Influence of Gestational Age, Cesarean Section and Hematocrit on Interleukin-8 Concentrations in Plasma and Detergent-Lysed Whole Blood of Noninfected Newborns

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Keywords

Interleukin-8 · IL-8 · Detergent-lysed whole blood · Hematocrit · Gestational age · Birth weight

Summary

Background: Sensitivity of interleukin-8 (IL-8) in detecting early-onset bacterial infection (EOBI) is high. A high percentage is bound to nonspecific receptors on erythrocytes which can be determined via cell lysis. We have shown detergent-lysed whole blood (DLWB) IL-8 to be superior to plasma IL-8 in detecting EOBI. **Methods:** To evaluate influence of pre- and perinatal factors on plasma and DLWB IL-8 concentrations, IL-8 was determined via ELISA (Immulate) in 146 noninfected newborns with risk factors for EOBI at two different time periods: 0–6 (group I) and 24–30 h (group II) after birth. The influence of gender, mode of delivery, gestational age and hematocrit was evaluated. **Results:** While we found no influence of gender or gestational age, hematocrit was positively correlated with IL-8 plasma concentration (group I: $r = 0.33$, $p < 0.001$; group II: $r = 0.30$, $p < 0.01$). IL-8 plasma concentrations after primary versus secondary cesarean section were lower ($p < 0.05$). Gestational age was correlated with DLWB IL-8 concentrations (group I: $r = 0.46$, $p < 0.001$; group II: $r = 0.28$, $p < 0.001$). **Conclusion:** Plasma IL-8 concentrations were positively correlated with hematocrit, whereas DLWB IL-8 concentrations increased with gestational age. This may be relevant to the interpretation of IL-8 in preterm infants and infants with anemia, polyglobulia or hemolytic diseases.

Schlüsselwörter

Interleukin-8 · IL-8 · Lysiertes Vollblut · Hämatokrit · Gestationsalter · Geburtsgewicht

Zusammenfassung

Hintergrund: Die Sensitivität von Interleukin-8 (IL-8) bei der Diagnose der frühen Form der neonatalen bakteriellen Infektion (early-onset bacterial infection; EOBI) ist hoch. Ein hoher Prozentsatz wird an nichtspezifische Rezeptoren auf Erythrozyten gebunden, welcher nach Zell-Lyse bestimmt werden kann. Wir haben gezeigt, dass Lysat-IL-8 (DLWB) bei der Diagnose der EOBI gegenüber Plasma-IL-8 überlegen ist. **Methoden:** Um den Einfluss von prä- und perinatalen Faktoren auf Plasma- und Lysat-IL-8 zu untersuchen, wurde bei 146 nichtinfizierten Neugeborenen mit Risikofaktoren für EOBI IL-8 mittels ELISA (Immulate) zu zwei unterschiedlichen Zeitpunkten bestimmt: 0–6 (Gruppe I) und 24–30 h (Gruppe II) nach Geburt. Der Einfluss von Geschlecht, Geburtsmodus, Gestationsalter und Hämatokrit wurde untersucht. **Ergebnisse:** Wir fanden keinen Einfluss von Geschlecht oder Gestationsalter auf Plasma-IL-8, der Hämatokrit korrelierte positiv mit Plasma-IL-8-Konzentrationen (Gruppe I: $r = 0,33$, $p < 0,001$; Gruppe II: $r = 0,30$, $p < 0,01$). IL-8-Konzentrationen in Plasma waren primärer niedriger als nach sekundärer Sectio caesarea ($p < 0,05$). Das Gestationsalter korrelierte positiv mit IL-8-Werten in Lysat: (Gruppe I: $r = 0,46$, $p < 0,001$; Gruppe II: $r = 0,28$, $p < 0,001$). **Schlussfolgerung:** Plasma-, jedoch nicht DLWB-IL-8-Konzentrationen, korrelierten positiv mit dem Hämatokrit, wohingegen IL-8 in Lysat mit dem Gestationsalter positiv korrelierte. Dieser Sachverhalt kann bei der Interpretation von IL-8-Werten von Frühgeborenen und von Kindern mit Polyglobulie, Anämie oder hämatologischen Erkrankungen relevant sein.

