Predictability Of Neonatal Infection In Cases Of Preterm Premature Rupture Of Membranes By Interleukin-6 Estimation: Antenatal Bed Side Test From Vaginal Fluid Versus Neonatal Serum Testing At Birth And After 24 Hours

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Abstract:

The Objective of this study was to assess the predictability of interleukin 6 (IL-6) for neonatal infection, in women with preterm premature rupture of membranes (PPROM) using a bed side test from vaginal fluid versus neonatal serum testing at birth and after 24 hours. The study was a cross sectional one and enrolled 73 women who were hospitalized for PPROM. The gestational age at study entry ranged between 28 and 35 completed weeks of gestation that was confirmed from history and/or by a first-trimester ultrasonography. Maternal routine investigations as serum C reactive protein (CRP) and IL-6 detection in vaginal fluid were done. Neonatal examination and assessment was done. Initial neonatal outcomes as Apgar score, neonatal infections and neonatal cranial ultrasound were assessed. Neonatal CRP and IL-6 were also estimated. The results showed that neonatal infections were more frequent for babies of women with IL-6-positive vaginal samples (29.7% vs 8.3%; P=.02). The sensitivity of vaginal testing of IL-6 for predicting neonatal infection was 79%; its specificity was 56%; its positive predictive value was 30%, and its negative predictive value was 92%. The mean neonatal IL-6 at 0 hour was significantly higher in babies of the vaginally positive than the vaginally negative mothers (P <0.01). The sensitivity of IL-6 at 0 hour for predicting neonatal infection was 99%; specificity was 91.4%; positive predictive value was 57.4%, and negative predictive value was 92%. The sensitivity at 24 hours was 67.4%; specificity was 69.3%; positive predictive value was 50%, and negative predictive value was 63%.

Conclusion: Estimation and detection of IL-6 with both the qualitative vaginal secretions bedside test and at birth neonatal serum are of great value in the prediction of neonatal infection in cases of PPROM. This cytokine may reflect the insult that the fetus had been exposed to; hence affection of the fetal brain. Antenatal detection and early at birth estimation are of medico legal importance, which may protect obstetricians and pediatricians from alleged intrapartum or early neonatal mismanagement.

Introduction:

Preterm premature rupture of membranes continues to be a leading cause of neonatal morbidity and mortality. PPROM occurs in approximately 2% of all pregnancies but is associated with 20% of all perinatal deaths. Complications associated with PPROM include preterm labor, maternal and fetal infection, asphyxia, and, if earlier in pregnancy, pulmonary hypoplasia and anatomic distortion as a result of amniotic bands. Chorioamnionitis occurs in 6% of preterm deliveries without premature rupture of membranes but is present in 27% of preterm deliveries with premature rupture of membranes; it is associated with a fourfold increase in neonatal mortality. Patients with PPROM are often managed expectantly with hospitalization and bed rest until there is clinical evidence of infection or documentation of fetal lung maturity. Clinical signs of infection are subtle and usually not present in early chorioamnionitis. Currently there are no reliable clinical markers to adequately indicate impending intrauterine infection in these patients. Microbial invasion of the amniotic cavity is frequently observed in patients with preterm premature rupture of membranes (PPROM). It is a major risk factor for neonatal infection and adverse neonatal outcomes, which include bronchopulmonary dysplasia and periventricular leukomalacia. No available, noninvasive tests currently provide early screening of fetal infection to help obstetricians determine the need for antibiotic treatment, cessation of tocolysis, or even early delivery. Antenatal detection of neonatal infection would also make it possible to target more
maternal rupture reduces the fluid volume. 6-8 complications; moreover, it is more difficult when amniocentesis, an invasive procedure that can cause amniotic fluid, however, can be studied only with complications than other markers that are studied in fluid IL-6 has a better diagnostic value for these membranes using a bedside test from vaginal fluid infection, in women with preterm premature rupture of the amniotic cavity and those who subsequently delivered preterm neonates. 5-8 High vaginal interleukin-6 (IL-6) concentrations are associated with microbial invasion of amniotic fluid, chorioamnionitis, and preterm delivery. 10-15 Amniotic fluid IL-6 has a better diagnostic value for these complications than other markers that are studied in amniotic fluid, which include Gram staining, glucose levels, white blood cell count, and leukocyte esterase. Amniotic fluid, however, can be studied only with amniocentesis, an invasive procedure that can cause complications; moreover, it is more difficult when membrane rupture reduces the fluid volume. 5-8 The aim of the present study was to assess the predictability of interleukin 6 (IL-6) for neonatal infection, in women with preterm premature rupture of membranes using a bedside test from vaginal fluid versus neonatal serum testing at birth and after 24 hours.

