What We Have Learned Regarding Antibiotic Therapy for the Reduction of Infant Morbidity After Preterm Premature Rupture of the Membranes

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Preterm premature rupture of the membranes (pPROM) is responsible for approximately one third of the over 450,000 preterm births occurring in the United States annually.1 Preterm PROM is associated with brief latency to delivery, maternal and neonatal infection, and umbilical cord compression due to oligohydramnios. Even with conservative management, the majority (70%-80%) of women with pPROM remote from term will deliver within 1 week of membrane rupture, placing the infant at risk for complications of immaturity in addition to those specifically related to premature membrane rupture.

Delivery before 32 weeks’ gestation is associated with a significant risk of neonatal complications, including severe acute morbidity and death. Because of this, the stable gravida with pPROM remote from term is generally best served by conservative management in an attempt to prolong pregnancy and reduce the risk of gestational age dependent morbidity in the newborn. Despite conservative efforts, many women will ultimately deliver after a brief latency. However, a subset of these women will remain pregnant for an extended period of time, allowing their fetus to mature in utero. Offsetting this potential benefit is the risk for development of amnionitis and fetal infection, abruptio placenta, and umbilical cord compression due to oligohydramnios.

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Over two-dozen controlled trials have been performed to determine if adjunctive antibiotic therapy during conservative management of pPROM could treat or prevent ascending intrauterine infection to prolong pregnancy and offer the opportunity for reduced neonatal infectious and gestational age dependent morbidity. Addressed in detail elsewhere, these studies have demonstrated significant pregnancy prolongation with antibiotic therapy, but the effect on infant mortality has been inconsistent. A significant number of women participating in these studies presented with pPROM near term and would not have benefited from brief pregnancy prolongation, regardless of antibiotic administration. Further, the broad range of antibiotics administered, differing routes and duration of administration, and the variability of concurrent tocolytic and corticosteroid administration make comparison of these studies difficult.

In 1990, the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network (NICHD-MFMU) began development of a randomized, placebo controlled trial to evaluate the impact of antibiotic therapy as adjunctive therapy to expectant management of pPROM remote from term. The purpose of this study was to determine if antibiotic therapy would reduce perinatal morbidity and mortality in a population at risk for these complications after pPROM. The steering committee accepted that antibiotics would likely prolong pregnancy and reduce amnionitis, based on the published data at that time, and proposed that adjunctive antibiotic treatment should not be given during conservative management of pPROM unless a significant neonatal benefit could be demonstrated with this intervention. To address this question, we chose the primary outcome for this study to be the occurrence of an infant with a major acute morbidity (“Composite” morbidity), including any of: fetal or postnatal death, respiratory distress syndrome (RDS), documented sepsis within 72 hours of delivery, grade 3 or 4 intraventricular hemorrhage (IVH), or stage 2 or 3 necrotizing enterocolitis. For twin pregnancies, an adverse outcome was considered to have occurred if one or both infant(s) suffered the evaluated morbidity or mortality while a good outcome was considered to have occurred only if neither infant suffered any of the complications included in the composite morbidity.

Methods

This multicenter study was performed at eleven clinical Centers with approval of the Human Research Committees of all participating institutions for the randomized interventional trial and for an observational study of pregnancy outcomes of eligible but nonrandomized women. Institutional Human Research Committee approval was also obtained by the Centers participating in ancillary studies of placental pathology and biologic fluid analyses. Women presenting with spontaneous premature rupture of the membranes at 24 weeks and 0 days to 32 weeks and 0 days gestation were considered eligible for entry if membrane rupture had occurred within 36 hours of randomization, cervical dilatation was less than or equal to 3 cm on visual examination, and if they had 4 or fewer contractions in a 60-minute monitoring period before randomization. Women with nonreassuring fetal testing, vaginal bleeding, maternal or fetal indication for delivery, cervical cerclage in place, antibiotic therapy within 5 days, corticosteroid therapy within 7 days, allergy to penicillins or erythromycin, bacteriuria, febrile illness requiring antibiotics, significant medical complications (class 2-4 cardiac disease, class D or F diabetes, endocrinopathy requiring medication, hematological disorders other than anemia, hypertensive disorders, acute and chronic liver disease, pulmonary hypertension and acute or chronic renal failure) were considered ineligible. Recent antibiotic use and corticosteroid administration were used as exclusion criteria because both interventions could potentially have a confounding effect on the study treatments and outcomes. Ultrasound was performed to evaluate fetal presentation, growth, placental location, amniotic fluid volume, and to exclude fetal malformations. Women with placenta previa, fetuses estimated to be below the 10th percentile of weight for gestational age, or fetal malformations were excluded.

Eligible and consenting women were assigned to receive either intravenous and oral antibiotics or indistinguishable placebos, using a randomization scheme provided to the research pharmacy at each clinical Center by an independent
data coordinating Center. The antibiotic regimen consisted of Ampicillin (2g IV q6h) and Erythromycin (250 mg IV q6h) for 48 hours, followed by Amoxicillin (250 mg PO q8h) (Warner Chilcott; Morris Plains, NJ) and Erythromycin-base E-Mycin (333 mg PO q8h) (Boots Laboratories; Lincolnshire, IL) for 5 days. Study medications were to be taken for 7 days unless delivery occurred sooner. Study participants with positive urine cultures or Neisseria gonorrhea cultures received appropriate antibiotic treatment in addition to their study medications. Women with positive group B streptococcus (GBS) cultures also received a 7-day course of oral Ampicillin (500 mg PO q6h) and received intrapartum intravenous Ampicillin prophylaxis (2 g IV q6h) in addition to their study medications.