Introduction

For the detection of both, early and late onset neonatal bacterial infection (EOBI; LOBI), cytokines play a predominant role [1]. Besides interleukin-6 (IL-6), IL-8 is a reliable, sensitive and easily accessible chemokine [2]. IL-8 is secreted within 1–3 h of endotoxin challenge [3]. As with most cytokines, its plasma half life is short, comprising less than 4 h [4, 5]. Circulating IL-8, which can be detected in plasma or serum via immunoassay, is bound immediately to two distinct high-affinity IL-8 receptors that are abundantly present on neutrophils before internalization and degradation [6, 7].

Circulating IL-8 only reflects a small portion of total blood IL-8 content, since 97% is cell-associated [7, 8] by chemokine-binding receptors such as the Duffy antigen-related chemokine receptors (DARC) on erythrocytes [8]. DARC-ligated IL-8 is biologically inactive to neutrophils but can re-circulate due to receptor ligation by other cytokines [9] or pathogens [10, 11]. Thereby, dissolved IL-8 may retain its biological activity. Since 85% of cell-associated IL-8 is bound on erythrocytes [8], this 'pool' may influence IL-8 plasma concentration. The sum of cell-associated plus circulating IL-8 can be determined by lysing blood cells with a detergent [8, 9].

We previously compared kinetics of plasma and detergent-lysed whole blood (DLWB) IL-8 in healthy newborns with those found in newborns with EOBI [12]. DLWB IL-8 was more sensitive during the first 6 h of infection than plasma IL-8. In contrast to plasma IL-8, it remains elevated for approximately 24 h [12], thereby partially bridging the diagnostic gap mentioned above. Also its negative predictive value to exclude EOBI was high. For preterm infants, this method appears particularly attractive as DLWB IL-8 only requires 50 µl EDTA blood.

Taking pre- and perinatal determinants of IL-8 into consideration, our patient population consisted of two main groups: late preterm (≥ 33 weeks of gestational age) and full-term infants. Mode of delivery was included, assuming IL-8 concentrations of infants born via secondary cesarean section are elevated in comparison to those delivered by primary cesarean section. We tested the hypothesis that red blood cell characteristics influence IL-8 plasma and DLWB kinetics in noninfected term newborns, which may influence the therapeutic value of plasma and DLWB IL-8 in neonatal blood disorders such as anemia, hemolysis or polyglobulia.

Patients and Methods

Patients

185 neonates were enrolled with institutional ethics committee approval and parental consent in this study. Inclusion criteria were suspicion of bacterial infection based on clinical signs compatible with infection (listed

below) and obstetric risk factors. Patients with co-morbidities causing elevated IL-8 concentrations such as chromosomal abnormalities or surgical intervention were excluded.

As previously described [12] indications for our observation program and blood screening were one or a combination of the following criteria: fever (rectal temperature ≥ 38.0 °C) or hypothermia, thermal instability, pallor, grey skin color, feeding difficulties, tachypnea or dyspnea, requirement of supplemental oxygen, respiratory insufficiency or apnea, capillary refill > 2 s, arterial hypotension, increased or decreased muscle tonus, irritability and lethargy [13]. Obstetrics risk factors included: peripartur maternal fever, maternal leukocytosis ($>12,000$ granulocytes/mm³) and/or CRP elevation > 10 mg/l after exclusion of other foci, history of amniotic infection, vaginal smear positive for group B streptococci, premature rupture of membranes ≥ 18 h, dystocia, foul smelling amniotic fluid, fetal tachycardia (>160 bpm).

Blood was drawn twice within the first 24–30 h of life, minimum time interval between the two measurements was 19 h. In analogy to previous studies [12, 13], EOBI was diagnosed in the presence of at least one positive clinical sign and evidence of the following criteria: a peak C-reactive protein (CRP) > 10 mg/l within 24 h after initial clinical symptoms, leukopenia ($<4,000$ /mm³) or leukocytosis ($>30,000$ /mm³ first day or $>20,000$ /mm³ second day), increased immature-to-total neutrophil ratio (>0.2) or positive blood culture. If EOBI could be excluded by clinical and laboratory monitoring (CRP levels ≤ 10 mg/l, no positive blood culture), neonates were diagnosed as not being infected and enrolled in our study. 27 neonates meeting criteria for EOBI were excluded. A further 5 neonates which were retrospectively diagnosed as non-infected, but initially received antibiotics for 48 h, were excluded.

