Interleukin-6: A Sensitive Parameter for the Early Diagnosis of Neonatal Bacterial Infection

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ABSTRACT. *Objective.* **Early recognition is important for the successful treatment and outcome of neonatal infections. As interleukin-6 (Il-6) plays a critical role in the induction of C-reactive protein (CRP) synthesis in the liver, it was hypothesized that this cytokine could be detected earlier in blood than the CRP during the course of bacterial infection.**

Design. **In a prospective study of 298 newborns who were admitted to the nursery unit, CRP levels, blood cell count with differential, and Il-6 levels were determined at the time of admission and 24 hours after admission. Seventy-six newborns were excluded from the study because of incomplete or incorrect blood sampling.**

Results. **The remaining 222 newborns were assigned to one of five groups: 11 newborns with blood culture-positive sepsis (sensitivity of Il-6 on admission 73%), 15 newborns with clinical sepsis (sensitivity of Il-6 on admission 87%), 41 newborns with infection (sensitivity of Il-6 on admission 68%), and 54 newborns without clinical and laboratory evidence of infection (specificity 78%). The remaining 101 newborns were defined as a mixed group because the diagnosis of neonatal infection could not clearly be made. Seventy-five percent of infected newborns had negative Il-6 levels 24 hours after admission. Of the 18 infected newborns with negative Il-6 levels on admission, 10 newborns had elevated CRP levels, suggesting that Il-6 was already negative because of the short half-life of Il-6. Sensitivity of Il-6 in CRP-negative newborns on admission was 100% in newborns with blood culture-positive and clinical sepsis. Il-6 was more sensitive than CRP in infected newborns on admission (73% vs 58%).**

Conclusion. **Il-6 is a sensitive parameter for diagnosing neonatal bacterial infection. The combination of CRP and Il-6 seems to be the ideal tool for the early diagnosis of neonatal infection.** ***Pediatrics* 1994;93:54–58; *interleukin-6, C-reactive protein, bacterial infection, newborn.***

ABBREVIATIONS. CRP, C-reactive protein; Il-6, interleukin-6.

Neonatal infection remains a major cause of morbidity and mortality in newborn infants. Because typical clinical features are missing in many patients with sepsis at an early stage of the illness, diagnosis and treatment may be delayed. Several indicators have been evaluated in the diagnosis of systemic bacterial infections and include various leukocyte indices[1,2] and acute-phase proteins. The major focus of attention has been directed to C-reactive protein (CRP). However, its value in the early diagnosis of infection is low.[3]

Recently, attention has been directed to the role of interleukin-6 (Il-6) as an important mediator of the inflammatory response. Il-6 is a pleiotropic cytokine involved in many aspects of the immune system.[4] It is synthesized and released in response to inflammatory stimuli by monocytes, endothelial cells, and fibroblasts and secondary to tumor necrosis factor and Il-1 production.[5] Il-6 acts as a signal in T-cell activation,[6] induces antibody secretion by human B cells,[7] and induces differentiation of cytotoxic T cells.[8] Il-6 is the major inducer of hepatic protein synthesis including CRP, fibrinogen, and serum amyloid A protein during acute-phase responses.[9] Bataille and Klein[10] showed that treatment with anti-Il-6 antibodies in patients with malignant diseases and high CRP levels resulted in a decrease of CRP levels to normal values.

In adults with sepsis, studies have demonstrated increased levels of Il-6 which correlated with mortality rates, suggesting that this mediator is important in the pathogenesis of sepsis in human beings.[11,12] In children with bacterial sepsis, Sullivan et al[13] could detect elevated levels of Il-6.

So far the significance of Il-6 as a diagnostic marker of systemic infection in newborns has not been investigated. Because Il-6 gives the main stimulus to hepatic CRP synthesis, we hypothesized that Il-6 provides an earlier indicator of neonatal bacterial infection than does CRP.

MATERIALS AND METHODS

Study Population

In a prospective study, all newborns admitted to the regular and intermediate-care wards from May 1991 to December 1991 were included in the study group. Also, newborns suspected of having infection during their hospital stay were included. Further, from January to March 1992 only newborns with the presumptive diagnosis of sepsis/neonatal infection were included in the study group.