Participating women were expectantly managed in hospital unless fluid leakage stopped and amniotic fluid volume returned to normal. Women underwent assessment for evidence of intrauterine infection including daily clinical assessment and examination while on study medications. Fetal well-being was evaluated by daily nonstress testing with biophysical profile scoring as needed, during the first week. Subsequent fetal evaluation was performed at least twice weekly until delivery unless leakage subsided and fluid returned to normal. Corticosteroid and tocolytic therapy were not permitted after enrollment. Elective delivery was prohibited prior to 34 weeks 0 days gestation and discouraged thereafter. The pediatric caregivers were masked as to the study arm and agreed not to alter neonatal management because of the possibility of prenatal antibiotic exposure. Infants were followed until death or until discharge to home or a chronic care facility. Neonatal head sonograms were performed routinely on infants with a birth weight less than 1,750 g, and on larger weight infants when clinically indicated. Neonatal management was otherwise left to the discretion of the neonatal caregiver.

At 7 Centers a single prechilled ethylene diamine tetra-acetate (EDTA)-tube of maternal blood was drawn and spun at 4C under 200xG for 5 minutes at the time of randomization and of delivery. The plasma supernatant was stored in aliquots at −70C, and subsequently shipped to a central storage facility. Umbilical cord blood was similarly collected and stored.

The sample size was based on an anticipated 35% incidence of primary outcome in the placebo group of the GBS-negative cohort, a one-third reduction with antibiotic treatment, a non-compliance rate of 10%, a two-tailed alpha of 0.05, and beta of 0.20. Because we planned to give Ampicillin to GBS carriers once identified in both the study antibiotic and placebo arms of the trial, we anticipated a lesser effect of antibiotic treatment in the GBS-positive stratum, and sample size was determined based on the GBS-negative stratum. It was determined that 600 women would be required in the GBS-negative cohort to have adequate power to assess the impact of antibiotics on infant morbidity. An external Data Monitoring Committee reviewed interim analyses for safety and efficacy. Statistical analyses included the Pearson chi-square test, Fisher’s exact test, the Wilcoxon rank sum test, and the Wei-Lachin test. The log-rank test was used to compare the survival distributions of the study groups. Stratified analyses (Mantel-Haenzel and Wei-Lachin) were used for the combined culture cohort after performing the Breslow-Day test for heterogeneity. In addition, Cox’s Proportional Hazards model was used to test the difference in latency between treatment groups, controlling for culture cohort. All patients were analyzed within their original study group, regardless of compliance to therapy or confounding treatments. The critical P value for the primary outcome in the final analysis was 0.048 (two-tailed).

### Results

Between February 1992 and January 1995, 614 women with pPROM at 24^0^-32^0^ weeks’ gestation consented to participation in this trial. Three were lost to follow-up, leaving 611 pregnancies for evaluation. There were 496 women within the GBS-negative cohort (83% of the anticipated recruitment goal). Because of changes in clinical practice regarding GBS prophylaxis and the 1994 NICHD consensus conference recommendation that corticosteroids be administered to patients with premature rupture of membranes, the Data Monitoring Committee recommended discontinuation of recruitment for the trial. Fortunately, because a higher incidence of primary outcome was seen in the placebo group than anticipated, the study had achieved adequate power to evaluate the primary outcome.
Primary Analysis

The primary analysis of this trial was published in the Journal of the American Medical Association in 1997.\textsuperscript{15} Analysis of outcomes for all 611 pregnancies demonstrated significant reductions in the incidences of Composite morbidity (44% \(v\) 53%, relative risk 0.84, \(P = .037\)), RDS (41% \(v\) 49%, relative risk 0.83, \(P = .037\)) and stage 2-3 necrotizing enterocolitis (2% \(v\) 6%, relative risk 0.40, \(P = .030\)) in the antibiotic group (Table 1). The relative risks of Grade 3-4 IVH (0.82) and early onset sepsis (0.83) after maternal antibiotic treatment were similar to those seen for primary outcome and RDS. In the GBS-negative cohort composite morbidity was significantly reduced in pregnancies assigned to antibiotic treatment (45% \(v\) 55%, relative risk 0.82, \(P = .030\)). Individually, respiratory distress syndrome was less frequently diagnosed among infants of mothers receiving antibiotic therapy (41% \(v\) 51%, relative risk 0.80, \(P = .025\)). Infants of mothers assigned to antibiotics had a 0.66 relative risk of having more than one component of the primary outcome when compared with those in the placebo group (95% confidence interval 0.43-1.04, \(P = .07\)). As anticipated, the incidences of Composite morbidity in both the antibiotic and placebo groups of the GBS-positive cohort were similar to that in the antibiotic group of the GBS-negative cohort as both study groups received antepartum and intrapartum Ampicillin in addition to their study medications. No significant improvement could be identified with additional study antibiotic treatment in the GBS-positive cohort. However, given the relatively small number of GBS carriers, the power of this finding was low.

Analysis of the total study population for other infant morbidities not comprising the composite morbidity revealed significant reductions in bronchopulmonary dysplasia, and patent ductus arteriosus in those assigned to antibiotics. In the GBS-negative cohort, maternal antibiotic therapy was also associated with a significant reductions in the incidences of documented neonatal sepsis and late onset sepsis (>72 hours after delivery), pneumonia and early onset pneumonia (within 72 hours of delivery), and noninfectious outcomes such as patent ductus arteriosus, bronchopulmonary dysplasia, and hyperbilirubinemia (67\% \(v\) 75\%, \(P = .042\), relative risk 0.89). Again, no improvement in other infant morbidities was identified with additional study antibiotic treatment in the GBS-positive cohort.

