold. In the first 24 h after birth, DLWB IL-8 concentrations increased from 9599 pg/mL (mean; SD 4433) after 6 h to 12179 pg/mL (SD 3849) after 24 h but decreased to 6289 pg/mL (SD 2104; *p* < 0.05 versus 24 h) after 48 h and to 5181 pg/mL (SD 2761) after 60 h (Fig. 1). For a cutoff of 60 pg/mL in plasma, ROC analysis

Figure 1. Kinetics of IL-8 in plasma (A) and DLWB (B) in 188 noninfected neonates.

revealed a sensitivity of 71% and a specificity 91%; for 18000 pg/mL in DLWB, a sensitivity of 97% and a specificity of 95% was found in the first 6 h after birth (Table 2).

Kinetics of IL-8 in EOBI. In the EOBI group, IL-8 concentrations in plasma (142.4 pg/mL; SD 111.6; *p* < 0.05 versus noninfected group) and DLWB (38620 pg/mL; SD 23197 pg/mL; *p* < 0.05 versus noninfected group) were already elevated immediately after birth (Fig. 2). Whereas plasma IL-8 declined after 18 h (NS versus noninfected group), the levels in DLWB showed a prolonged period of elevation, lasting for another ~12 h (*p* < 0.05 versus noninfected group). The sensitivity, specificity, PPV, and NPV for DLWB IL-8 at first clinical suspicion of infection are shown in Table 2 in comparison with plasma IL-8, CRP, and I/T ratio. ROC curves for IL-8 and CRP at 6, 12, and 24 h are displayed in Figure 3. Correlation between plasma and DLWB IL-8 was poor (*r* < 0.35, NS).

In neonates with EOBI, the sensitivity of IL-8 in DLWB (>18,000 pg/mL) was 97% (34 of 35), compared with 71% (>60 pg/mL; 25 of 35) in plasma. The CRP had a sensitivity of only 14% (5 of 35) in the first 6 h. The best sensitivity for EOBI (100%; 35 of 35) was achieved by the combination of DLWB IL-8 and CRP. In neonates who were evaluated during the first 12 h, DLWB IL-8 detected EOBI with a sensitivity of 93% (95% confidence interval: 87–99%) versus plasma IL-8 of 70% (60–80%) and a specificity of 89% (82–96%) versus 93% (87–99%). After 18 h, the sensitivity of DLWB IL-8 was 79% (15 of 19) versus 32% (6 of 19) in plasma, and the specificity was 87% (79–95%) versus 96% (91–100%). Sensitivity and specificity of CRP to detect EOBI increased over time. I/T ratio showed a low sensitivity of 14% (8–20%) to detect EOBI after 6 h, increasing to 40% (30–50%) after 12 h but declining again to 20% (10–30%) after 24 h (Table 3, Fig. 3). In our study group (prevalence 0.245), the NPV of DLWB IL-8 after 24 h was higher than that of plasma IL-8 (80 versus 66%; Table 3).

DISCUSSION

Investigating the suitability of DLWB compared with plasma IL-8 as an early marker of EOBI, we found 200- to 300-fold increased levels for the former compared with the

Table 1. Patient characteristics

	EOBI	Control
Neonates (<i>n</i>)	61	188
Postnatal age at time of clinical suspicion (h)	7.53	
	SD 6.21	
Male	41 (67.2%)	92 (48.9%)
Gestational age [d; median (range)]	282 (231–288)	275 (231–294)
Birth weight [g; median (range)]	3567 (2390–4850)	3921 (2490–4460)

Table 2. Diagnostic accuracy of plasma and DLWB IL-8, CRP, and I/T ratio within 6 h after first suspicion of EOB

	DLWB IL-8 >18 000 pg/mL	IL-8 plasma >60 pg/mL	CRP >10 mg/L	I/T ratio ≥0.2
Sensitivity (%)	97 (94–100)	71 (63–79)	14 (8–20)	14 (8–20)
Specificity (%)	95 (91–99)	90 (85–95)	100 (90–100)	10 (97–100)
PPV (%)	87 (81–93)	74 (66–82)	100 (97–100)	71 (63–79)
NPV (%)	99 (97–100)	89 (86–92)	75 (67–83)	75 (67–83)

Numbers in parentheses are exact 95% confidence intervals based on binomial sampling process.