In all newborns a white blood cell count with differential, CRP, and Il-6 were determined at the time of admission and 24 hours after admission. In babies with clinical signs of infection, blood cultures and local cultures were also taken. Chest roentgenograms were performed when clinically indicated. Data recorded included age, initial diagnosis, maternal risk factors (fever, early rupture of membranes, early onset of labor), and clinical signs in the baby (tachypnea, apnea, nasal flaring, retractions, cyanosis, capillary

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refilling time, poor skin color, fever, reduced muscle tone, bradycardia, and tachycardia).

White Blood Cell Count and Differential

Total white blood cell count was measured using the model S Coulter counter and corrected for the number of red blood cell precursors seen in the differential count. The differential count was made by the same three experienced hematological technicians. A band neutrophil was defined as one in which the connection between the nuclear segments was greater than one third of the width of the nuclear lobes. Pathological neutrophil indices used to support a diagnosis of neonatal infection were defined according the criteria of Manroe et al[1] and Lloyd and Oto.[2]

CRP Determination

Heparinized blood (500 μL) was obtained and CRP was analyzed by a rate nephelometry method.[14] According to the study of Mathers and Pohlandt[3] a CRP value ≥10 mg/L was defined as increased.

Il-6 Determination

Blood (200 μL) was collected in EDTA-plastic tubes and immediately centrifuged. Plasma was stored at −20°C before measurement. Il-6 determination was performed with an enzyme-linked immunosorbent assay (limit of detection ≥10 pg/mL). The Il-6 standard solutions and the samples were incubated with monoclonal (mouse) Il-6 antibodies adsorbed to the microtiter plate and simultaneously with (sheep) Il-6 antibodies labeled with horseradish peroxidase. After a washing step the bound peroxidase was measured enzymatically and the Il-6 concentration in the samples was calculated. The coefficient of variation was 3% to 7% and day-to-day precision was 5% to 10%. Duplicate determinations were performed for each plasma sample. Il-6 levels greater 10 pg/mL were regarded as abnormal.

Classification of Newborns

The following five groups were prospectively defined:

Group 1—Blood Culture-Positive Sepsis. Newborns with positive blood culture, abnormal differential blood cell count, and elevated CRP level on admission and/or 24 hours after admission. All three items were mandatory for inclusion in group 1.

Group 2a—Clinical Sepsis. Newborns with abnormal differential blood cell count, elevated CRP on admission and/or 24 hours after admission, and clinical signs of infection but negative blood culture. All four items were mandatory for inclusion in group 2a. Clinical signs of infection were defined as three or more of the following categories of clinical signs:

- Apnea/tachypnea/nasal flaring/retractions/cyanosis/respiratory distress
- Bradycardia/tachycardia
- Hypotonia/seizures
- Poor skin color/capillary refilling time ≥3 seconds
- Irritability/lethargy

Group 2b—Infection. Newborns with negative blood culture, abnormal differential blood cell count, elevated CRP on admission and/or 24 hours after admission, and fewer than three clinical signs. All four items were mandatory for inclusion in group 2b.

Group 3—Control Newborns. Newborns with normal or abnormal differential blood cell count, normal CRP level on admission and 24 hours after admission, and no clinical signs of infection. All three items were mandatory for inclusion in group 3.

Group 4—Mixed Group. All newborns who could not be included in group 1, 2a, 2b, or 3. All newborns had a negative CRP value on admission and/or 24 hours after admission. CRP has a very high specificity for neonatal infection. To be sure to include only children with "proven" infection in group 1, 2a, or 2b, all CRP-negative newborns with clinical signs and normal or abnormal differential blood cell count were separated in the mixed group. In this group neonatal infection could be neither confirmed nor excluded.

RESULTS

Study Population

A total of 298 newborns were admitted to the study, but 76 were excluded because of incorrect or incomplete blood sampling. Of the remaining 222 newborns, 11 met the criteria of group 1; 15, group 2a; 41, group 2b; 54, group 3; and 101, group 4. Clinical details of newborns with blood culture-positive sepsis (group 1) are shown in Table 1.