Survival analysis for the total study cohort

Table 1. Infant Mortality and Selected Morbidities (%) According to Study Group Assignment and the Presence or Absence of Positive GBS Carrier Status

<table>
<thead>
<tr>
<th></th>
<th>GBS-negative Stratum</th>
<th>GBS-positive Stratum</th>
<th>Total Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antibiotics Placebo</td>
<td>Antibiotics Placebo</td>
<td>Antibiotics Placebo</td>
</tr>
<tr>
<td>N</td>
<td>238 257 61 55 299 312</td>
<td>238 257 61 55 299 312</td>
<td>238 257 61 55 299 312</td>
</tr>
<tr>
<td>Composite Morbidity</td>
<td>44.5 54.5 0.030 0.82</td>
<td>42.6 45.5 0.759 0.94</td>
<td>44.1 52.9 0.037 0.84</td>
</tr>
<tr>
<td>RDS</td>
<td>40.8 50.6 0.025 0.80</td>
<td>39.3 40.0 0.879 0.97</td>
<td>40.5 48.7 0.037 0.83</td>
</tr>
<tr>
<td>PDA</td>
<td>11.8 21.0 0.005 0.56</td>
<td>11.5 16.4 0.424 0.69</td>
<td>11.7 20.2 0.004 0.58</td>
</tr>
<tr>
<td>BPD</td>
<td>14.3 21.0 0.048 0.68</td>
<td>8.2 18.2 0.101 0.44</td>
<td>13.0 20.5 0.014 0.64</td>
</tr>
<tr>
<td>IVH</td>
<td>18.9 23.7 0.191 0.80</td>
<td>19.7 12.7 0.394 1.52</td>
<td>19.1 21.8 0.418 0.88</td>
</tr>
<tr>
<td>Grade 3–4</td>
<td>5.9 8.2 0.321 0.72</td>
<td>8.2 5.5 0.721 1.48</td>
<td>6.4 7.7 0.515 0.82</td>
</tr>
<tr>
<td>Sepsis ≤72 hours</td>
<td>8.4 15.6 0.014 0.54</td>
<td>23.0 12.7 0.166 1.77</td>
<td>11.4 15.1 * *</td>
</tr>
<tr>
<td>Death ≤72 hours</td>
<td>4.6 7.0 .025 0.66</td>
<td>8.2 3.6 .445 2.21</td>
<td>5.4 6.4 .563 .83</td>
</tr>
<tr>
<td>Pneumonia ≤72 hours</td>
<td>3.8 9.7 0.099 0.39</td>
<td>14.8 9.1 0.368 1.59</td>
<td>6.0 9.6 * *</td>
</tr>
<tr>
<td>NEC</td>
<td>2.9 7.0 .038 0.42</td>
<td>8.2 3.6 .445 2.21</td>
<td>4.0 6.4 .173 .61</td>
</tr>
<tr>
<td>NEC &gt;72 hours</td>
<td>0.4 4.7 .003 0.09</td>
<td>6.3 0.0 .121 1.7</td>
<td>1.7 3.8 * *</td>
</tr>
<tr>
<td>NEC ≥72 hours</td>
<td>2.5 2.3 .089 1.08</td>
<td>1.6 3.6 .600 0.44</td>
<td>2.3 2.6 .846 .91</td>
</tr>
<tr>
<td>Stage 2–3</td>
<td>8.0 7.8 .933 1.03</td>
<td>8.2 12.7 .404 0.63</td>
<td>8.0 8.7 .745 .92</td>
</tr>
<tr>
<td>Death</td>
<td>2.1 5.4 .053 0.39</td>
<td>3.3 7.3 .417 0.44</td>
<td>2.3 5.8 .030 0.40</td>
</tr>
</tbody>
</table>

* Breslow-Day test for heterogeneity: \(P < .05\), no stratified relative risk or \(P\) value calculated.

Abbreviations: GBS, Group B streptococcus; R.R., relative risk; RDS, respiratory distress; PDA, patent ductus arteriosus; BPD, bronchopulmonary dysplasia; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis.
revealed significant improvement in latency ($P < .001$) in the group assigned to antibiotics. There was also significant prolongation of latency for those assigned to antibiotics in the GBS-negative cohort ($P < .001$; Fig 1). This group had a prolonged median time to delivery (6.1 v 2.9 days, $P < .001$). Significantly more women remained pregnant at each day between 2 days and 3 weeks after randomization. Particularly striking is that the survival curve of the antibiotic treated group did not return to baseline after completion of the antibiotic regimen on day 7, suggesting antibiotics to effectively treat or prevent infection rather than to just suppress infection until treatment is stopped. Infant birth weight ($1,549 \pm 497$ g v $1,457 \pm 508$ g, $P = .029$) was also higher with antibiotics in the GBS-negative cohort. No improvement in latency or birth weight was seen with additional antibiotics in the GBS-positive cohort. The antibiotic group had a lower incidence of clinical amnionitis in the total population (23% v 33%, $P = .010$) and the GBS-negative cohort (24 v 34%, $P = .012$). There were no cases of pseudomembranous enterocolitis, maternal sepsis, or maternal deaths. One maternal yeast infection was identified in the antibiotic group. There were eight cases of neonatal candida sepsis; five in the placebo group (1.6%) and 3 in the antibiotic group (0.7%, $P = .446$). Compliance with intravenous therapy was acceptable in both study groups, but was significantly lower in the antibiotic group (84 v 89%, $P = .046$). Oral therapy was associated with infrequent side effects, excellent compliance, and no reduction in compliance in the antibiotic group (93% v 94%, $P = .660$).