Data collected included obstetric information, mode of delivery (spontaneous birth, primary cesarean section [section due to maternal indication; normal cardiotocography (CTG) without labor pain], secondary cesarean section [section due to pathologic CTG; existing or increased labor pain] and vacuum extraction, gender, gestational age, birth weight, clinical and laboratory information (plasma IL-8, DLWB IL-8, CRP, white and differential blood count). Blood samples were processed and analyzed within 2 h. A prerequisite was a technically smooth venipuncture. Blood samples which were macroscopically hemolytic or processed with a delay of more than 2 h were excluded ($n = 19$). In order to receive detailed information on the first 24–30 h post partum, we grouped the patients as follows: Group I includes IL-8 concentrations measured within the first 6 h, group II at approximately 24–30 h, mostly to confirm or exclude EOBI.

Biochemical and Hematologic Determinations

Circulating IL-8 was detected in plasma, sampled in lithium-heparin coated tubes, and results are available after 50 min. 25 µl plasma were diluted with 100 µl (1:5) sample diluent (I8; DPC Biermann, Bad Nauheim, Germany). The detection limit of plasma IL-8 was 2 pg/ml (standardized in accordance with the National Institute for Biological Standards and Controls Reference Preparation 89/520). Inter- and intra-assay variations were $< 5\%$ at 100 pg/ml. Native EDTA blood, obtained for routine hematologic measurement, served as a source for DLWB IL-8. For a single determination a total of 50 µl whole blood was required. A blood cell lysate was prepared immediately from each EDTA blood sample: a 0.05 ml aliquot was mixed well with 0.05 ml of lysis solution (buffered solution with detergent; Milena Biotec, Bad Nauheim, Germany) and incubated in stoppered 1.5 ml polypropylene tubes for 5 min at room temperature [12]. The resulting lysate was used for IL-8 measurements without further centrifugation via chemiluminescence immunoassay (Immulate test code I8, DPC Biermann). Hematocrit, and hemoglobin were detected via an automated assay (Sysmex XE-2100, Norderstedt, Germany). CRP was measured by enzyme sandwich immunoassay (Vitros 250, Ortho Diagnostics, Rochester, NY, USA). Intra- and inter-assay variations were $< 5\%$.

Table 1. Patient characteristics

	Group I	Group II
Number of neonates	95	51
Male	41 (43.2%)	27 (52.9%)
Median birth weight, g (range)	3,320 (1,100–4,250)	3,400 (1,790–4,400)
Median gestational age, days (range)	276 (232–297)	276 (235–292)
Preterm	248 (232–259)	251 (235–259)
Term	278 (260–297)	278 (260–292)

Table 2. IL-8 concentrations in plasma and DLWB

	IL-8 concentrations, pg/ml (range)					
	group I			group II		
	n	IL-8 plasma	IL-8 DLWB	n	IL-8 plasma	IL-8 DLWB
Mode of delivery						
Spontaneous birth	38	34.0 (10–96)	14,333 (400–36,480)	29	24.9 (2–48)	12,461 (2,560–27,560)
Cesarean section	45	24.7 (10–78)	8,787 (400–23,040)	14	19.6 (10–38)	11,314 (4,640–20,000)
Vacuum extraction	12	27.4 (10–34)	12,813 (3,240–28,800)	8	25.9 (10–38)	12,480 (8,200–16,680)
Gender						
Male	41	28.3 (10–63)	11,266 (400–36,480)	27	26.0 (10–42)	12,042 (2,560–19,680)
Female	54	30.8 (10–93)	11,702 (400–28,800)	24	22.0 (2–45)	12,268 (4,640–27,560)

Table 3. IL-8 concentrations in plasma and DLWB in newborns delivered by primary versus secondary cesarean section

	Group I		Group II	
	primary	secondary	primary	secondary
Number of neonates	16	29	5	9
Median IL-8 plasma, pg/ml (range)	19.1 (10–42)	32.0 (10–79)	20.2 (13–30)	28.1 (10–96)
Median IL-8 EDTA, pg/ml (range)	6,227 (400–15,240)	10,199 (3,680–23,040)	12,400 (6,600–19,680)	10,711 (4,640–16,680)

Statistical Analysis

Data were expressed as median and ranges. For data analysis, Mann-Whitney U test was applied. To examine the relationship between IL-8, gestational age and hematocrit, scatter plots were used and Spearman's rank correlation coefficients were calculated. All charts were created with SigmaPlot (SPSS, Chicago, IL, USA).

