Maternal Antibiotics and Decreased Periventricular Leukomalacia in Very Low-Birth-Weight Infants

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Objective: To investigate the effect of maternal antibiotics, given in the predelivery period, on neonatal outcomes.

Design: Retrospective cohort study.

Setting: A single level 3 neonatal intensive care unit.

Patients: All infants with birth weights 1500 g or less cared for from July 1994 to July 2000 (n=834) were included in the study. Mothers were classified as receiving antibiotics if they received any parenteral antibiotics in the predelivery period. Infants whose mothers received antibiotics were compared with infants whose mothers received no antibiotics.

Main Outcome Measures: The main outcome variables studied included intraventricular hemorrhage (IVH), cystic periventricular leukomalacia (PVL), sepsis, and mortality.

Results: Of 834 mothers, 374 (45%) received antibiotics prior to delivery. On univariate analysis, there were no differences in the relative risk (RR) of mortality (1.26; 95% confidence interval [CI], 0.86-1.79) or grades 3 to 4 IVH (RR, 1.39; 95% CI, 0.82-1.90) between the antibiotics and no-antibiotics groups. Infants born to mothers receiving antibiotics had an increased risk of culture-proven sepsis (RR, 1.4; 95% CI, 1.02-1.64) and a decreased risk of cystic PVL (RR, 0.26; 95% CI, 0.09-0.79) compared with infants whose mothers did not receive antibiotics. After controlling for confounding variables, maternal antibiotics were not associated with a decrease in the risk of mortality (adjusted risk [AR], 1.0; 95% CI, 0.5-2.1), grades 3 to 4 IVH (AR, 1.0; 95% CI, 0.5-1.9), or sepsis (AR, 0.9; 95% CI, 0.7-1.4). However, the use of maternal antibiotics was associated with a decreased risk of developing cystic PVL (AR, 0.09; 95% CI, 0.02-0.5).

Conclusions: In our population of very low-birth-weight infants, maternal antibiotics were associated with a decreased risk of cystic PVL. Maternal antibiotics do not change the risk of mortality, sepsis, or severe IVH.
The study was approved by the Investigational Review Board at Christiana Care Health System, Newark, Del. All consecutive live-born infants 23 weeks’ gestational age or older who were offered intensive care, had birth weights of 1500 g or less, and were born between July 1, 1994, and July 1, 2000, were included in the analysis (n=834). The neonatal intensive care nursery is a regional level 3 unit caring for both inborn (90%) and outborn infants.

 Mothers were classified as receiving antibiotics if they received any parenteral antibiotics during the birth hospitalization but before delivery. Mothers who received parenteral antibiotics during another hospitalization were not classified as receiving antibiotics. Antibiotics were further broken down by classification. The penicillin family of antibiotics included penicillin G, ampicillin, and ampicillin/clavulanic acid. Aminoglycoside coverage included gentamicin or tobramycin. Anaerobic coverage included clindamycin and metronidazole. Other macrolide coverage included erythromycin. Cephalosporins were also classified separately. Mothers received antibiotics for clinical infections such as chorioamnionitis, for suspected infections such as prolonged rupture of membranes, or due to preterm labor with unknown group B streptococcus status. Christiana Hospital, Newark, uses a culture-based approach at 35 to 36 weeks’ gestation to prevent group B streptococcus infection. Mothers presenting before this time received antibiotic prophylaxis at the discretion of the attending obstetrician based on clinical risk factors. However, infants transferred from other institutions may have been treated according to other protocols.

 For the purposes of this study, IVH was classified using the definition of Papile et al.10 Grades 3 to 4 IVH were considered to be severe. Cystic PVL was diagnosed only in the presence of echolucent cysts in the periventricular white matter. For the purposes of this study, echodense lesions in the periventricular white matter without subsequent cyst formation were not classified as PVL unless there was subsequent formation of echolucent cysts. Infants with ventricular expansion were also not classified as having cystic PVL unless there was formation of echolucent periventricular cysts. Infants who developed cystic PVL after developing IVH were classified as having both IVH and cystic PVL. Ultrasonography was performed using standard sagittal and coronal images through the anterior fontanel with portable ultrasonography machine (Acuson 128; Acuson, Mountain View, Calif) using a 7.5-MHz transducer. Cranial sonograms were obtained on the fourth day of life and then monthly until hospital discharge. Cranial sonograms were obtained more frequently if clinically indicated. All cranial sonograms were interpreted by a pediatric radiologist who was not aware of maternal antibiotic status.