Subjects and Methods:
This study was conducted from September 2002, through September 2004, in Tanta University Hospital. The study included 73 preterm neonates delivered for women who were hospitalized for PPROM at the Inpatient Ward of Obstetrics and Gynecology Department. These neonates were assessed at the Neonatology Unit of Pediatrics Department and All the Biochemical tests were done at the Clinical Pathology Department. The inclusion criteria were neonates with gestational age at study entry between 28 and 35 completed weeks of gestation that was confirmed from history and/or by a first-trimester ultrasonography and assessed after birth. The included women provided informed consent for themselves and their neonates. Women who met the following criteria were excluded: clinical chorioamnionitis, fetal distress, fetal malformation, a maternal disease that contraindicate continuation of pregnancy, or true labor. In this prospective study, the physicians in charge of both obstetric and neonatal care were blinded to the biochemical results.

Maternal assessment:
Initial assessment and sampling:
Complete history taking and thorough clinical examination were done to ensure inclusion and exclusion criteria. Assessment of ruptured membranes was done through sterile speculum examination with patient at rest and with straining. Vaginal secretions were collected at admission from each patient who was hospitalized with preterm labor and ruptured membranes; after insertion of a sterile speculum into the vagina, 15 to 35 µL of secretions were aspirated from the posterior fornix with sterile plastic pipettes. Samples were kept in -5°C until processing.

Clinical assessment of outcomes:
We assessed the following maternal outcomes: delivery within 7 days of admission, delivery at ≤32 completed weeks of gestation, time elapsed between admission and delivery, chorioamnionitis (defined by a temperature of >38°C and fetal tachycardia of >160 beats/min), intrapartum fever of >38°C, and fetal tachycardia during labor.

Chorioamnionitis assessment:
Any tissue samples (amnion, chorion-decidua, umbilical cord, and chorionic plate) were collected for histopathological examination. Histologic chorioamnionitis was defined in the presence of acute inflammatory changes in as described by Salafia et al.13 Four grades of inflammation were used to assess the amnion, chorion-decidua, umbilical cord, and chorionic plate. Grade 2 inflammation, characterized by multiple foci of 5 or more polymorph nuclear leukocytes or a larger focus in the subchorionic fibrin, was used as the cutoff for clinically important placental inflammation because this grade has been shown to be a sensitive indicator of culture-proven amniotic infection.

Neonatal assessment:
Initial neonatal outcomes:
Neonatal outcomes were assessed using: Apgar score at 5 minutes; cord artery pH; transfer to neonatal intensive care unit; duration of stay in the neonatal intensive care unit; postnatal antibiotic therapy; intraventricular hemorrhage that was graded 1 to 4 on the basis of the cranial ultrasound finding by Papile's criteria;17 periventricular leukomalacia, defined as hyper echoic lesions that persist through day 7 of life or any hypo echoic lesions; necrotizing enterocolitis, defined by the presence of abdominal distention, gastric retention, heme-positive stools, length of time for withholding enteral feeding, antibiotic treatment, and radiographic presence of pneumatosis intestinalis, portal air, or free intraperitoneal air; and neonatal death.
Neonatal infection:
In Neonatal Unit, every newborn infant who is born at <37 weeks of gestation with PPROM, was subjected to the following procedures: Complete physical examination, Bacteriologic examination of respiratory secretions (either pharyngeal or tracheal as appropriate if the baby is intubated), meconium, and peripheral blood samples, CRP: Normal value, <5.0 mg/L, White blood cell count: normal range, 5 to 30 × 10⁹/mL, Chest radiography: All results were available within 1 hour. Neonatal infections were classified as proven or probable neonatal infection.