Results

Patients

Follow-up was completed in 146 non-infected neonates with pre- or perinatal risk factors and/or symptoms compatible with EOBI. 39 additional neonates were excluded: 27 of them showed clinical signs for EOBI and an elevated CRP concentration > 10 mg/l for more than 24 h, one had a positive blood culture, 5 received ex iuvantibus therapy with antibiotics and 7 had an incomplete data record. Patient characteristics are

presented in table 1. Group I comprised 95 neonates (43% male, 73 term, 22 preterm), group II 51 neonates (53% male, 46 term, 5 preterm).

Influence of Gender and Mode of Delivery

In noninfected neonates, median DLWB IL-8 concentrations were 400–500% higher than mean IL-8 concentrations in plasma in group I and II. Gender did not influence plasma or DLWB IL-8 concentrations ($p > 0.05$; table 2).

Dividing mode of delivery into spontaneous births, vacuum extractions, or cesarean sections, no differences in IL-8 concentrations were evident ($p > 0.05$; table 2), suggesting that the delivery mode did not influence the IL-8 concentrations.

Regarding cesarean sections, median plasma IL-8 concentrations from neonates delivered by primary section in group I were 19.1 pg/ml. These values were lower than those

Table 4. Plasma and DLWB IL-8 in preterm and term neonates

	Group I		Group II	
	preterm	term	preterm	term
Median gestational age, days (range)	248 (232–259)	278 (260–297)	251 (235–259)	278 (260–292)
Number of neonates	22	73	5	46
Median IL-8 plasma, pg/ml (range)	27.6 (10–63)	29.3 (10–93)	22.8 (10–45)	24.1 (2–96)
Median IL-8 DLWB, pg/ml (range)	5,934 (400–17,520)	13,199 (400–36,480)	6,456 (4,920–16,320)	12,768 (2,560–27,560)

Fig. 1. Influence of gestational age on IL-8 concentrations in plasma in group I (upper panel left; $r = 0.199$; $p = 0.054$) and group II (upper panel right; $r = 0.173$; $p = 0.223$) and DLWB in group I (lower panel left; $r = 0.499$; $p < 0.001$) and group II (lower panel right; $r = 0.251$; $p < 0.01$).

from neonates delivered by secondary section (32.0 pg/ml ($p < 0.001$; table 3). The same phenomenon held true in group II with 20.2 pg/ml versus 28.1 pg/ml ($p < 0.05$; table 3).

Median DLWB IL-8 concentrations in group I showed differences with 6,227 pg/ml following primary and 10,199 pg/ml following secondary section ($p < 0.001$; table 3). In contrast to plasma IL-8, this difference was no more evident after 24–30 h, with median DLWB IL-8 concentrations of 12,400 pg/ml versus 10,711 pg/ml (table 3).

Influence of Gestational Age

DLWB IL-8 concentrations were influenced by gestational age in group I ($r = 0.499$; $p < 0.001$) and group II ($r = 0.251$; $p < 0.01$) as shown in figure 1. Median values for preterm neonates in group I were 5,934 pg/ml versus 13,199 pg/ml for term neonates and in group II 6,456 pg/ml versus 12,768

pg/ml (table 4). We found no influence on plasma IL-8 concentrations for gestational age ($p > 0.05$). Median values for preterm neonates in group I were 27.6 pg/ml versus 29.3 pg/ml for term neonates and in group II 22.8 pg/ml versus 24.1 pg/ml (table 4).

Influence of Hematocrit

In both groups, plasma IL-8 concentrations were positively correlated with the hematocrit (group I: $r = 0.331$, $p < 0.001$; group II: $r = 0.332$, $p < 0.05$; fig. 2); in group I and group II median plasma IL-8 concentrations were 29.7 mg/dl 24.2 pg/ml, respectively; the respective median hematocrit values were 52.3% and 50.2%. DLWB IL-8 concentrations were not influenced by the hematocrit ($p > 0.05$), with median DLWB IL-8 concentrations of 11,513 pg/ml in group I and 12,149 pg/ml in group II.

Fig. 2. Influence of hematocrit on IL-8 concentrations in plasma in group I (upper panel left; $r = 0.331$; $p < 0.001$) and group II (upper panel right; $r = 0.332$; $p < 0.05$) and DLWB in group I (lower panel left; $r = 0.172$; $p = 0.095$) and group II (lower panel right; $r = -0.106$; $p = 0.459$)

Discussion

We found the hematocrit to be positively correlated with plasma IL-8 concentrations (group I: $r = 0.331$, $p < 0.001$; group II: $r = 0.332$, $p < 0.05$). Plasma concentrations were lower if newborns were delivered via primary cesarean sections. Of the parameters investigated, gestational age was correlated with DLWB IL-8 concentrations (group I: $r = 0.499$, $p < 0.001$; group II: $r = 0.251$, $p < 0.01$).