Interleukin-6

Group 1. Eight of 11 newborns with blood culture-positive sepsis had increased levels of Il-6 on admission (median 65 pg/mL, range 20 to 3500 pg/mL), whereas 24 hours after admission Il-6 was negative in all but one baby. The 3 newborns who had no detectable Il-6 levels on admission had raised CRP levels on admission with low CRP levels 24 hours after admission (CRP 5 mg/L, 13 mg/L, 16 mg/L) (Table 1, Fig 1). Eighty-two percent of the newborns had a pathological differential blood cell count on admission.

Group 2a. In group 2a, 13 of 15 newborns had initially elevated Il-6 levels (median 40 pg/mL, range 10 to 2400 pg/mL) which decreased to undetectable levels in 12 of 15 newborns 24 hours after admission (median 0 pg/mL) (Fig 2). The two Il-6-negative newborns had increased CRP levels on admission and low CRP levels after 24 hours (8 mg/L, 21 mg/L). Eighty

TABLE 1. Clinical Details of Newborns With Blood Culture-Positive Sepsis*

Patient No.	GA, wk	Age†	CRP, mg/L		Il-6, pg/mL		Blood Culture
			On Adm	24 h After	On Adm	24 h After	
1	39	10	18	24	149	0	GBS
2	40	83	19	5	0	0	CNS
3	40	1	<2	‡	130	‡	*Escherichia coli* GBS
4	35	1	32	9	65	0	CNS
5	42	73	104	50	36	0	*Proteus mirabilis*
6	41	9	3	110	>1000	0	GBS
7	38	26	<2	86	20	0	CNS
8	42	63	55	13	0	0	CNS
9	35	86	12	16	0	0	Enterococcus
10	31	1	58	88	3500	0	*E. coli*
11	26	64	105	170	2300	270	*Klebsiella oxytoca*

* Abbreviations: GA, gestational age; Adm, admission; CRP, C-reactive protein; IL-6, interleukin-6; GBS, Group B β-hemolytic streptococci; CNS, coagulase-negative staphylococci.
† Postnatal age in hours.
‡ Child died within the first 24 hours.

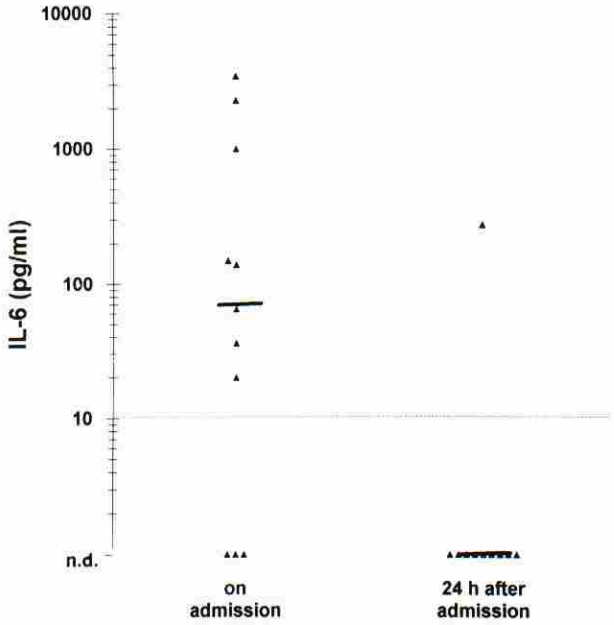

Fig 1. Plasma levels of interleukin-6 (Il-6) in newborns with blood culture-positive sepsis (group 1) at the time of admission and 24 hours later. Horizontal lines show median. Below dotted line: range of normal value. n.d., no Il-6 detectable.