Proven neonatal infection was suggested by a culture of a sample that was collected from a normally sterile site was positive and was associated with clinical signs of infection or with elevated neonatal CRP or with a chest radiograph that was compatible with a pulmonary infection.

Probable neonatal infection was suggested by clinical, radiographic, and laboratory findings, despite negative bacteriologic cultures. Clinical signs were attributed to infection when they could not be explained otherwise. Newborn infants were considered uninfected when there were no clinical, radiographic, or laboratory findings that were linked to infection. This group included colonized newborn infants without any sign of infection. The group of infected infants included all newborn infants with proved or probable infection.

Laboratory Investigations:
A: Maternal
Routine investigations were assayed as required for the routine monitoring of patients with PPROM. They included C-reactive protein (CRP) and leukocytic count.

CRP was determined by a nephelometric method. A level of 15 mg/L or greater was considered abnormal. Leukocyte count: The leukocyte count was measured with a Coulter Counter (Coulter Electronics, Hialeah, Fla.); the differential count was made by hematologic technicians; leukopenia was defined as a leukocyte count of less than 5000 cells/mm³; neutropenia as a count of less than 1500/mm³, and leukocytosis as a count of more than 28,000/mm³.

IL-6 detection: To meet clinical requirements for speed, a strip test bedside assay was designed to test for IL-6 in vaginal secretions. Immunochromatographic membranes (Pall Gelman Science, Paris, France) were coated with a mouse anti-human IL-6 monoclonal antibodies (clone B-E4; Diaclone, Besançon, France) and a goat anti-human immunoglobulin G Fc monoclonal antibodies (Diaclone) to make a test line and a control line, respectively. The sample, which was diluted 1:2, was applied to a pad that was coated with gold-labeled mouse anti-human IL-6 (clone B-E4; Diaclone) and affixed to the membrane. In case of positivity, IL-6/gold-labeled anti–IL-6 antibody complexes bound to the test line. In the absence of IL-6, the free gold-labeled antibody bound to the control line. The detection threshold of IL-6 was 100 pg/mL in vaginal secretions. The result was available in <20 minutes.

When the test result is positive, 2 bands appear after 15 minutes: the test band (T) reveals the presence of IL-6. The band C is used as a control. In the absence of IL-6, only the control band is observed. The complete absence of bands means the failure of the test.

B: Neonatal
CRP was determined by a nephelometric method. A level of 15 mg/L or greater was considered abnormal. The cut off values for significant levels were variable; starting from 5 mg/L. To increase the specificity, we increased the cut off value to be as 15 mg/L.

Neonatal IL-6 detection: Interleukin-6. Blood (100 µl, allowing a double determination) was collected in ethylenediaminetetraacetic acid-coated plastic tubes, centrifuged, and stored at -20° C. Determination of IL-6 was performed with an enzyme-linked immunosorbent assay technique whose limit of detection is 5 pg/ml (Hoffmann-La Roche, Basel, Switzerland). 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 being washed, the bound peroxidase was measured enzymatically and the IL-6 concentration in the samples was calculated. The coefficient of variation was 3% to 7%. The cut off values for significant levels of IL6 started from 50 pg/ml. The cut off value for levels was considered as 100 pg/ml to increase the specificity.

Neonatal cranial ultrasound:
Cranial sonography was performed for all the neonates using Aloka LD 630 Realtime Scanner with a 5 MHZ mechanical sector transducer (Japan). Brain imaging was done through the anterior and posterior fontanelles and sutures and the brain anatomy was visualized in the coronal, sagittal and axial planes.
Statistical Analysis:
Data entry and statistical analysis were accomplished with the use of SPSS software (version 9, SPSS Inc, Chicago, USA). We compared variables with the X² or Fisher's exact test, as appropriate. Odds ratios for maternal infectious marker and factors related to neonatal infection were calculated, with their 95% CIs. Adjusted odds ratios and 95% confidence intervals were calculated only for the significant variables (P < 0.05).

Results:
Table I portrays a summary of the characteristics of the study population of 73 women. Mean gestational age at inclusion was 28.4 ± 3.2 weeks, and mean term at birth 31.2 ± 3.2 weeks.