In summary, antibiotic treatment of expectantly managed women with pPROM at 240-320 weeks' gestation will reduce infectious and gestational age dependent infant morbidity. Treatment leads to less frequent clinical amnionitis and significantly enhanced pregnancy prolongation. The patient presenting with pPROM and unknown GBS culture status can be counseled that her fetus could benefit from such intervention.

This trial provided us with an opportunity to consider a number of additional issues. While benefit of treatment is evident in those who are not GBS carriers and in the overall population, it is not apparent from this study that benefit can be accrued by GBS carriers. However, this study was not designed to have adequate power to address the potential benefits of additional broad spectrum therapy over-and-above Ampicillin therapy alone in this subgroup, and the power of this finding is low. Because of this, we do not currently recommend that GBS carriers be treated differently than non-carriers or those of unknown status, other than the need for repeated intrapartum prophylaxis of those without a recent negative ano-vaginal culture for GBS, regardless of prior treatment.16

We chose a composite morbidity as the primary outcome for this trial. It is fortunate that we found similar trends towards a reduction in morbidity for all outcomes studied with antibiotic treatment. Had antibiotics reduced gestational age dependent morbidity through pregnancy prolongation but increased infectious morbidity through increased infectious morbidity, an overall benefit might not have been seen. In choosing a composite morbidity, it is important to consider that all components may not be equally common and that a relatively minor morbidity might overshadow others. In this study, and although RDS was by far the most common morbidity, it was fortunate that a similar trend towards reduced morbidity for this and virtually all other morbidities in the total population and GBS-negative stratum. The inconsistent findings in the GBS-positive stratum may reflect the small sample size in this subgroup, but could also be related to an undetermined cause in this group.
The efficacy and safety of corticosteroid administration for fetal maturation in the setting of pPROM has been questioned because of the brief latency and concern regarding the potential for increased neonatal infection. When this study was initiated, the majority of physicians in the United States and the majority of physicians in the participating clinical Centers did not give corticosteroids to women with pPROM. By prohibiting corticosteroid treatment within this protocol, we were able to show a direct correlation between antibiotic therapy and less frequent RDS. This effect is likely caused by the significant prolongation of pregnancy with antibiotic treatment. The NICHD consensus conference on the use of corticosteroids recommended corticosteroid administration in order to reduce intraventricular hemorrhage after pPROM at less than 30 to 32 weeks.\(^1\) The propagation of antibiotic therapy reduced over- dence interval; 1.95, 0.83–4.59) or neonatal infection (7.0 v 6.6%, odds ratio, 95% confidence interval 1.05, 0.66–1.68) after pPROM.\(^8\) Similarly, it is not clear that tocolytic therapy in the setting of pPROM offers significant neonatal benefit. It could be hypothesized that tocolytic therapy during initial antibiotic treatment and corticosteroid administration, could suppress short-term uterine activity to allow more adequate antibiotic and antenatal corticosteroid effects. The utility of concurrent antibiotic treatment and tocolysis in the setting of pPROM should be evaluated further in prospective clinical trials.

Ancillary Studies

Maternal and Fetal Systemic Inflammation, Amnionitis, and Perinatal Outcomes

Regardless of antibiotic treatment, infection is common after pPROM remote from term. In this study, amnionitis (at least 2 of: unexplained uterine tenderness, fever >100.4°F, foul vaginal discharge, with blood cell count >20,000/mL, maternal or fetal tachycardia), early neonatal sepsis, total sepsis, and early pneumonia complicated 28.1%, 5.7%, 12.8%, and 2.7% of pregnancies, respectively. To evaluate the impact of amnionitis on neonatal infection after conservative management of pPROM, and to determine the impact of antibiotic treatment before amnionitis on infant morbidity, we evaluated neonatal infectious outcomes based on the occurrence of amnionitis as well as antibiotic treatment group.\(^9\) While antibiotic treatment reduced the incidence of amnionitis from 32.8% to 23.2% (\(P = .01\)), amnionitis was associated with increased neonatal sepsis (19.4 v 10.2%, \(P = .004\), relative risk = 1.8), and early neonatal sepsis (9.7 v 4.1%, \(P = .01\), relative risk = 2.2). Study antibiotic treatment reduced neonatal sepsis in GBS negative gravids not developing amnionitis (5.9 v 13.4%, \(P = .01\), relative risk = 0.43) but not in those developing amnionitis or in GBS carriers. A similar trend was seen for early sepsis (2.5% v 5.8%, \(P = .07\), relative risk = 0.43). Study antibiotic treatment reduced overall pneumonia after amnionitis (2.9% v 11.4%, \(P = .04\), relative risk = 0.28), but not early onset pneumonia (1.4% v 4.8%, \(P = .23\), relative risk = 0.61). While study antibiotics do decrease
the risk of amnionitis and neonatal sepsis, the risk of neonatal sepsis should amnionitis occur is similar to that of infants not exposed to study antibiotics before birth. This may in part be due to the fact that most women with clinical amnionitis receive antibiotics in labor, potentially negating the impact of prior exposure in this high-risk population. The lack of impact in the GBS-positive stratum may again reflect confounding antibiotic treatment in the placebo arm due to the study policy of routine ante- and intrapartum treatment with Ampicillin.