A positive blood culture was required for a neonate to be assigned to the sepsis group. Culture-negative sepsis or sepsis-like syndromes were not considered in this analysis. Early-onset sepsis was defined as a positive blood culture in the first 72 hours of life. Late-onset sepsis was defined as a positive blood culture after the first 72 hours of life. Because of the infrequent occurrence of early-onset sepsis, early- and late-onset sepsis were also combined into a single variable of total sepsis for further analysis. Patent ductus arteriosus was diagnosed by echocardiography. Necrotizing enterocolitis was diagnosed in the presence of an abnormal radiograph and clinical symptoms with or without the need for surgical intervention. All infants had total thyroxine levels measured on the fifth day of life as part of the routine state of Delaware newborn screen. Thyroxine values were investigated because of the previous association of transient hypothyroxinemia with cystic PVL, IVH, and death.11,12 Bronchopulmonary dysplasia was defined as an oxygen requirement at 28 days of life.13 Clinical chorioamnionitis was diagnosed by the attending obstetrician in the presence of maternal fever, uterine tenderness, or foul-smelling fluid. Prolonged rupture of membranes was considered longer than 24 hours’ duration. The best obstetric estimate was used to classify gestational age. The modified Ballard examination was used as an estimate of gestational age only in the absence of obstetrical dating.14

Univariate analysis included 1-way analysis of variance for continuous variables, the χ² test for categorical variables, and the Mann-Whitney U test for ordinal variables and continuous variables with nonnormal distribution. Multivariate analysis was done using logistic regression. Odds ratios were converted to risk ratios using the method described by Zhang and Yu.15 Independent variables entered into the model included known confounders for the dependent variable and any variable with a P value less than .15 on univariate analysis. For each dependent variable, 2 models were created. In the first model, antenatal and postnatal variables were entered. In the second model, postnatal variables were eliminated. This was done because in a theoretical randomized study of antenatal antibiotic use, postnatal outcomes would not be known before randomization. Furthermore, it is possible that some of the postnatal variables tested may be part of the causal pathway of the tested dependent outcomes and were thus eliminated.16

Power analysis revealed that approximately 800 patients would be required to show a 30% reduction in mortality, severe IVH, or sepsis with 80% power and an α of .05. All data are expressed as mean±SD. P<.05 was considered significant. All statistics were calculated on commercially available software (Statistica; StatSoft, Inc, Tulsa, Okla).

## RESULTS

### UNIVARIATE ANALYSIS

A total of 834 infants were analyzed. Of the total cohort, 374 mothers (45%) received antibiotics. Of the mothers receiving antibiotics, 321 (86%) received at least one β-lactam antibiotic. Further breakdown included 318 mothers who received antibiotics in the penicillin family and 3 who received a cephalosporin; 99 mothers received erythromycin, 60 received anaerobic coverage, and 33 received an aminoglycoside. Many mothers received a combination of antibiotics in different categories, so the numbers sum to more than 100%.

Infants in the antibiotics group were more likely to be inborn, but they were less likely to be born by cesarean delivery or be small for their gestational age compared with infants whose mothers did not receive antibiotics. There were no differences in gestational age, birth weight, sex, race, or maternal age between groups (Table 1).

Infants in the antibiotics group were more likely to be born to a mother who had a fever, chorioamnionitis, or premature prolonged rupture of membranes and who had received antenatal steroids or magnesium sulfate compared with infants in the no-antibiotics group. Mothers who received antibiotics were less likely to have preeclampsia than were mothers in the no-antibiotics group. There were no differences in the occurrences of multiple gestations or oligohydramnios between groups (Table 2).

There was no difference in mortality between groups. Infants born to mothers who had received antibiotics were more likely to have any grade of IVH, but there was no...
difference between groups in the occurrence of severe IVH. However, infants in the antibiotics group were less likely to have cystic PVL and more likely to have sepsis (Table 3). There were 6 infants in the antibiotics group (1.6%) and 8 infants in the no-antibiotics group (1.7%) who died before undergoing head ultrasonography ($P = .83$). These infants were classified as not having IVH or PVL. Because the number of infants in this category was small and did not differ between groups, the data were not recalculated excluding these infants.