Figure 2. IL-8 concentrations in plasma (A), DLWB (B), and CRP concentration (C) in 61 term neonates with EOB. Lines represent the ROC-determined cutoff of 60 pg/mL (plasma; A), 18,000 pg/mL (DLWB; B), and 10 g/L (CRP; C). * $p < 0.05$ vs noninfected group measured at the same time, using two-sample t test for the logarithm of the values.

latter parameter in noninfected full-term neonates (Fig. 1). The rise in DLWB IL-8 with EOB occurred as early as in plasma, but whereas plasma IL-8 started to decrease again after 12 h, DLWB IL-8 concentrations remained elevated for another 18 h, thereby resulting in a higher sensitivity for EOB (Figs. 2 and 3). Most important, sensitivity for EOB was considerably higher (0.97 versus 0.71 at 6 h) for DLWB than for plasma IL-8, making this parameter seemingly well suited for an early detection of EOB.

Lysing blood cells with detergents is a simple method of detecting cell-associated IL-8 (22). Recently, a cell lysis solution became commercially available, which allows for a fully automated enzyme immunoassay analysis on the IMMULITE System. The methodologic advantages of the system include a fast sample processing, so the results are available ~50 min after blood sampling, and the small volume (50 μ L) of blood required. Both features are prerequisites for successful use in neonates.

Consecutive *in vivo* kinetics of plasma and DLWB IL-8 were investigated in adult patients who underwent IL-2 immunotherapy. IL-2-induced plasma IL-8 was detectable after 2–4 h and declined rapidly to undetectable within 8 h. Compared with plasma, DLWB IL-8 concentrations were higher, increased as rapid as in plasma, and remained elevated for a longer interval (26), characteristics also found in the present study.

Previous studies on IL-8 in DLWB revealed a critical dependence on preanalytic sample handling (17,22). Much more

than circulating IL-8, DLWB IL-8 is affected by blood storage conditions between time of collection and further processing. Whereas storage up to 48 h on ice caused no increase in DLWB IL-8, storage at room temperature led to a significant increase *ex vivo*, which amounted to ~20% after 3 h (17) and up to 45% after 12 h, whereas the increase in plasma was undetectable (17) or moderate (unpublished observations). Therefore, a delay in sample handling may cause false-positive results.

Induction of IL-8 expression does not necessarily lead to elevated plasma concentrations, as shown during cardiopulmonary bypass (18). Compared with adult blood, in which 94–96% of exogenous IL-8 was cell associated (22), we found 99% of detectable IL-8 as detergent lysable and <1% as plasma IL-8 in neonates. Red blood cells therefore act as a sink for IL-8, thereby potentially influencing its activity. To what extent cell-bound IL-8 may serve as an “IL-8 memory” remains to be investigated.

Previous studies on the diagnostic value of plasma IL-8 in the prediction of EOB reported this cytokine to be more sensitive but less specific than CRP at the onset of suspected infection (7,23,27). Combining serum IL-8 with CRP improved the sensitivity from 83 to 93% without losing much specificity (7). Our data show a somewhat lower sensitivity (71%) for plasma IL-8.

CRP concentrations in serum/plasma increase several hundred-fold in response to bacterial infection but only after 18–24 h (24,28,29). Serial CRP levels are useful to test the probability of suspected infection. However, a normal CRP (28) and IL-8 concentration at the time of clinical suspicion cannot guide the decision whether to treat, especially if symptoms have a short history. The result could be a constellation whereby EOB cannot be detected by plasma IL-8 any more because it already has declined but CRP levels have not yet increased. DLWB IL-8 may close this diagnostic gap.

Although highly predictive, determining plasma IL concentrations as diagnostic tools for the detection of EOB may be challenging, particularly if the sampling does not occur in the initial phase. Messer *et al.* (30) observed six patients with negative IL-6 plasma levels in his set of infected neonates, probably after IL-6 had already peaked and returned to basal levels. Similar results were obtained by Buck *et al.* (31), whereby IL-6 decreased to undetectable levels after 24 h in the majority of infected neonates. IL-6 and IL-8 kinetics in newborns were found to be similar (11). In contrast to CRP levels, IL-6 (32) and IL-8 (33) concentrations may continuously decrease, even in the presence of an ongoing septic infection.