Amniotic fluid cytokines, particularly Interleukin-6 (IL-6) and granulocyte colony stimulating factor (G-CSF), have been shown to correlate with intrauterine infection and neonatal sepsis after pPROM. Given the high risk of infectious morbidity after pPROM, we correlated maternal and neonatal plasma cytokines with perinatal morbidity and evaluated the impact of antibiotic treatment on plasma cytokine levels. Plasma samples were obtained from 228 women at randomization, 125 women at delivery, and from 196 umbilical cord blood samples at delivery. Tumor necrosis factor-alpha (TNF-alpha), IL-6, and IL-10 (Endogen Inc, Woburn, MA), and G-CSF (R & D Systems, Minneapolis, MN) were analyzed in batch fashion after storage at −70°C. Results were evaluated based on the presence or absence of clinical amnionitis, neonatal sepsis, neonatal pneumonia, or composite neonatal morbidity. For those with paired samples, cord blood levels were significantly higher than maternal levels at randomization and delivery for G-CSF, and for IL-6, P ≤ .0001 for each. Maternal IL-10 levels were low on admission, and significantly higher at delivery than in cord blood, P = .0001. TNF-alpha levels were rarely positive. Maternal G-CSF, IL-6, and IL-10 levels at delivery and cord blood levels were not significantly decreased with antibiotic treatment. Maternal cytokine levels at randomization were not predictive of amnionitis or neonatal morbidity. Maternal delivery and cord blood levels of G-CSF, IL-6, and IL-10 were elevated in the presence of amnionitis, P < .003. G-CSF and IL-6 levels were higher in cord bloods of infants subsequently suffering composite morbidity. G-CSF levels were elevated in cord blood of infants developing sepsis, P = .001, and cord blood IL-6 levels were also higher with subsequent early sepsis but not over-all sepsis. Because adhesion molecules play an important role in the interaction between neutrophils and the vascular endothelium in response to inflammation, and because levels of circulating adhesion molecules are increased in adults and neonates with sepsis, we sought to evaluate the relationships between circulation intercellular adhesion molecule-1 (cICAM-1) pPROM, antibiotic treatment and perinatal morbidity. cICAM-1 levels (R&D systems) were determined in batch fashion, concurrent to cytokine analysis of samples (as described above). Maternal cICAM-1 levels were higher than those in cord blood. Similarly to cytokine levels, maternal cICAM-1 levels on admission were not associated with adverse maternal or neonatal outcomes and study antibiotic treatment had no apparent impact on maternal cICAM-1 levels at delivery. Umbilical cord blood cICAM-1 levels were higher in those with amnionitis, but were not predictive of infant morbidity. Serum ferritin in asymptomatic women has been found to be predictive of subsequent preterm birth and may be elevated in response to an acute-phase reaction to systemic inflammation. Based on these observations, we hypothesized that maternal ferritin levels might be elevated in those destined to develop clinical amnionitis and/or neonatal morbidity. Analysis of plasma samples revealed maternal ferritin levels to double between randomization and delivery (18.6 v. 38.5 ng/mL, P < .001). Ferritin levels did not rise as much in the antibiotic study group (16.8 v. 21.4 ng/dL), a finding that did not reach statistical significance (P = .11). Ferritin levels were not predictive of or altered in the presence of amnionitis, and did not predict brief latency. However, mothers whose infants subsequently developed sepsis had significantly higher plasma ferritin levels at randomization (23.9 v. 18.3 ng/mL, P = .004) and at delivery (68.5 v. 32.5 ng/mL, P = .008). Plasma ferritin levels at randomization and delivery did not correlate strongly with the previously described cytokines or cICAM-1, other than a weak association with IL-6 at randomization (r = .17, P = .01). Ferritin levels at randomization did correlate significantly with hemoglobin and hematocrit levels (r = .25, P = .0002), but maternal ferritin levels at delivery did not.
In summary, we found antibiotic treatment to reduce the incidence of clinical amnionitis and that amnionitis was significantly associated with an increased risk of neonatal sepsis. Antibiotic treatment reduces neonatal sepsis in the GBS-negative stratum, but not those developing amnionitis or in the GBS-positive stratum. Early pneumonia was decreased with antibiotics for those with amnionitis. Because subsequent development of amnionitis cannot be predicted accurately on admission and those developing amnionitis do benefit from a reduction in neonatal pneumonia, because GBS carrier status is generally unknown on admission, and because our findings regarding the GBS-positive stratum lack adequate power, we recommend initiation of broad spectrum treatment on all patients treated conservatively with pPROM remote from term. Maternal IL-6 and G-CSF levels are increased in the presence of clinical amnionitis, as is neonatal cICAM-1. Neonatal IL-6 and G-CSF levels are higher than maternal levels, and increased in cord blood when composite morbidity subsequently occurs. Cord blood IL-6 levels are associated with early sepsis. This study supports and further develops data from previous work demonstrating a correlation between perinatal infection after pPROM and the presence of maternal and neonatal systemic inflammation, and provides new insight regarding the link between plasma ferritin and subsequent neonatal sepsis. Although maternal plasma evaluation does not carry adequate predictive value to be used clinically, umbilical cord blood sampling for inflammatory cytokines may be helpful in predicting subsequent neonatal morbidity. There are accumulating data linking perinatal infection to neurologic complications. Cerebral palsy and cystic periventricular leukomalacia have been linked to the presence of amnionitis, and elevated amniotic fluid cytokines. Fetal systemic inflammation, which may accompany or reflect maternal or fetal infection, has been associated with brain lesions such as periventricular leukomalacia and/or the subsequent development of cerebral palsy.25-28 Currently, there are no data to suggest that immediate delivery of the candidate for conservative management after pPROM will prevent these sequelae.