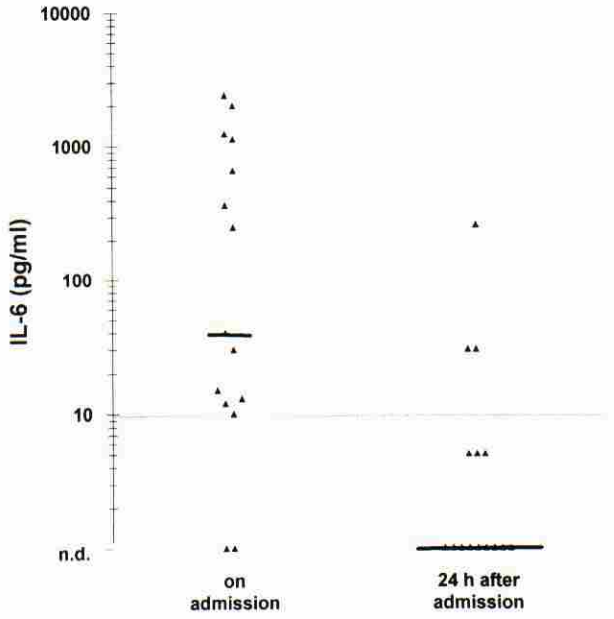

Fig 2. Plasma levels of interleukin-6 (Il-6) in newborns with clinical sepsis (group 2a) at the time of admission and 24 hours later. Horizontal lines show median. Below dotted line: range of normal value. n.d., no Il-6 detectable.

percent of the newborns had a pathological differential blood cell count on admission.

Group 2b. Twenty-eight of 41 newborns had increased Il-6 levels on admission (median 30 pg/mL, range 10 to 3050 pg/mL) (Fig 3). Of 13 newborns with negative Il-6 levels on admission, 4 newborns who also had negative CRP levels had elevated Il-6 levels 24 hours after admission (range 16 to 2500 pg/mL). Of the remaining 9 newborns, 7 had only very slightly elevated CRP levels (maximum value 16 mg/L). In 2

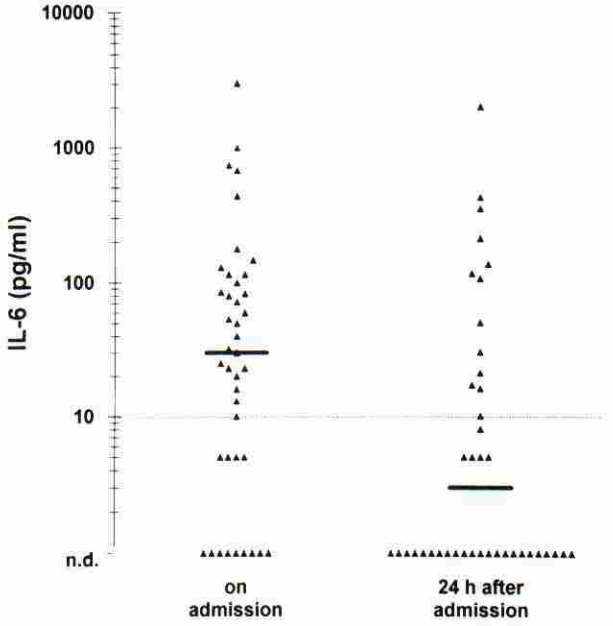

Fig 3. Plasma levels of interleukin-6 (Il-6) in newborns with infection (group 2b) at the time of admission and 24 hours later. Horizontal lines show median. Below dotted line: range of normal value. n.d., no Il-6 detectable.

newborns, elevated CRP levels found on admission declined within 24 hours to normal values.

Seventy-five percent of infected newborns had negative Il-6 levels 24 hours after admission. Of 18 infected newborns with negative Il-6 levels on admission, 10 already had elevated CRP levels. Sixty-three percent of the newborns had a pathological differential blood cell count on admission.

Group 3. Forty-two of 54 control newborns had negative Il-6 levels on admission and 24 hours after admission (specificity 78%). However, 12 newborns had elevated Il-6 levels (median 20 pg/mL, 10 to 510 pg/mL) on admission and/or 24 hours after admission (Fig 4). Eight of the 12 newborns had an abnormal differential blood cell count. In 3 of the 12 newborns maternal risk factors for infection were present. Three newborns were admitted because of low birth weight. No correlation of low Apgar score or low arterial umbilical cord pH with elevated Il-6 levels in the remaining newborns could be detected (data not shown). Twenty-four percent of all newborns had a pathological differential blood cell count on admission.