At the time of clinical suspicion in late-onset neonatal infections, Franz *et al.* (7) predominantly found increased serum IL-8 levels before CRP elevations became detectable.

Figure 3. ROC curves for IL-8 in plasma and DLWB, CRP, and I/T ratio after 6, 12, 18, and 24 h. Sensitivity is plotted against 1 – specificity for IL-8 level thresholds between 55 and 70 pg/mL (plasma) and 17,500 and 19,000 pg/mL (DLWB), CRP plasma level thresholds between 8 and 13 mg/L, and I/T ratio thresholds between 0.15 and 0.18 for patients with culture-proven and clinical EOBi.

Table 3. Diagnostic accuracy of plasma and DLWB IL-8, CRP, and IT ratio after 24 h

	DLWB IL-8 >18 000 pg/mL	IL-8 plasma >60 pg/mL	CRP >10 mg/L	I/T ratio ≥0.2
Sensitivity (%)	70 (59–81)	33 (22–44)	100 (97–100)	20 (10–30)
Specificity (%)	92 (85–99)	100 (97–100)	95 (90–100)	98 (96–100)
PPV (%)	88 (80–96)	100 (97–100)	94 (88–100)	100 (97–100)
NPV (%)	80 (70–90)	66 (55–77)	100 (97–100)	61 (49–73)

Numbers in parentheses are exact 95% confidence intervals based on binomial sampling process.

They reported, however, about occasionally normal IL-8 concentrations with (already) elevated CRP, again suggesting delayed detection of infection.

Our investigation again reveals the problem of defining EOBi. Although the clinical course was compatible with EOBi, blood cultures were positive in only two (3.2%) patients. This may be due to the consequent implementation of peripartum maternal antibiotic treatment, which makes the diagnostic value of neonatal blood cultures uncertain (34), and that we used very small amounts of blood (<0.5 mL) in only one culture. The sensitivity of blood cultures in EOBi is low (35) and depends on the number and timing of cultures taken, blood volume, culture medium dilution, technique, tempera-

ture, and organism density (36,37). Although only one had a positive blood culture, 24.5% of our neonates with EOBi were positive for group B streptococci. Estimates of the incidence of group B streptococci EOBi revealed that the true burden of the disease as indicated by culture-proven cases is underestimated, because the latter can be falsely negative in at least 50% of infants (38).

As in various other studies (7,35,38), the majority of our EOBi group, therefore, consisted of patients defined as “clinically infected neonates” with one or more clinical symptoms, which may, however, be nonspecific, plus alterations in blood parameters, *i.e.* increased plasma IL-8 or CRP, leukocytosis, leukopenia, or increased I/T ratio. Although the clinical workup was mostly per-

formed by experienced neonatologists, we are aware of the intrinsic restrictions of this approach, related to heterogeneity of patients, limited comparability to other studies, or dependence on physicians' experience. The CRP cutoff applied in this study (10 mg/L) is also somewhat arbitrary but has been used by several other investigators (7,24,39). Thus, strictly speaking, one could use the term "suspected EOBI" or "clinical EOBI" in the non-culture-proven cases.

In conclusion, the diagnostic value of DLWB IL-8 was superior to plasma IL-8 in this group of neonates with suspected EOBI and combined the advantage of a fast response with a long-lasting difference to noninfected neonates, thereby possibly widening the diagnostic window for EOBI detection by another 12 h. In addition, compared with plasma IL-8, the requested blood volume was reduced by 50%, which makes this parameter particularly attractive for use in preterm neonates.

