

Early Detection of Bacterial Sepsis in Newborns

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Abstract

Background: Neonatal infections are the most common cause of morbidity and mortality in infancy.

Objectives: To determine the usefulness of interleukin-6 and blood culture in the diagnosis of suspected bacterial infections in neonatal intensive care unit.

Subjects and Methods: This retrospective case record analysis was conducted over two years 2009-2010. Data was collected using patient's records from the pediatric and neonatal care unit of the hospital. The ages of the new born ranged from 28 to 42 weeks of gestation. Blood samples were collected at the time of admission, before the initiation of the first dose of antibiotic. Tests included; complete blood count, blood culture, and interleukin-6 (IL-6), C-reactive protein estimation. The results of blood culture and inflammatory markers, i.e. interleukin-6 (IL-6) and C-reactive protein were analysed.

Results: A total of 73 blood samples were taken from neonates who were clinically suspected to be having sepsis. Twenty two samples revealed positive blood cultures for bacteria. The level of interleukin in first hour was very high (>1000 pg/ml) in 17 samples while its median value was 100 pg/ml. Normal or minimal elevation of C-reactive protein was seen in these cases. Low level of (<350 pg/ml) was seen in 5 newborns and C-reactive protein in these cases was around 12 mg/l.

Conclusions: High IL-6 levels show some correlation with positive bacterial blood culture and it can help in supplementing the diagnosis of neonatal sepsis.

Policy message: For an early diagnosis of neonatal sepsis, IL-6 levels may be used to treat these cases especially in settings where blood culture facilities are remote.

Key words: C-reactive protein, interleukin-6, sepsis.

Introduction

Neonatal sepsis refers to a systemic infection with positive blood or other central culture. Clinical diagnosis of sepsis in newborn infants is not easy because, symptoms and signs are non-specific. There is no laboratory test with 100% specificity and sensitivity, to detect bacteraemia in neonate but search has continued for a reliable test. The neonates with "risk factors" for neonatal sepsis are thus treated with broad spectrum antibiotics and require prolonged hospitalization¹. Mortality has decreased in recent years for term, low-birth weight, and very-low-birth weight infants, possibly due to the shift in predominant organisms from gram-negative to gram-positive². Blood cultures are the gold standard for diagnosis of sepsis; however, the results of the test are available only after 48-72 hours. The

positivity rates vary widely, ranging from 30 to 87%³. Interleukin-6 is a rapid-response inflammatory protein with a short plasma half-life⁴. The proinflammatory cytokine interleukin-6 consist of a series of phosphoglycoproteins with a molecular weight ranging from 21 to 45 kDa and the serum reference limit of IL-6 is less than 10 pg/ml in healthy individuals⁵⁻⁶. In response to inflammatory tissue injury, IL-6 is a mediator of the acute phase response. Previous clinical studies demonstrated consistently elevated IL-6 level in patients with sepsis, and that IL-6 levels above 1000 pg/ml are generally associated with an increased mortality rate in critically ill patients⁷⁻⁹. Undetectable concentrations may be measured in septic neonates at the time of suspected infection onset because the interleukin-6 concentration has already returned to baseline. By contrast, C-reactive protein, an acute phase protein stimulated by interleukin-6 rises to abnormal concentrations in neonates 24-48 h after the onset of infection-time when interleukin-6 concentrations may have already fallen to within the normal range. Levels of C-reactive protein, an acute phase protein associated with tissue injury, are elevated at some point in 50-90% of infants with systemic bacterial

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infections¹⁰. So the value of C-reactive protein in sepsis workup is questionable. Therefore, the combination of interleukin-6 an early marker of infection, with C-reactive protein, a later sepsis marker, may allow the clinician to monitor the evolution of neonatal infection and detect more accurately infection among neonates. This study intends to measure both markers although interleukin is more accurate in early diagnosis of bacterial sepsis in neonatal critical care unites. Therefore, the purpose of this study was to evaluate the high level of IL-6 in early detection of positive bacterial blood culture in first hour of newborn admission with clinical sepsis to intensive care unit.