Group 4. The mixed group could be divided mainly into three subgroups. Forty-five newborns had one clinical sign, negative CRP level, and normal or abnormal differential blood cell count. In this subgroup 26 newborns (58%) had an elevated Il-6 level on admission (n = 15) and/or 24 hours after admission (n = 17). The second subgroup included 31 newborns with two clinical signs, negative CRP, and normal or abnormal differential blood cell count. Nineteen (61%) of the 31 newborns had elevated Il-6 levels on admission (n = 17) and/or 24 hours after admission (n = 8). The third subgroup included 19 newborns with three or more clinical signs, negative CRP, and normal or abnormal differential blood cell count. Among these newborns, 13 (68%) had increased Il-6

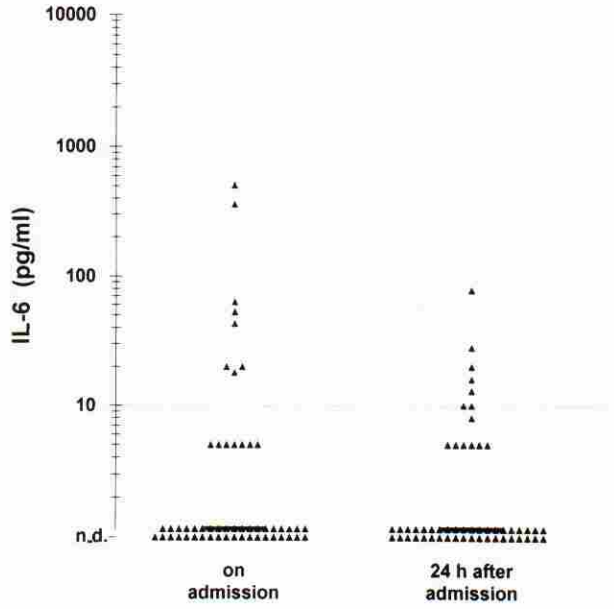

Fig 4. Plasma levels of interleukin-6 (Il-6) in control newborns (group 3) at the time of admission and 24 hours later. Horizontal lines show median. Below dotted line: range of normal value. n.d., no Il-6 detectable.

levels on admission (n = 13) and/or 24 hours after admission (n = 6). The remaining 6 newborns had elevated CRP levels with normal differential blood cell count (3 newborns had elevated Il-6 levels). Because the diagnosis of neonatal infection in the mixed group could be neither confirmed nor excluded, sensitivity and specificity were not calculated.

CRP

In group 1, all three newborns with negative CRP levels on admission had elevated Il-6 levels on admission. In group 2a, all newborns (n = 6) with negative CRP levels on admission had elevated Il-6 levels on admission. Of 19 newborns in group 2b with negative CRP levels on admission, 14 had initially elevated Il-6 levels. Il-6 was more sensitive than CRP in infected newborns on admission (73% vs 58%).

Sensitivity values of Il-6 and CRP for early detection of bacterial infection in group 1, 2a, 2b, and 3 are shown in Table 2. Sensitivity of Il-6 in infected newborns with initially negative CRP was 100% in group 1 and group 2a and 74% in group 2b.

Relationship of Antibiotic Treatment With Il-6 Levels

To investigate whether the decline of Il-6 within the first 24 hours was dependent on antibiotic treatment, Il-6 levels of antibiotic-treated newborns were compared with those of untreated newborns. All newborns of group 1, 93% of group 2a, and 78% of group 2b were treated with antibiotics within the first 24 hours of admission. The median Il-6 level 24 hours after admission in newborns treated with antibiotics was 0 pg/mL in group 1, group 2a, and group 2b. The median Il-6 values 24 hours after admission in newborns who were not treated with antibiotics were less than 10 pg/mL in group 2a (n = 1) and 0 pg/mL in group 2b (n = 3).