There were 183 infants in the combined cohort with one or more episodes of culture-proven sepsis during their hospital stay. Coagulase-negative staphylococci were the most common organism, accounting for sepsis in 110 infants (60%); 27 (15%) cases were caused by a Candida species, 23 (13%) were caused by gram-negative rods, including Escherichia coli and Pseudomonas species, and the remaining 23 (13%) cases were caused by other organisms, including group B streptococcus and Staphylococcus aureus. Of the infants with gram-negative sepsis, 70% of the mothers had received antibiotics compared with 46% of mothers whose infants had sepsis caused by all other organisms ($P = .05$). Of the infants with fungal sepsis, 32% of the mothers had received antibiotics compared with 51% of mothers with sepsis caused by bacteria ($P = .91$). There was no difference in the occurrence of early-onset sepsis in the antibiotics (3 [0.8%] of 374) and the no-antibiotics (6 [1.3%] of 456) groups ($P = .23$).

## MULTIVARIATE ANALYSIS

Results of the multivariate analysis are presented in Table 4. Antenatal variables controlled for included gestational age, intrauterine growth restriction, mode of delivery, premature prolonged rupture of membranes, maternal fever, preeclampsia, mother taking magnesium sulfate, chorioamnionitis, prenatal steroids, inborn status, and year of birth. When postnatal variables were controlled for, the following variables were included in the model: mechanical ventilation, bronchopulmonary dysplasia, patent ductus arteriosus, and thyroxine level on initial newborn screen. Sepsis, IVH, and PVL were also used as dependent variables. Maternal antibiotics were not associated with a decreased risk of mortality, sepsis, all IVH, or severe IVH. However, maternal antibiotics were associated with a decreased risk of cystic PVL when all variables or antenatal variables only and antenatal as well as postnatal variables.

## COMMENT

The main finding of our investigation is that very low-birth-weight infants born to mothers who receive parental antibiotics have a decreased risk of developing cystic PVL compared with infants whose mothers did not re-
Maternal antibiotics had no effect on the overall risk of neonatal sepsis, neonatal mortality, or IVH. Cystic PVL and cerebral palsy have both been associated with maternal chorioamnionitis. Similarly, rabbits injected with E. coli antenatally have been shown to develop brain injury. These epidemiologic and animal data suggest that antenatal infection and/or inflammatory changes can be a cause of brain injury in infants. Antibiotics are frequently indicated in the antenatal period for clinical conditions such as chorioamnionitis, urinary tract infection, prolonged premature rupture of membranes, and preterm labor. Antibiotics may also be used in the antenatal period to prevent infection with group B streptococcus. The effect of maternal antibiotics on neonatal outcomes remains controversial. In one recent meta-analysis, maternal antibiotics given to mothers in preterm labor with intact membranes demonstrated a prolongation of the latency period but an increase in neonatal mortality and no reduction in IVH. Another meta-analysis by Egarter et al showed a reduced risk of IVH when antibiotics were given to mothers with rupture of membranes. However, this meta-analysis excluded mothers who had received steroids prior to delivering. In a recent large prospective trial, Kenyon et al showed that oral erythromycin or amoxicillin/clavulanic acid given separately or in combination to mothers in preterm labor with intact membranes did not prolong the latency period and did not decrease neonatal mortality, sepsis, or ultrasonographically evident brain injury. In another study of mothers with preterm labor and ruptured fetal membranes, Kenyon et al showed that oral erythromycin prolonged the latency period, reduced sepsis, and reduced the rate of major cerebral abnormality. These findings were only present when multiple-gestation pregnancies were excluded. Furthermore, PVL was not analyzed separately from other cranial ultrasound abnormalities. In investigating risk factors for PVL, other studies have explored the association of neonatal white matter injury and maternal antibiotics and have shown increased maternal antibiotic usage in infants with PVL compared with infants without PVL. In these two studies, maternal antibiotic usage was not the primary focus of the investigations. Furthermore, maternal antibiotics were not included as part of the final multivariate analyses.