Subjects and Methods

This study used the retrospective approach to review medical records for newborns who were admitted to intensive care unit of Carl Gustav Carus university hospital in Dresden-Germany, between January 2009 and December 2010 with the provisional diagnosis of neonatal sepsis.

A total of 778 newborns were admitted in the unit over the two years period. Case records of all newborns presenting with signs and symptoms of septicemia with/without pneumonia and/or meningitis were further analysed for blood culture, interleukin-6 levels and C-reactive proteins results which were compared. Neonates were excluded if they had: a) major congenital anomaly; b) inborn errors of metabolism, c) hemolytic jaundice or respiratory distress syndrome (due to surfactant deficiency). Informed consent was obtained from the parents of each newborn and placed in the file of each newborn at time of admission

There were 73 newborns who had suspected septicaemia and 22 blood samples showed a positive blood culture for bacteria.

Medical records reviewed maturity, age at onset, sex, birth weight, symptoms and signs along with the maternal risk factors. The cases with suspect sepsis were screened for interleukin-6, C- reactive protein, and blood culture. Other investigations were done previously as required. Some of these neonates were asymptomatic but were evaluated for sepsis because of maternal intrapartum sepsis risk factors like prolonged rupture of membranes, maternal urinary tract infection, maternal intrapartum fever >38°C, chorioamnionitis, and excessive vaginal discharge. The criterion standard for diagnosing sepsis is the positive organism-specific blood culture, at least in the absence of maternal intrapartum antibiotic prophylaxis. Samples for blood culture, Interleukin-6 and C-reactive protein were obtained from peripheral or umbilical vein for every newborn admitted in critical care unit for routine culture of aerobic and anaerobic bacteria. Cultures of cerebrospinal fluid and urine were performed when appropriate. In addition, tracheal samples from

intubated patients were cultured for bacteriology and screening for IgM antibodies to Toxoplasma, rubella virus, cytomegalovirus, herpes simplex virus, and *Treponema pallidum* was done in the first few postnatal days if congenital infection seemed likely. The demographic characters, blood culture results and IL-6 with C-reactive protein levels of patients, were compared especially with the positive blood culture for bacteria. The clinical sepsis was defined according to the standard parameters on clinical, laboratory or cultural screen¹⁰⁻¹¹. We used IBM SPSS version 19 multilingual-EQUINOX for statistical analysis. Correlation between variables and statistical differences were analyzed using Fisher exact, Monte Carlo and Wilcoxon tests. *p*-values of <0.05 were considered to be significant.

Results

A total of 778 newborns that were screened retrospectively represented all hospitalized neonates in critical care. Of these 254(32.6%) were full term (>37 gestational age) and 524(67.4%) preterm (>28week gestational age). Among the total number of newborns, 73(9.4%) met inclusion criteria. Twenty two (30%) newborn had positive and 51(70%) negative blood culture. Of newborns suspected of neonatal sepsis with negative blood culture and negative C-reactive protein, three had slightly elevated level of IL-6. Statistical analysis showed no significant difference with regards to gender and gestational age of both groups (*p*>.05). In addition, for those 22 newborns diagnosed with sepsis, the mean IL-6 was 100 pg/ml. High level groups (IL-6 >1000 pg/ml) included 17 newborns, and low level group (IL-6 <350 pg/ml) 5 newborns. C-reactive protein maximum level was 12 mg/l in eight newborns.

Table 1: Most common isolate microorganism from blood culture in 73 infants with clinical suspected sepsis.

Microorganism	Frequency	Percentage
No microorganism	51	69.9
<i>E.coli</i>	7	9.6
<i>Coagulase Neg. Staph</i>	5	6.8
<i>Streptococcus pneumonia</i>	4	5.5
<i>Pseudomonas aeruginosa</i>	4	5.5
<i>Klebsiella spp.</i>	2	2.7
Total	73	100

