Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes

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OBJECTIVE: The purpose of this study was to compare intraamniotic infection vs microbial invasion of the amniotic cavity (MIAC) as predictors of adverse outcome in preterm labor with intact membranes.

STUDY DESIGN: Interleukin-6 (IL-6) was measured in prospectively collected amniotic fluid from 305 women with preterm labor. MIAC was defined by amniotic fluid culture and/or detection of microbial 16S ribosomal DNA. Cases were categorized into 5 groups: infection (MIAC; IL-6, ≥11.3 ng/mL); severe inflammation (no MIAC; IL-6, ≥11.3 ng/mL); mild inflammation (no MIAC; IL-6, 2.6-11.2 ng/mL); colonization (MIAC; IL-6, <2.6 ng/mL); negative (no MIAC; IL-6, <2.6 ng/mL).

RESULTS: The infection (n = 27) and severe inflammation (n = 36) groups had similar latency (median, <1 day and 2 days, respectively) and similar rates of composite perinatal morbidity and mortality (81% and 72%, respectively). The colonization (n = 4) and negative (n = 195) groups had similar outcomes (median latency, 23.5 and 25 days; composite morbidity and mortality rates, 21% and 25%, respectively). The mild inflammation (n = 47) groups had outcomes that were intermediate to the severe inflammation and negative groups (median latency, 7 days; composite morbidity and mortality rates, 53%). In logistic regression adjusting for gestational age at enrollment, IL-6 ≥11.3 and 2.6-11.2 ng/mL, but not MIAC, were associated significantly with composite morbidity and mortality rates (odds ratio [OR], 4.9; 95% confidence interval [CI], 2.2–11.2, OR, 3.1; 95% CI, 1.5–6.4, and OR, 1.8; 95% CI, 0.6–5.5, respectively).

CONCLUSION: We confirmed previous reports that intraamniotic inflammation is associated with adverse perinatal outcomes whether or not intraamniotic microbes are detected. Colonization without inflammation appears relatively benign. Intraamniotic inflammation is not simply present or absent but also has degrees of severity that correlate with adverse outcomes. We propose the designation amniotic inflammatory response syndrome to denote the adverse outcomes that are associated with intraamniotic inflammation.


BACKGROUND AND OBJECTIVE
Intrauterine infection and inflammation are well-documented causes of preterm labor with intact fetal membranes, especially at very early gestational ages. Cultures for microorganisms in amniotic fluid demonstrate microbial invasion of the amniotic cavity (MIAC) in 20-60% of women with preterm labor at <28 weeks of gestation. Even with culture-negative amniotic fluid, women in preterm labor often have intraamniotic inflammation that is evidenced by elevated amniotic fluid levels of inflammatory markers such as interleukin-6 (IL-6). Whether or not the amniotic fluid culture is positive, intraamniotic inflammation is associated with preterm labor, especially at very early gestational ages. Cul

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short latency and high rates of perinatal morbidity and mortality. Four states of amniotic fluid are possible: infection (MIAC and inflammatory response both present), inflammation (inflammatory response present, MIAC absent), colonization (MIAC present, inflammatory response absent), and negative (both absent).

The aims of the present investigation were to compare the outcomes of preterm labor in women with intraamniotic infection, inflammation, or colonization and to examine whether those outcomes are related to the severity of the inflammatory response as defined by intraamniotic IL-6 levels.

**Materials and Methods**

Women with singleton pregnancies in spontaneous preterm labor with intact fetal membranes underwent amniocentesis for intraamniotic infection and to measure amniotic fluid IL-6. Amniotic fluid was sent for assessment of glucose, white blood cell count, Gram stain, and aerobic and anaerobic culture.

Amniotic fluid IL-6 concentration was assayed with immunoassay. Polymerase chain reaction for amniotic fluid 16S recombinant DNA (rDNA) was performed. MIAC was defined by a positive 16S rDNA result and/or a positive culture. Cases were divided into 5 groups based on amniotic fluid IL-6 results: (1) infection group: MIAC plus IL-6 concentration of ≥11.3 ng/mL; (2) severe inflammation group: no MIAC, IL-6 concentration of ≥11.3 ng/mL; (3) mild inflammation group: no MIAC, IL-6 concentration of 2.6-11.2 ng/mL; (4) colonization group: MIAC, IL-6 concentration of <2.6 ng/mL; and (5) negative group: no MIAC, IL-6 concentration of <2.6 ng/mL.

The Table summarizes perinatal outcomes. The infection and severe inflammation groups had shorter latency periods (median, <1 and 2 days, respectively) than those cases with low IL-6 concentrations (colonization and negative fluid; median, 23.5 and 25 days, respectively). The group with intermediate IL-6 concentration (mild inflammation) had intermediate latency (median, 7 days).