Table I. Characteristics of the seventy three women or the study population

<table>
<thead>
<tr>
<th>Characteristic (mean ± SD)</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother's age (y):</td>
<td>31.8 ± 4.9</td>
</tr>
<tr>
<td>Nulliparous (n)</td>
<td>22 (58.9%)</td>
</tr>
<tr>
<td>First pregnancy (n)</td>
<td>24 (30.1%)</td>
</tr>
<tr>
<td>Gestational age at delivery (wk):</td>
<td>31.2 ± 3.2</td>
</tr>
<tr>
<td>Birth weight &lt;1500 g (n/N)</td>
<td>29/73 (39.7%)</td>
</tr>
</tbody>
</table>

Table II shows the gestational age at admission and at delivery (that is, the period of time that elapsed between admission and delivery) were similar for the groups that were positive and negative for IL-6. More women in the group who were positive for IL-6 (46.0%) had fever during labor than did women in the IL-6-negative group (25.0%; P = .04). There was no association between IL-6 results and fetal tachycardia. Histologic chorioamnionitis tends to be more frequent in the placenta of women with IL-6-positive vaginal sample (57.7% vs 44.0%; P = .02).

Table II. Pregnancy outcome according to IL-6 findings in vaginal secretions at admission

<table>
<thead>
<tr>
<th>Variable (mean ± SD)</th>
<th>IL-6-positive (n=37)</th>
<th>IL-6-negative (n=36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery (wk)</td>
<td>30.7 ± 3.2</td>
<td>31.6 ± 3.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Admission - delivery interval (d)</td>
<td>16.9 ± 17.4</td>
<td>22.1 ± 17.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Delivery &lt;5 d (n/N)</td>
<td>12/37 (32.4%)</td>
<td>18/36 (50.0%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Delivery &lt;32 wk (n/N)</td>
<td>23/37 (62.2%)</td>
<td>17/36 (47.2%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Chorioamnionitis (n/N)</td>
<td>15/37 (40.5%)</td>
<td>10/36 (27.8%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Intrapartum fever (n/N)</td>
<td>17/37 (46.0%)</td>
<td>9/36 (25.0%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Fetal tachycardia during labor (n/N)</td>
<td>16/37 (43.2%)</td>
<td>11/36 (30.6%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Histologic chorioamnionitis (n/N)</td>
<td>15/37 (40.5%)</td>
<td>10/36 (27.8%)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

n/N = testing will be done with X² test

Table III demonstrates that neonatal infections were more frequent for babies of women with IL-6-positive vaginal samples (29.7% vs 8.3%; P = .02). Immediate neonatal morbidity (Apgar score at 5 minutes, pH at birth, transfer to the neonatal intensive care unit), postnatal antibiotherapy, and neonatal morbidity at 2 months did not differ according to the presence of IL-6.

Table III. Neonatal morbidity and mortality rates, according to prenatal IL-6 results in maternal vaginal secretions

<table>
<thead>
<tr>
<th>Variable (n/N)</th>
<th>IL-6-positive (n=37)</th>
<th>IL-6-negative (n=36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal infection (n/N)</td>
<td>11/37 (29.7%)</td>
<td>3/36 (8.3%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>5-Min Apgar score &lt;7 (n/N)</td>
<td>14/37 (37.8%)</td>
<td>13/36 (36.1%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Cord artery pH &lt;7.15 (n/N)</td>
<td>10/37 (26.3%)</td>
<td>3/36 (8.3%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Transfer to neonatal intensive care unit (n/N)</td>
<td>12/37 (32.4%)</td>
<td>8/36 (22.2%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Days of stay in neonatal care unit (n/N)</td>
<td>19 ± 14.9</td>
<td>17 ± 21.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Postnatal antibiotherapy (n/N)</td>
<td>26/37 (70.3%)</td>
<td>28/36 (77.8%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Oxygen dependency &gt;28 d (n/N)</td>
<td>4/36 (11.1%)</td>
<td>6/35 (17.1%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>At 36 weeks postmenstrual (n/N)</td>
<td>2/16 (5.0%)</td>
<td>0/35 (0.0%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Periventricular leukomalacia (n/N)</td>
<td>0/36</td>
<td>0/35</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Intraventricular hemorrhage grade 1 - 2 (n/N)</td>
<td>8/36 (22.2%)</td>
<td>5/35 (13.9%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Intraventricular hemorrhage grade 3 - 4 (n/N)</td>
<td>2/36</td>
<td>0/35</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Neonatal death (n/N)</td>
<td>1/37 (2.8%)</td>
<td>1/36 (2.7%)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