The importance of the association of maternal antibiotics and cystic PVL is highlighted by the close correlation between cystic PVL and the development of cerebral palsy. The association between maternal antibiotics and a reduced rate of cystic PVL is complex. From our data, we cannot determine whether maternal antibiotics are part of the protective pathway that reduces the occurrence of cystic PVL or are simply an associated factor. It is possible that treating mothers with antibiotics attenuates or prevents the fetal/maternal inflammatory response and leads to a decrease in brain injury. It is also possible that maternal antibiotics are acting as direct fetal anti-inflammatory agents. Some antibiotics, such as the macrolide class, have been shown to have direct anti-inflammatory properties. In our patient population, multiple antibiotic types were used for numerous medical indications. Therefore, maternal antibiotics may be a marker for other neuroprotective obstetrical factors. Although we controlled for different conditions such as preeclampsia, medications such as steroids and magnesium sulfate, and mode of delivery, among other variables, we were unable to control for other factors such as time of labor, cerebral blood flow changes, or associated changes in fetal heart rate. We also attempted to control for as many antenatal conditions as possible in which antibiotics are used, such as chorioamnionitis, premature prolonged rupture of membranes, and maternal fever. However, because maternal antibiotics were not randomly assigned, the association between cystic PVL and predelivery antibiotics may have resulted from confounding by indication. Confounding by indication has been described in other cohort studies in which the clinical indications for prescribing a drug, in this case maternal antibiotics, may have been potential confounders for the main outcome variables studied.

Despite the potential to alter neonatal colonization, our data do not indicate an overall increased risk of neonatal sepsis with maternal antibiotics. Because early sepsis occurs infrequently, our study was of insufficient power to detect the effect of prenatal antibiotics on developing early sepsis in a statistically meaningful way. Antibiotic therapy for group B streptococcus has been suggested to increase the risk of resistant gram-negative bacterial infection in the postnatal period. We did not explore resistance patterns in this study, but the rate of maternal antibiotic usage was higher among infants with gram-negative sepsis compared with those who developed sepsis caused by other organisms. Although there was no overall increase in the rate of sepsis with maternal antibiotics, the increased occurrence of maternal antibiotic usage associated with late-onset neonatal gram-negative infection provided indirect evidence that antenatal antibiotics may alter neonatal colonization. This potential to influence the type of neonatal infection must be considered when considering any future studies of maternal antibiotics. Neonatal fungal infection has been linked to postnatal antibiotic coverage. However, in our patient population, mothers of infants with fungal infection were not more likely to have received antibiotics in the predelivery period compared with mothers of infants with sepsis caused by bacterial infection.

Our study has a number of important limitations. We were unable to consider important information regarding the timing and length of antibiotic coverage because of the inconsistent availability of these data. However, our data are from a single center with a relatively uniform obstetrical practice. Mothers in our population were treated with antibiotics for heterogenous indications that included proven infection such as chorioamnionitis, suspected infection such as fever or prolonged rupture of membranes, or simply for prophylaxis against infections such as group B streptococcus. Because it was difficult to determine the exact indication for antibiotic administration retrospectively, we were unable to determine whether any differences in the indication for antibiotic usage influenced outcome. Nevertheless, because preterm labor and neonatal brain injury have both been linked to subclinical and proven infections, it is pos-
Previous research has shown a relationship between antenatal infection and postnatal brain injury. To date, studies exploring the relationship between antenatal antibiotics and postnatal brain injury have been inconclusive. Our study demonstrates a decrease in cystic PVL among infants born to mothers who have received antibiotics. These data add to the association between antenatal infection and neonatal brain injury and allow for hypothesis generation for future studies directed at reducing brain injury in the preterm infant.

In conclusion, our data show that maternal use of antibiotics for prophylaxis as well as proven infection may provide some neuroprotection to the fetus by treating or modifying subclinical infection. Our analysis was limited to the cystic form of PVL. Infants who may have had white matter injury manifesting with cranial sonographic findings of ventricular dilatation or periventricular echodensities were not included in this analysis. We limited our analysis to cystic PVL because of the certainty of establishing this diagnosis and the strong correlation of the cystic form of PVL with the outcome of cerebral palsy. Therefore, it is important to consider that there may have been infants with white matter injury in our study population who did not have ultrasonographic evidence of cystic PVL.

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