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<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Amniotic fluid interleukin-6, ≥11.3 ng/mL</th>
<th>Amniotic fluid interleukin-6, 2.6-11.2 ng/mL</th>
<th>Amniotic fluid interleukin-6, &lt;2.6 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected</td>
<td>Severe inflammation</td>
<td>Mild inflammation</td>
<td>Colonization</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>1165 ± 614</td>
<td>1335 ± 839</td>
<td>2083 ± 858</td>
<td>2206 ± 1102</td>
</tr>
<tr>
<td>Composite perinatal morbidity and death: ≥1 of the following, n (%)</td>
<td>22 (81)</td>
<td>26 (72)</td>
<td>25 (53)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Perinatal death</td>
<td>5 (19)</td>
<td>7 (19)</td>
<td>3 (6)</td>
<td>0</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory distress syndrome, n (%)</td>
<td>17 (63)</td>
<td>20 (56)</td>
<td>23 (49)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Intraventricular hemorrhage, grade 3 or 4, n (%)</td>
<td>2 (7)</td>
<td>3 (8)</td>
<td>2 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Necrotizing enterocolitis, n (%)</td>
<td>1 (4)</td>
<td>2 (6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Culture proven neonatal sepsis, n (%)</td>
<td>6 (22)</td>
<td>6 (17)</td>
<td>4 (9)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Comparison of the subjects in the 5 groups by analysis of variance (birthweight) or Fisher exact test (morbidity); † Data are given as mean ± SD.

inflammation groups had high rates of perinatal morbidity and mortality; the mild inflammation group had an intermediate rate. With adjustment for gestational age at enrollment, both MIAC and IL-6 levels had significant associations with latency of <72 hours, but only IL-6 levels had significant associations with composite perinatal morbidity and mortality.

COMMENT

Our results confirm that women in preterm labor have high rates of intra-amniotic infection and inflammation, especially at early gestational ages. We also confirm that intraamniotic inflammation, as evidenced by high IL-6 levels, is associated with short latency and high rates of perinatal morbidity and mortality whether or not microbes are detected in the amniotic fluid.

We present 4 novel findings. First, in the inflammation groups, the absence of MIAC was shown not only by negative amniotic fluid cultures, as in previous reports, but also by negative 16S rDNA polymerase chain reaction. Second, the degree of inflammation (mild or severe) as categorized by a single biomarker (IL-6 concentration) was correlated with the rates of perinatal morbidity and mortality. Third, outcomes in the amniotic fluid colonization and negative groups were similar. Fourth, amniotic fluid IL-6 level was stronger than MIAC as a predictor of composite perinatal morbidity and death.

Recent evidence challenges the traditional view that the normal intrauterine environment is sterile. Bacteria have been found in fetal membranes, placenta, and amniotic fluid in cesarean delivery without labor.

The interplay between intraamniotic bacteria and the inflammatory response can be summarized with a model that involves 4 stages: homeostasis, incitement, evolution, and resolution. We propose that some microbes may exist at low levels in the intrauterine milieu in many normal human pregnancies and may colonize the decidua, placenta, fetal membranes, and, occasionally, the amniotic fluid. If the organisms are kept in check by a low-level inflammatory response (homeostasis), colonization has no adverse sequelae. However, in some cases, the balance may be upset. Such imbalance could trigger a more vigorous inflammatory response (incitement). We propose that the inflammatory response, not the microbial invasion, triggers the release of prostaglandins, which causes contractions and cervical change, the clinical hallmarks of preterm labor. Once established, severe intraamniotic inflammation almost always progresses rapidly to delivery. It is unknown precisely what factors drive the transitions between these stages.

We propose the term amniotic inflammatory response syndrome to describe the relationship between elevated amniotic fluid inflammatory markers such as IL-6 and a spectrum of adverse outcomes that include early preterm birth and perinatal morbidity and death. The term amniotic inflammatory response syndrome focuses attention on intraamniotic inflammation rather than infection because the inflammatory response may be more directly responsible than the presence of microbes for a short latency and the resultant perinatal morbidity and death.

CLINICAL IMPLICATIONS

- Controlled clinical trials are needed to address whether targeted antibiotic therapy is beneficial for the subgroup of women in preterm labor with known microbial invasion of the amniotic cavity and to assess the possible benefits of the treatment of intraamniotic inflammation with steroids, nonsteroidal antiinflammatory drugs, and/or other immune modulators.
- We hypothesize that antibiotic and/or antiinflammatory treatment would be more successful in the presence of mild inflammation rather than severe inflammation.