Histologic Features and Impact of Antibiotics on Placental Histology after pPROM

Concurrent to the clinical trial, 2 evaluations of placental histology were undertaken. First, data were collected from specimens collected for routine histopathologic assessment based on clinical indication. Second, an ancillary study of placental histopathology was performed by perinatal pathologists masked to study arm and clinical findings at delivery.

Data from a total of 488 clinical specimens (79% of participants) were available for review.29 In this evaluation, there was no evident difference in the frequency of placental (24% vs 32%) or membrane (84% vs 85%) inflammation with antibiotic exposure. Latency was shorter when membrane inflammation was present (6.7 vs 14.6 days, \( P < .001 \)) but no correlation with placental histology was identified. Clinical amnionitis was 5-fold more common for those women with membrane and placental inflammation (odds ratio 4.9, 95% confidence interval 1.9–2.4, \( P = .0009 \)). However, after controlling for study drug exposure, latency to delivery, GBS carriage, and gestational age at delivery, there was no association between placental or membrane inflammation and composite morbidity, RDS, IVH, necrotizing enterocolitis, sepsis, or perinatal death.

In the first phase of the ancillary study, the membrane rupture site was sampled by inking the open sac margin and rolling a membrane strip in 4 quadrants from the ink to the placental margin.30 Specimens were sent for central masked review by 4 pathologists who initially used a provisional feature list to score the slides from 15 placentas. After review of the preliminary scoring results, it was determined that 29 features of membrane histology could be objectively described with agreement among the pathologists (Table 2). The feature list allows both novel and commonly recognized histologic features of fetal membranes to be recorded with objectivity, and could be a useful tool for clinical correlation in studies of membrane rupture, especially those evaluating preterm PROM.

Subsequently, the ancillary study evaluated the hypotheses that membrane histology would show distinct differences between women with term and preterm rupture of membranes, and
that antibiotic therapy would alter the histologic appearance of fetal membranes in pPROM. \(^{31}\) Placental membranes were sampled from 268 women participating in the placebo-controlled trial, and from 4 control groups who were not in the trial, including: 1) preterm labor without pPROM (n = 21); 2) term labor (n = 65); 3) term PROM (n = 21);and 4) term cesarean section (n = 27). The cases and controls were scored for histologic features by pathologists masked to the identity of each sample (case or control). Histopathologic evidence of acute inflammation was seen more frequently with pPROM than in other preterm or term controls. Comparison of samples from patients participating in the randomized trial revealed no histological differences between pPROM cases treated with antibiotic and those receiving placebo. Further there was no evident difference in membrane histology for those with membrane rupture greater or less than 48 hours.

In summary, it is apparent that pregnancy complicated by pPROM is more frequently associated with acute inflammation than matched controls, that membrane histology does not correlate to duration of membrane rupture, and that membrane inflammation is associated with shorter latency. Membrane or placental inflammation is associated with amnionitis in the setting of pPROM, but not with the frequency of infant morbidities. Aggressive antibiotic treatment of pPROM remote from term does not alter the incidence of acute placental or membrane inflammation. Because the clinical benefits regarding amnionitis with antibiotic treatment are not evident at the histopathologic level, it is likely that pre-existing acute inflammation is not reversed by antibiotic treatment, or that antibiotic treatment does not prevent the cascade of histopathologic inflammation from progressing in the setting of pPROM. In either case, it appears that antibiotic treatment during conservative management of pPROM remote from term does not reduce the likelihood of fetal exposure to intrauterine inflammatory milieu. While such exposure might predispose to long-term neurologic sequelae (see above), the lack of correlation between acute inflammation and duration of membrane rupture suggests that early delivery after pPROM will not necessarily prevent exposure to this environment or these outcomes.

### Secondary Analyses

#### Factors Altering Outcome after pPROM Remote from Term

Because antibiotics reduce but do not eliminate the risk of perinatal morbidity after pPROM, and because conservative management of the initially asymptomatic patient carries the risk of subsequent complications such as clinical amnionitis, abruptio placentae, and fetal loss or distress due to umbilical cord compression, we...
sought to identify factors that might influence latency, infection, and perinatal morbidity after conservative management.\textsuperscript{32} Multivariable regression analysis was used to evaluate the impact of medical and obstetric factors on outcomes including: latency, amnionitis, composite infant morbidity, RDS, sepsis, and pneumonia. As anticipated, increasing gestational age at randomization was associated with less amnionitis and infant morbidity. Antibiotic treatment continued to significantly reduce infant morbidity after controlling for other potentially confounding factors. Antibiotic use before membrane rupture was also associated with improved latency ($P = .01$). Regarding amnionitis and infant morbidity: socioeconomic, infectious, and racial factors played significant roles. Gravidas with prior preterm deliveries had less composite morbidity and RDS ($P < .001$ for each). Antibiotics, increasing gestational age at pPROM, and marriage were significantly associated with decreasing neonatal sepsis. Antibiotic treatment significantly reduced amnionitis in uninsured gravidas, odds ratio: 0.44, $P < .01$). However, significant confounding interactions were identified. Subsequent analysis of singleton gestations revealed that vaginal bleeding before pPROM was not associated with shorter latency, amnionitis or perinatal death, but was associated with a significantly higher frequency of RDS (odds ratio = 3.1; 95% confidence interval 1.5-6.7; $P = .004$) and a trend towards increased abruptio placentae after pPROM (odds ratio = 2.6; 95% confidence interval 0.9-7.4; $P = .07$).\textsuperscript{33} Although these analyses revealed statistical associations with subsequent outcome, they did not allow us to identify characteristics of such high predictive value that they could be used to guide treatment.