REFERENCES

- Berner R, Niemeyer CM, Leititis JU, Funke A, Schwab C, Rau U, Richter K, Tawfeek MS, Clad A, Brandis M 1998 Plasma levels and gene expression of granulocyte colony-stimulating factor, tumor necrosis factor- α , interleukin (IL)-1 β , IL-6, IL-8, and soluble intercellular adhesion molecule-1 in neonatal early onset sepsis. *Pediatr Res* 44:469–477
- Stoll BJ, Gordon T, Korones SB, Shankaran S, Tyson JE, Bauer CR, Fanaroff AA, Lemons JA, Donovan EF, Oh W, Stevenson DK, Ehrenranz RA, Papile LA, Verter J, Wright LL 1996 Early-onset sepsis in very low birthweight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. *J Pediatr* 129:72–80
- Krediet T, Gerards L, Fleer A, van Stekelenburg G 1992 The predictive value of CRP and I/T-ratio in neonatal infection. *J Perinat Med* 20: 479–485
- Lehrnbecher T, Schrod L, Rutsch P, Roos T, Martius J, von Stockhausen HB 1996 Immunologic parameters in cord blood indicating early-onset sepsis. *Biol Neonate* 70:206–212
- Mehr S, Doyle LW 2000 Cytokines as markers of bacterial sepsis in newborn infants: a review. *Pediatr Infect Dis J* 19:879–887
- Fowlie PW, Schmid B 1998 Diagnostic tests for bacterial infection from birth to 90 days—a systematic review. *Arch Dis Child Fetal Neonatal Ed* 78:F92–F98
- Franz AR, Steinbach G, Kron M, Pohlandt F 1999 Reduction of unnecessary antibiotic therapy in newborn infants using interleukin-8 and C-reactive protein as markers of bacterial infections. *Pediatrics* 104:447–453
- de Winter RJ, Manten A, de Jong YP, Adams R, van Deventer SJ, Lie KI 1997 Interleukin 8 released after acute myocardial infarction is mainly bound to erythrocytes. *Heart* 78:598–602
- Hata T, Kawamura T, Inada K, Fujiwaki R, Ariyuki Y, Hata K, Kitao M 1996 Interleukin-6, interleukin-8, and granulocyte elastase in newborns with fetal distress. *Gynecol Obstet Invest* 42:174–177
- Kalfin RE, Engelman RM, Rousou JA, Flack JE 3rd, Deaton DW, Kreutzer DL, Das DK 1993 Induction of interleukin-8 expression during cardiopulmonary bypass. *Circulation* 88:401–406
- Martich GD, Danner RL, Ceska M, Suffredini AF 1991 Detection of interleukin-8 and tumor necrosis factor in normal humans after intravenous endotoxin: the effect of anti-inflammatory agents. *J Exp Med* 173:1021–1024
- Redl H, Schlag G, Bahrami S, Schade U, Ceska M, Stutz P 1991 Plasma neutrophil-activating peptide-1/interleukin-8 and neutrophil elastase in a primate bacteremia model. *J Infect Dis* 164:383–388
- Redl H, Schlag G, Bahrami S, Dinges HP, Schade U, Ceska M 1991 Markers of endotoxin related leukocyte activation and injury mechanisms. *Prog Clin Biol Res* 367:83–100
- Holmes WE, Lee J, Kuang WJ, Rice GC, Wood WI 1991 Structure and functional expression of a human interleukin-8 receptor. *Science* 253:1278–1280
- Murphy PM, Tiffany HL 1991 Cloning of complementary DNA encoding a functional human interleukin-8 receptor. *Science* 253:1280–1283
- Darbonne WC, Rice GC, Mohler MA, Apple T, Hebert CA, Valente AJ, Baker JB 1991 Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. *J Clin Invest* 88:1362–1369
- Reinsberg J, Dembinski J, Dorn C, Behrendt D, Bartmann P, van Der Ven H 2000 Determination of total interleukin-8 in whole blood after cell lysis. *Clin Chem* 46:1387–1394
- Kalfin RE, Engelman RM, Rousou JA, Flack JE 3rd, Deaton DW, Kreutzer DL, Das DK 1993 Induction of interleukin-8 expression during cardiopulmonary bypass. *Circulation* 88:401–406
- Peiper SC, Wang ZX, Neote K, Martin AW, Showell HJ, Conklyn MJ, Ogborne K, Hadley TJ, Lu ZH, Hesselgesser J, Horuk R 1995 The Duffy antigen/receptor for chemokines (DARC) is expressed in endothelial cells of Duffy negative individuals who lack the erythrocyte receptor. *J Exp Med* 181:1311–1317