As seen in table IV, markers of maternal infection were assessed in relation to the presence of IL-6. It shows that elevated CRP > 15 mg/mL and positive IL-6 were both associated with neonatal infection. However, mean CRP was not different between the infected and not infected group (10.0 ± 17.4 mg/L vs 13.8 ± 17.3 mg/L; P = .46). After a logistic regression, only IL-6 and maternal CRP > 15 remained associated with neonatal infection (odds ratio, 4.5; 95% CI, 1.1-18.5).

Table IV. Odds of neonatal infection as function of maternal markers, based on logistic regression

<table>
<thead>
<tr>
<th>Variable (n/N)</th>
<th>IL-6-negative (n=36)</th>
<th>IL-6-positive (n=37)</th>
<th>Odds ratio (95% CI)</th>
<th>Adjusted odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serum CRP (mg/L)</td>
<td>5-20</td>
<td>2.1 (0.6-6.8)</td>
<td>4.5 (1.1-18.5)</td>
<td></td>
</tr>
<tr>
<td>Maternal white blood cell count (×1000)</td>
<td>5-20</td>
<td>0.21 (0.03-1.75)</td>
<td>5.3 (0.8-36.1)</td>
<td></td>
</tr>
<tr>
<td>Term of birth: gestational age (wk)</td>
<td>&gt;32</td>
<td>0.55 (0.17-1.79)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The dependent variable is neonatal infection. Adjusted odds ratios and 95% CIs were calculated only for the significant variables (P < 0.01). There was no case of maternal white blood cell count > 20 × 10³/mL in the case of neonatal infection.

As seen in table V, the sensitivity of IL-6 for predicting neonatal infection was 79%; its specificity was 56%; its positive predictive value was 30%, and its negative predictive value was 92%. The sensitivity of a CRP > 15 mg/L for predicting neonatal infection was 21%; its specificity was 95%; its positive predictive value was 50%, and its negative predictive value was 84%.

As seen in table VI, markers of neonatal infection were assessed in relation to the presence of IL-6 positivity in their mothers, at both 0 hour (admission) and at 24 hours.
The mean neonatal CRP at 0 hour was not different between babies of the vaginally positive and the vaginally negative mothers (P >0.05). Significant correlation was present between vaginal IL6 and neonatal CRP. (r= 0.56 and 0.63 respectively).

At 24 hours: The mean neonatal CRP was significantly higher in babies of the vaginally positive than the vaginally negative mothers (P <0.01). Significant correlation was present between vaginal IL6 and neonatal CRP. (r= 0.72 and 0.67 respectively). Comparison showed only a significantly higher CRP values in babies of the vaginally positive mothers at the 24 hours compared to 0 hour (P <0.01**) (table VI-A).

Table VI: Markers of neonatal infection in relation to the presence of vaginal IL-6 positivity in their mothers, at both 0 hour (admission) and at 24 hours

| Variable | Vaginal IL-6-positive result | Vaginal IL-6-negative result | P value
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>At 0 hr</td>
<td><strong>5 mg/L (0-26)</strong></td>
<td>3 mg/L (0-19)</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td></td>
<td>&gt;15 mg/L</td>
<td>7 (19%)</td>
<td>r = -0.56*</td>
</tr>
<tr>
<td></td>
<td>&lt;15 mg/L</td>
<td>30 (81%)</td>
<td>r = 0.63*</td>
</tr>
<tr>
<td>At 24 hrs</td>
<td><strong>27 mg/L (3-64)</strong></td>
<td>5 mg/L (0-21)</td>
<td>P &lt;0.01**</td>
</tr>
<tr>
<td></td>
<td>&gt;15 mg/L</td>
<td>32 (86.4%)</td>
<td>r = 0.73*</td>
</tr>
<tr>
<td></td>
<td>&lt;15 mg/L</td>
<td>5 (13.6%)</td>
<td>r = 0.67*</td>
</tr>
<tr>
<td>(0 vs 24)</td>
<td><strong>P &lt;0.01</strong></td>
<td>P &gt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