**Impact of Oligohydramnios after pPROM Remote from Term**

Prior studies have suggested that oligohydramnios is associated with adverse outcomes after pPROM. We attempted to further elucidate these associations, evaluate whether the four quadrant amniotic fluid index (AFI) or a single maximum vertical amniotic fluid pocket (MVP) was more useful in predicting outcome, and determine if amniotic fluid assessment on admission has adequate predictive value to identify those who should be candidates for expeditious delivery despite early gestational age at membrane rupture.\textsuperscript{34} Two-hundred and ninety eight gravidas had both an AFI and MVP obtained by ultrasound prior to randomization. For this analysis, we defined oligohydramnios as an AFI $< 5$ cm or an MVP $< 2$ cm. The impact of oligohydramnios on latency, amnionitis, composite morbidity, RDS, over-all sepsis, and pneumonia was analyzed by univariate and multivariate analysis, with logistic regression controlling for gestational age, GBS culture status, and antibiotic study group assignment. Sixty-six point eight percent of patients had an AFI $< 5$ cm and 47.3% had an MVP $< 2$ cm. Delivery occurred within 48 hours, 1 and 2 weeks in 32.9%, 63.8%, and 82.2% of women, respectively. Both low AFI and low MVP were associated with increased likelihood of delivery at each time interval ($p < 0.01$), but neither were significantly associated with amnionitis. Low AFI was not associated with increased composite morbidity, RDS, sepsis, or pneumonia. Univariate analysis revealed low MVP to be associated with increased composite morbidity (55.3% $v$ 38.9%, $P = .005$), and RDS (51.8% $v$ 34.0%, $P = .002$), and logistic regression revealed a statistically significant association between low MVP and latency, composite morbidity, RDS, and pneumonia ($P < .05$ for each). However, while oligohydramnios based on MVP is associated with shorter latency and increased infant morbidity after pPROM, the test result is not highly discriminative of those who will or will not suffer complications (Table 3). We do not recommend that decisions be made regarding

<table>
<thead>
<tr>
<th>AFI</th>
<th>MVP</th>
</tr>
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<tbody>
<tr>
<td><strong>Sensitivity (%)</strong></td>
<td>74.5</td>
</tr>
<tr>
<td><strong>Specificity (%)</strong></td>
<td>37.0</td>
</tr>
<tr>
<td><strong>Positive predictive value (%)</strong></td>
<td>36.7</td>
</tr>
<tr>
<td><strong>Negative predictive value (%)</strong></td>
<td>74.8</td>
</tr>
</tbody>
</table>

**Table 3.** Predictive Value of Low Amniotic Fluid Index (AFI $< 5$ cm) or Maximum Vertical Fluid Pocket (MVP $< 2$ cm) for Latency after Conservative Management of pPROM $\leq$32 weeks 0 days.
the management of pHROM remote from term solely based on the presence of oligohydramnios.

Impact of Digital Examination after pHROM

In 2000, Alexander et al³⁵ performed an analysis of all women participating in the randomized trial and the 183 eligible women participating in the observational study to determine the effects of digital cervical examination on maternal and neonatal outcomes. Women with either 1 (n = 161) or 2 digital cervical examinations (n = 27) were compared with those having no digital examinations (n = 606) on initial assessment after pHROM. While latency from rupture to delivery was shorter in the digital examination group (median 3 vs 5 days; \( P < .009 \)), there were no evident differences in amnionitis (27% v 29%; \( P = .69 \)), endometritis (13% v 11%; \( P = .5 \)), or wound infection (0.5% v 1%; \( P = 1.000 \)). Infant outcomes including composite morbidity (56% v 48%; \( P = .10 \)), early sepsis (6% v 5%; \( P = .68 \)), respiratory distress syndrome (51% v 45%; \( P = .18 \)), intraventricular hemorrhage (7% v 7%; \( P = .67 \)), necrotizing enterocolitis (5% v 3%; \( P = .19 \)), and perinatal death (7% v 5%; \( P = .45 \)) were also similar in the two groups. Multivariable analysis adjusting for antibiotic study group, group B streptococcal culture status, race, and maternal transfer did not modify these results. Thus, while digital examinations are discouraged due to their effect on latency, the performance of 1 or 2 examinations should not be considered criteria for expeditious delivery in the absence of other findings.

Estimation of Birth Weight after pHROM Remote from Term

Oligohydramnios after membrane rupture has also been implicated as a factor leading to inaccurate assessment of fetal weight. We performed an analysis of 237 women delivering within 72 hours of a ultrasound estimating fetal weight, lie, and placental location to determine if these factors would alter the predictive accuracy of ultrasound for infant birth weight.³⁶ Other study parameters included Center of care, AFI and MVP. With a mean birthweight of 1377 ± 453 g, the mean percentage absolute error between estimated weight and actual birth weight was 10.3% ± 7.8% (Table 4). Ultrasound accuracy did not vary significantly with birthweight (\( P = .22 \)), estimated gestational age (\( P = .19 \)), or between Centers (\( P = .76 \)). Nor was accuracy affected by fetal presentation (Vertex, 10.2%; Breech, 10.6%; Transverse, 10.1%; \( P = .96 \)). Ultrasound was not less accurate with an AFI < 5 cm (10.6% v 10.3%, \( P = .85 \)) or an MVP < 2.0 cm (11.3% v 9.3%, \( P = .12 \)). Given the average error in ultrasound estimation of birth weight of ~10%, particular attention should be given to counseling based on this finding near the limit of fetal viability.