Table-1 shows that the most common isolated microorganism from blood culture was *E.coli* seen in 7 cases (9.6%) while the least common was *Klebsiella spp.* seen in two cases (2.7%). In all newborns with bacterial sepsis. IL-6 plasma levels were significantly elevated (>30000pg/ml) at the time of admission and the bacterial sepsis was mainly due to gram negative bacteria. There was no correlation between the gestational age (*r*=0.24; *p*>0.05) and gender (*r*=0.27; *p*>0.05) and the IL-6 plasma

levels among newborns with positive blood culture. In addition, Serum IL-6 levels were higher in neonates with positive blood culture especially in gram negative bacteria compared to *coagulase negative staphylococcus* than in the non-infected neonates and those with negative blood culture ($p<0.001$ and $p<0.01$, respectively). The level of C-reactive protein in first hour of admission was not significantly elevated. Table-2 shows the levels of IL-6 which ranged from 20000 to 45000 pg/ml in cases having gram negative bacterial culture especially *E.Coli* and *Pseudomonas* and it was also seen in *Strep. Pneumoniae*. In *staphylococcus* infection the levels of interleukin-6 were not significantly elevated and they reached a maximum level of 1000 pg/ml. When the level of IL-6 was less than 50 pg/ml the culture was negative in all cases.

Table 2: Levels of interleukin-6 (IL-6) and C-reactive protein (CRP) in 73 infants with clinical suspected sepsis.

Variable	IL-6 (pg/ml) <50pg/ml and number of patient	CRP (mg/l) <5mg/l
Blood culture positive (<i>E.coli.</i> , <i>Streptococcus pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella</i> .)	1,200-50,000(17)	5-22
<i>Coagulase Neg. Staph.</i>	200-1000(5)	1-5
Blood culture negative	10-100(51)	negative<1

Discussion

This retrospective study showed that IL-6 was highly reactive and showed peak plasma concentrations at the time when the infants were suspected of having sepsis. C-reactive proteins reacted late and their maximum concentrations appeared at 24 hours. It was also noted that when the cytokine values were decreasing, the values of C-reactive protein were just starting to increase.

Early recognition of sepsis and prompt initiation of appropriate antibiotic therapy is essential for the successful treatment of bacterial infections. Clinical symptoms and signs of neonatal sepsis are very non-specific and, therefore, can easily mislead the physician. The major problem in neonatal infections is the identification of the infected infant and an equally important task is identifying the non-infected infant¹². The incidence of sepsis in neonatal intensive care units (NICU) is high due to inadequate management and transplacental transfer of antibiotics which are administered to the mothers during the pre and intrapartum period as their use might increase the likelihood of culture-negative sepsis^{13,14}. It is in this group of patients that a test is required that can rapidly and reliably identify the presence of sepsis. Such test would radically alter neonatal prescribing practices and

limit the unnecessary administration of antibiotics to uninfected infants. In the present study all newborns whose mother had received antibiotics before delivery were excluded because of this reason.

In the present study it was found that IL6 is an important marker which could be used to detect early host response to infection. Its concentration increased sharply after exposure to bacterial products and regressed subsequently with an increase in C-reactive protein values. Many studies have tried to find reliable early reacting cytokines for the detection of bacterial sepsis in neonates¹⁵. Evaluation of existence of possible infection is sometimes extremely difficult, therefore, antibiotics are used more often than necessary in the NICU, increasing the risk of antibiotic resistance¹⁶.

The inflammatory process in sepsis is biochemically very complex. Various studies have shown that some pro-inflammatory cytokines, peak very fast i.e within 1-4 hours of a sepsis stimulus¹⁵⁻¹⁶ while, C-reactive proteins peak after 24 hours of the septic stimulus^{17,18}. The present study showed that the IL-6 concentrations were significantly elevated in the neonates who were admitted with suspected sepsis and ultimately had bacterial infections on blood culture, while, IL-6 levels were not increased in those who were admitted with suspected sepsis but later had negative blood culture. Another study showed umbilical cord blood IL6 as a sensitive marker for diagnosing neonatal infection within 72 hours of birth, the sensitivities and negative predictive values being 87–100% and 93–100% respectively^{8,19}. This study also showed that IL-6 is a highly sensitive marker of sepsis in the immediate postnatal period because of its rapid response time, which is much faster than that of C-reactive proteins; therefore, this marker could be used as an early diagnosing marker for bacterial sepsis and starting appropriate therapy. Neonates with viral infection, bacterial colonization, or respiratory distress due to various causes, including hypoxemia, had normal or only slightly increased serum interleukin-6 levels.

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