A: Neonatal CRP

B: Neonatal IL-6

As seen in table VII, the sensitivity of a CRP >15 mg/L at 0 hour for predicting neonatal infection was 34.6%; its specificity was 76.5%; its positive predictive value was 38.7%, and its negative predictive value was 73.4%. The sensitivity at 24 hours was 86.3%; its specificity was 88.2%; its positive predictive value was 77.8%, and its negative predictive value was 84%. The sensitivity of IL-6 >100 pg/ml at 0 hour for predicting neonatal infection was 99%; its specificity was 91.4%; its positive predictive value was 57.4%, and its negative predictive value was 92%. The sensitivity at 24 hours was 67.4%; its specificity was 69.3%; its positive predictive value was 50%, and its negative predictive value was 63%.

Discussion:

Patients of preterm premature rupture of membranes are routinely managed expectantly unless infection or labor ensues. Unfortunately, clinical signs of intrauterine infection are either absent or appear late in patients with preterm premature rupture of membranes. Several authors have studied the relationship between IL-6 concentrations in cervical or vaginal secretions, preterm labor, and amniotic infection.9,18-20 Amniotic fluid tests including Gram stain, cell counts, and concentrations of glucose and amniotic fluid IL-6 have been evaluated in patients with preterm premature rupture of membranes. Of these, amniotic fluid IL-6 has been shown to be the most sensitive predictor of infection of the amniotic cavity in patients with preterm labor and premature rupture of membranes and those at term.6, 21,22 All of these studies have involved enzyme-linked immunosorbent assays (ELISA) of IL-6, and only one of them dealt with the relation between IL-6 levels and neonatal infection in case of PROM.9 ELISAs generally require several hours to provide results, whereas other tests that depend on immunochromatography take approximately 20 minutes. This rapidity may be useful in the case of emergency. In addition, ELISAs in vaginal secretions lack reproducibility because of the presence of fibrin debris that may cause elevated optical density backgrounds. Under such conditions, the cutoff level
may be difficult to determine; and discrimination between infected and non-infected patients was difficult. In contrast, we observed that these immunochromatographic tests in cervical secretions were always clearly positive or negative, as already observed in comparisons with reverse transcriptase–polymerase chain reaction.23

A noninvasive method of testing for maternal-fetal infection involves the detection of proinflammatory cytokines in vaginal secretions. Some studies have suggested that their presence in cervical or vaginal secretions is associated with microbial invasion of amniotic fluid. In particular, IL-6 has been associated with neonatal infection and adverse neonatal outcome in cases of PPROM.24 The results of IL-6 assayed with bedside test were correlated with results for IL-6 messenger RNA in vaginal secretions and predicted preterm birth in a population of women with preterm labor with intact membranes.23

In this study, the detection of IL-6 by immunochromatography in the vaginal secretions of women with PPROM is correlated with neonatal infection. In a previous study among women with PPROM, IL-6 concentrations in cervical secretions were found to be correlated with amniotic fluid levels and with either neonatal infections or adverse neonatal outcome.25 IL-6 was associated with neonatal infection with positive (30%) and negative (93%) predictive values similar to the results of this study.

Various mechanisms may explain the association between IL-6 in vaginal secretions and neonatal infection. High cytokine levels in cervical secretions may reflect overall increased production throughout the maternal genital tract in response to intra-amniotic infection. It may also be a sign of a primary inflammation process at the amnio-chorionic-decidual surface.22 Another possibility is that IL-6 levels in vaginal secretions may mirror the IL-6 in amniotic fluid after membrane rupture.