Prediction of Pulmonary Maturity after pHROM Remote from Term

While a “mature” fetal pulmonary maturity study result is generally reliable when obtained at or near term, the predictive value of such testing remote from term is less well defined. To evaluate the reliability of transvaginally collected amniotic fluid assessment for fetal pulmonary maturity, we evaluated the outcomes of 91 singleton gestations delivered before 33 weeks’ gestation and within seven days of admission.³⁷ Thirty-five samples were assessed by foam stability test (FSI), 25 by lecithin/sphingomyelin (L/S) ratio, and 45 were tested using the FLM assay (Fluorescence polarization); 12 women had more than one test performed. A “mature” result was

<p>| Table 4. Mean Absolute Error (%) in Ultrasound Estimation of Birth Weight Based on Actual Birth Weight and Best Obstetrical Estimate of Gestational Age at Delivery |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Birth weight (grams)</th>
<th>Mean Absolute Error (%)</th>
<th>Standard Deviation</th>
<th>Gestation (weeks)</th>
<th>Mean Absolute Error (%)</th>
<th>Standard Deviation</th>
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<tr>
<td>&lt;750</td>
<td>9.5</td>
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<td>10.8</td>
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<tr>
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<td>7.4</td>
<td>26–27</td>
<td>8.0</td>
<td>6.6</td>
</tr>
<tr>
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<td>6.2</td>
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<td>8.5</td>
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<td>30</td>
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<tr>
<td>&gt;1750</td>
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</table>
defined as an FSI ≥ 47 an L/S ratio ≥ 2.0, or and FLM result ≥ 55 mg/g albumin. Testing carried a false “maturity” rate of at least 14% for each test (Table 5). Alternatively, with an “immature” result the risk of RDS was between 44% and 58%. All false positive results (infant with RDS after a mature test) occurred before 32 weeks gestation. Based on these findings, results from vaginal pool specimens should be interpreted with caution when delivery is anticipated before 32 weeks.

The ORACLE Trial

The NICHD-MFMU study should be considered in the context of a subsequent trial of adequate power to assess an antibiotic intervention for women with pPROM. In the ORACLE trial, Kenyon et al38 performed a large multi-arm, multicenter placebo controlled trial of oral antibiotic therapy for women with pPROM before 37 weeks’ gestation. Women were randomized to receive oral Erythromycin, Amoxicillin-clavulonic acid, both, or placebo for up to 10 days. Several hundred statistical analyses were performed in addition to the primary analysis of oral therapy given to women with pPROM before 37 weeks’ gestation. In summary, oral Erythromycin led to brief pregnancy prolongation (not significant at 7 days), decreased supplemental oxygen need (31% v 36%, P = .02), less frequent positive blood cultures (6% v 8%, P = .02), but no significant reduction in composite morbidity (one or more of: death, chronic lung disease, or major cerebral abnormality on ultrasonography), (13% v 15%, P = .08). Secondary analysis of singleton gestations revealed a reduction in oxygen dependence at 28 days (7% v 9%, P = .03), positive blood cultures (3% v 7%, P = .04), abnormal cerebral ultrasonography (3% v 5%, P = .04) and composite morbidity (11% v 14%, P = .02) with Erythromycin. Oral Amoxicillin-clavulonic acid increased latency (43% v 37% pregnant at 7 days, P = .005) and reduced supplemental oxygen need (30% v 36%, P = .05), but was associated with increased necrotizing enterocolitis (2% v 0.5%, P = .001) without reducing other neonatal morbidities. The combination of oral Amoxicillin-clavulonic acid and Erythromycin yielded similar findings. The authors suggested but did not present similar findings for the subgroup with pPROM remote from term. Based on these results, we conclude that oral antibiotic therapy with Erythromycin reduces perinatal morbidity when given to women with pPROM before 37 weeks gestation, but given the small differences in the actual incidences of morbidity in the study groups, many women would need to be treated to prevent one adverse outcome. While the finding regarding increased necrotizing enterocolitis with oral Amoxicillin-clavulonic acid is concerning, it is contrary to the NICHD-MFMU trial finding of reduced stage 2-3 necrotizing enterocolitis with aggressive antibiotic therapy in a higher risk population. Given this inconsistency, and the lack of a consistent trend toward a positive or negative effect of antibiotics on necrotizing enterocolitis in the published literature, further study is necessary.

The findings of the ORACLE trial regarding oral antibiotic therapy are not inconsistent with those of the NICHD-MFMU study. While oral erythromycin may offer some benefit, and there may be some risk associated with oral Amoxicillin-clavulonic acid treatment of women with pPROM, aggressive broad-spectrum intravenous and oral therapy of women with pPROM remote from term reduces gestational age dependent and infectious morbidity. As adequate markers are absent for those destined to deliver quickly regardless of conservative management or for subsequent intra-amniotic infection, we recommend broad spectrum intravenous and oral antibiotic treatment of women undergoing conservative management of pPROM remote from term. While the NICHD-MFMU trial does not address management of women with pPROM after 32 weeks 0 days gestation, the risks and limited benefits of conservative management near term, and gestational age appropriate outcome data regarding the consequences of preterm birth should be carefully considered before conservative management is undertaken.

Table 5. Predictive Value of Vaginally Collected Amniotic Fluid Specimens for Pulmonary Maturity after pPROM Remote from Term

<table>
<thead>
<tr>
<th>Method</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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<tr>
<td>FSI</td>
<td>35</td>
<td>86</td>
<td>43</td>
<td>86</td>
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<tr>
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<tr>
<td>FLM</td>
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<td>11</td>
<td>94</td>
<td>75</td>
<td>42</td>
</tr>
</tbody>
</table>

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References