- Lachgar A, Jaureguierry G, Le Buenac H, Bizzini B, Zagury JF, Rappaport J, Zagury D 1998 Binding of HIV-1 to RBCs involves the Duffy antigen receptors for chemokines (DARC). *Biomed Pharmacother* 52:436–439
- Hesselgesser J, Chitnis CE, Miller LH, Yansura DG, Simmons LC, Fairbrother WJ, Kotts C, Wirth C, Gillece-Castro BL, Horuk R 1995 A mutant of melanoma growth stimulating activity does not activate neutrophils but blocks erythrocyte invasion by malaria. *J Biol Chem* 270:11472–11476
- Marie C, Fitting C, Cheval C, Losser MR, Carlet J, Payen D, Foster K, Cavaillon JM 1997 Presence of high levels of leukocyte-associated interleukin-8 upon cell activation and in patients with sepsis syndrome. *Infect Immun* 65:865–871
- Franz AR, Kron M, Pohlandt F, Steinbach G 1999 Comparison of procalcitonin with interleukin 8, C-reactive protein and differential white blood cell count for the early diagnosis of bacterial infections in newborn infants. *Pediatr Infect Dis J* 18:666–671
- Mathers NJ, Pohlandt F 1987 Diagnostic audit of C-reactive protein in neonatal infection. *Eur J Pediatr* 146:147–151
- Zweig MH, Campbell G 1993 Receiver-operating characteristics (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 39:561–577
- Tilg H, Shapiro L, Atkins MB, Dinarello CA, Mier JW 1993 Induction of circulating and erythrocyte-bound IL-8 by IL-2 immunotherapy and suppression of its in vitro production by IL-1 receptor antagonist and soluble tumor necrosis factor receptor (p75) chimera. *J Immunol* 151:2399–23307
- Edgar JD, Wilson DC, McMillan SA, Crookard AD, Halliday MI, Gardiner KR, Rowlands BJ, Halliday HL, McNeill TA 1994 Predictive value of soluble immunological mediators in neonatal infection. *Clin Sci (Lond)* 87:165–171
- Benitz WE, Han MY, Madan A, Ramachandra P 1998 Serial serum C-reactive protein levels in the diagnosis of neonatal infection. *Pediatrics* 102:E41
- Berner R, Tuxen B, Clad A, Forster J, Brandis M 2000 Elevated gene expression of interleukin-8 in cord blood is a sensitive marker for neonatal infection. *Eur J Pediatr* 159:205–210
- Messer J, Eyer D, Donato L, Gallati H, Matis J, Simeoni U 1996 Evaluation of interleukin-6 and soluble receptors of tumor necrosis factor for early diagnosis of neonatal infection. *J Pediatr* 129: 574–2580
- Buck C, Bundschuh J, Gallati H, Bartmann P, Pohlandt F 1994 Interleukin-6: a sensitive parameter for the early diagnosis of neonatal bacterial infection. *Pediatrics* 93:54–258
- Hack CE, De Groob ER, Felt-Bersma RJ, Nuijens JH, Strade Van Schijndel RJ, Eerenberg-Belmer AJ, Thijs LG, Aarden LA 1989 Increased plasma levels of interleukin-6 in sepsis. *Blood* 74:1704–21710
- Hack CE, Hart M, van Schijndel RJ, Eerenberg AJ, Nuijens JH, Thijs LG, Aarden LA 1992 Interleukin-8 in sepsis: relation to shock and inflammatory mediators. *Infect Immun* 60:2835–22842
- Hsu KK, Pelton SI, Shapiro DS 2003 Detection of group B streptococcal bacteremia in simulated intrapartum antimicrobial prophylaxis. *Diagn Microbiol Infect Dis* 45:23–227
- Ottolini MC, Lundgren K, Mirkinson LJ, Cason S, Ottolini MG 2003 Utility of complete blood count and blood culture screening to diagnose neonatal sepsis in the asymptomatic newborn. *Pediatr Infect Dis J* 22:430–2434
- Kumar Y, Qunibi M, Neal TJ, Yoxall CW 2001 Time to positivity of neonatal blood cultures. *Arch Dis Child Fetal Neonatal Ed* 85:F182–F186
- Buttery JP 2002 Blood cultures in newborn and children: optimising an everyday test. *Arch Dis Child Fetal Neonatal Ed* 87:F25–F228
- Luck S, Torny M, d'Agapeyeff K, Pitt A, Heath P, Breathnach A, Russell AB 2003 Estimated early-onset group B streptococcal neonatal disease. *Lancet* 361:1953–21954
- Hengst JM 2003 The role of C-reactive protein in the evaluation and management of infants with suspected sepsis. *Adv Neonatal Care* 3:3–213