Shalak et al.,30 in 2002, found that the concentrations of specific cytokines were elevated in the umbilical cord samples from neonates with brain damage as compared with healthy control infants. A direct relationship was present between an abnormal
neurologic examination and IL-6 concentrations at 6 hours of age. Moreover, infants with the most abnormal neurologic examination, had the highest concentration of IL-6 at 6 hours of age.

Chiesa et al.,31 had found in 2003, that the median cord interleukin-6 concentrations in the infants who developed encephalopathy was 376-fold as high as the values in the normal infants and 5.5-fold as high as those in the infants who did not develop encephalopathy. Regardless of outcome, in the asphyxiated infants the interleukin-6 values were significantly lower at both 24 and 48 h of life than at birth, with a significant decline from 24 to 48 h of life.

Adinolfi et al.32 reported that maternal production of cytokines in response to infection during pregnancy also may also be an important factor in initiating or supporting brain damage during fetal development.32 Gonzalez et al.33 reported that IL-6 levels on days 0 and 1 were significantly higher in infants with confirmed sepsis, prior to the blood culture being positive. IL-6 levels may be useful in the initiation as well as early termination of antibiotic therapy in late-onset neonatal sepsis.33 In this study, the IL-6 was evident to be present early at 0 hour, more earlier than the appearance of CPR. So, CRP is not valuable for an early diagnosis of infection. Also, the IL-6 had attained much higher sensitivities and specificities. Concomitantly going with the results of this study, previous studies had already evaluated the interest of IL-6, which increases rapidly after the onset of infection, several hours before the increase in the concentration of CRP, the most commonly used reference marker. Additionally, IL-6 has a good sensitivity and a good specificity when used as a marker of infection.34-37 Indeed, Lencki et al.36 suggested that cord blood IL-6 levels could be used to identify neonates at risk of sepsis. The negative predictive value of 97% seems interesting because it would allow the exclusion of infection, with the result that unnecessary antibiotic treatment could be avoided.

Buck et al.38 observed that IL-6 decreased in 24 hours to undetectable levels in the majority of their infected infants. In contrast to CRP levels, IL-6 levels can become normal even if the infection continues, as shown by Hack et al.,39 who reported falling levels of IL-6 even in patients with persistent symptoms of endotoxic shock. The half-life of IL-6 is short for different reasons: binding to plasma proteins such as α2-macroglobulin, early storage in the liver, or inhibition by other cytokines. This probably explains the reduction in sensitivity with increasing postnatal age.

Wu and Colford,40 in 2000, had reported an association between intrauterine infection in the form of clinical and/or histologic chorioamnionitis and adverse outcome, particularly adverse neurologic outcome in preterm infants.

In a prospective study of 309 preterm infants born at less than 32 weeks’ gestation, observed higher umbilical cord plasma IL-6 levels were present in infants with neonatal systemic inflammatory response syndrome (SIRS), periventricular leukomalacia (PVL), Necrotizing enterocolitis (NEC), Bronchopulmonary dysplasia (BPD) and composite neonatal morbidity.41 Yoon et al.,41 in 2003, reported that the diagnosis of periventricular leukomalacia (PVL) is one of the highest risk factors for subsequent development of cerebral palsy and other long-term neurologic handicap.

The primary concern of Obstetricians and pediatricians regarding intrapartum fever has been that an infection is present in the mother that may be passed to the infant during labor and delivery. This infection may be associated with elevated fetal, amniotic and vaginal fluid cytokines. Cytokines may have a direct toxic effect via increased production of nitric oxide synthase, cyclooxygenase, and free radicals and excitatory amino acid release. Another mechanism may be via hypoxia ischemia caused by funisitis which is known to be a potent stimulus for brain production of cytokines.

**Conclusion:**

1. Estimation and detection of IL-6 with both the qualitative vaginal secretions bedside test and at birth neonatal serum are of great value in the prediction of neonatal infection in cases of PPROM.
2. The earlier detection of IL-6 whether by antenatal testing or by at birth neonatal sampling may represent a continuous effort to get the best benefits and to try to manage cases of PPROM as early as possible to avoid such hidden infection.
3. Antenatal detection and early at birth estimation are of medico legal importance, which may protect obstetricians from alleged intrapartum complications and pediatricians from mismanaged neonates.
References