TABLE 2. Sensitivity of Interleukin-6 (Il-6) and C-Reactive Protein (CRP) on Admission in Infected Newborns*

	Il-6	CRP	Il-6 (Negative CRP on Admission)
Group 1 (n = 11)	73	73	100
Group 2a (n = 15)	87	60	100
Group 2b (n = 41)	68	54	74
Total (n = 67) (1 + 2a + 2b)	73	58	82

* Values are percentages.

Local Cultures

In approximately 60% of all newborns, cultures of smears from the ears and gastric secretions were performed on admission. Since bacteria could be isolated in about 50% of all cultures, this unspecific finding was not used for classification of the newborns (data not shown).

DISCUSSION

We demonstrated that newborns with systemic bacterial infection were able to produce Il-6. Previous studies have shown that Il-6 is increased in adult patients with sepsis.[11,12,15] Compared with adults, newborns have an increased susceptibility for severe and life-threatening infections. As a cause, it has been discussed whether a diminished production of IL-6 could be partly responsible for the increased risk of severe infection in newborns.[16,17] Few studies have examined Il-6 production in children with blood culture-positive sepsis and detected elevated levels of Il-6 compared to a control group.[13,18,19] In newborns with perinatal infectious complications, Miller et al[20] detected elevated cord plasma Il-6 concentrations.

Il-6 levels have so far only been investigated in patients with blood culture-positive sepsis. In newborns with obvious clinical and laboratory signs of bacterial infection, however, we often fail to isolate bacteria from the blood culture. As demonstrated in our study, the largest subgroup of newborns with bacterial infection is that with negative blood culture. The lack of reliable clinical signs and/or laboratory tests often causes diagnostic problems leading to anticipatory antibiotic treatment. In newborns of group 4 (mixed group) we could not establish the diagnosis of neonatal infection. Therefore, to investigate the relevance of Il-6 as an early diagnostic marker in neonatal infection, these newborns were considered separately.

In most cases Il-6 peaked at the time of admission and decreased within 24 hours to undetectable levels. This observation confirms the very short half-life of Il-6, as demonstrated by Castell et al.[21] The short half-life could be due to binding to specific plasma proteins, eg, α_2-macroglobulin,[22] rapid accumulation in the liver,[21] or inhibition through other cytokines.[23,24] In some of the infected newborns with initially elevated CRP levels we could not detect Il-6 levels. This is in line with the short half-life of Il-6 and the cascade of induction of acute-phase proteins where CRP synthesis is induced by Il-6.

Because there were too few untreated newborns, we could not investigate the influence of antibiotic treatment on Il-6 kinetics. Previous studies in adults

with bacterial sepsis tried to correlate clinical outcome with the increase in Il-6.[12,13,15] We did not find a correlation of Il-6 level with clinical signs (data not shown). Because of the short half-life we do not think that the absolute level is of any prognostic value, since it is uncertain at which stage of infection blood was taken for Il-6 determination.

So far the detection of Il-6 in plasma or serum is performed by commercially available Il-6 enzyme-linked immunosorbent assays. The measurement of Il-6 takes approximately 2 to 4 hours and the costs are approximately $20 for one sample. Considering that differential blood cell count, CRP, and Il-6 will distinguish between infected and uninfected newborns earlier and more reliably, the cost of care will be diminished because not all neonates with suspected infection need to be admitted to the hospital, and some receive anticipatory but unnecessary antibiotic treatment.

As shown in our results, the consecutive determination of Il-6 shows the real significance of this acute-phase protein in the clinical management of systemic infection. We have shown that Il-6 is a very early marker in the diagnosis of neonatal infection. Also, differential blood cell count is a very sensitive test but its specificity for neonatal infection is low. On admission CRP is a less sensitive but specific parameter. Thus in the clinical setting the combination of CRP and Il-6 may be the ideal tool for the early diagnosis of neonatal bacterial infection.

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MILITARY METAPHORS IN MEDICAL LANGUAGE

Contemporary medicine's focus on fighting disease (in contrast to caring for sick patients) has made war metaphors much more viable.

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Submitted by Student