Conventional tests have limitations in identifying infections in newborns. There is a rapidly increasing demand for standardized tests, including flow cytometric techniques, for the detection of neonatal infection requiring minimal amounts of blood. A variety of parameters, including receptors on monocytes [15–17], cytokines and proteins, are expressed or secreted differently in full or preterm neonates and are influenced by perinatal confounders [18, 19].

Therefore the aim of our study was to evaluate the influence of mode of delivery and hematocrit on IL-8 plasma and DLWB concentrations in term and preterm neonates. Compared with plasma IL-8, DLWB IL-8 offers the advantages that tests require smaller sample volumes and are not altered by hemolysis [20]. In agreement to other studies [19, 21, 22] we found that IL-8 concentrations in plasma and DLWB were not influenced by gender.

In the first 6 h post partum there were significant differences in plasma and DLWB IL-8 concentrations between neonates delivered by primary and those delivered by secondary cesarean section (table 3). In plasma this difference was still evident 24 h post partum, but not for DLWB. Labor pain is known to influence IL-8 production [23]; in a recent study Jokic et al. [24] reported that IL-8 concentrations are less influenced by mode of delivery than by the existence and duration of labor pain. In our group of spontaneous births (table 2), we also registered the duration of labor and found no correlation between either DLWB or plasma IL-8 concentrations (not shown); however, we did not analyze or standardize labor pain. Dembinski et al. [28] found more evident neonatal proinflammatory response to labor stress in concentrations of IL-8 in whole blood than in serum.

Besides the influence of labor pain, which is also described by others [25, 26], delivery as such may lead to elevated plasma IL-8 concentrations via mediators of stress and anxiety. van Gool et al. [27] showed a significant increase in early postnatal plasma IL-8 concentrations if delivery was recognized as a traumatic event by the mother.

As practical consequences of our results, one would have to assume that the threshold of values for plasma IL-8 would be lower if newborn were delivered via primary section.

In agreement with observations of Dembinski et al. [28], plasma IL-8 was not influenced by gestational age, in contrast to DLWB IL-8; late preterm neonates had lower DLWB IL-8 concentrations (fig. 1). This might be attributed to immaturity [29], but could also be seen as an epiphenomenon for reasons leading to preterm delivery (e.g. HELPP syndrome, gestational diabetes mellitus, placental dysfunction). Hematocrit values were not different between preterm and term infants (not shown). Thus, in analogy to reduced expression of blood type antigens, binding receptors, including Duffy antigen related chemokine receptors (DARC) on erythrocytes [8], might as well be reduced. Our data suggest that the hematocrit influences plasma IL-8 concentrations in noninfected newborns in the first 24 h (fig. 2).

Kinetics of DLWB IL-8 is not completely understood. DLWB IL-8 concentrations were higher than plasma IL-8 concentrations, ranging from 400 to 36,680 pg/ml. Our results were similar to those of Franz et al. [13] and reflected the fact that only 1–4% of IL-8 is dissolved in plasma [12]. Another reason for this finding is the fact that DLWB IL-8 is not only bound to erythrocytes but also to a smaller amount of about 15% to blood cells such as granulocytes, monocytes etc. [30]. Our previous studies showed normal levels for DLWB IL-8 concentrations of <18,000 pg/ml [12].

Our investigation again reveals the problem of an ideal population and the definition of infected versus noninfected newborns: Despite existing risk factors and indefinite short-lasting clinical signs, we excluded EOBI via CRP and the clinical course. Although the clinical work-up was mostly performed by experienced neonatologists, we are aware of intrinsic restrictions related to heterogeneity of patients, limited comparability to other studies or dependence on physicians' experience. The CRP cut-off applied in our study (10 mg/l) is arbitrary but has been used by other investigators [31].

Plasma IL-8 concentrations increase with hematocrit and with secondary cesarean section as compared to primary section. In contrast, DLWB IL-8, a highly specific and sensitive parameter to detect EOBI [12] and LOBI [20] is less influenced by the hematocrit. This has to be considered in the interpretation of plasma IL-8 of newborns with anemia, polyglobulia or hemolytic diseases such as blood incompatibility of mother and child, icterus praecox or hyperbilirubinemia and should be investigated prospectively.

Disclosure Statement

The authors declare no conflict of interest.

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