Intrauterine Exposure to Infection and Risk of Cerebral Palsy in Very Preterm Infants

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Objective: To evaluate exposure to intrauterine infection as an independent risk factor for spastic cerebral palsy (CP) among very prematurely born infants.

Study Design: Retrospective case-control study.

Methods: Singleton children with gestational ages less than 32 weeks and birth weights less than 1999 g who survived to age 2 years and were born from 1988 to 1994 in a level 2 or 3 hospital in California were included in the study. Cases were children with congenital spastic CP (n=170). Controls were children randomly sampled within 250-g birth weight intervals (n=270). Gestational age was controlled through multiple logistic models. Major analyses were controlled for preeclampsia and short time between admission and delivery.

Results: Neither clinical nor histologic indicators of intrauterine infection were associated with total spastic CP or spastic diplegia in these infants. Although not predicted by prior hypothesis, we observed an approximate doubling of risk for infants of infected mothers among children born to white women, whereas no association was noted among children born to women of other races/ethnicities. White controls had lower frequency of all measured infection indicators compared with white cases and cases and controls of other races/ethnicities.

Conclusion: Exposure to intrauterine infection was not an independent risk factor for CP in very premature infants when gestational age and other confounders were tightly controlled.


The risk of cerebral palsy (CP) is substantially higher among infants born preterm than in more mature infants: the younger the gestational age, the greater the risk.1,2 The reasons for the increased risk among prematurely born infants remains elusive. A possible, and commonly offered, explanation is intrauterine exposure to infection.3,5

Among prematurely born children, studies have consistently demonstrated increased risk of CP for children born to mothers with preterm labor or premature rupture of membranes compared with those born early because of preeclampsia.1,2,6-7 This relationship has been observed despite a variety of approaches to treatment and in the presence of appropriate control for gestational age.2 One possibility is that preeclampsia itself is “protective” against neurologic damage. Alternatively, preterm labor or premature rupture of membranes may increase risk. Intrauterine infection is frequently observed in association with preterm labor or premature rupture of membranes but only infrequently with preeclampsia, and it is tempting to hypothesize that intrauterine infection or the fetal response to it may contribute to long-term neurologic damage and CP. The gestational age group at highest risk for intrauterine infection is also the group at highest risk for CP, which is compatible with this hypothesis. In addition, reports of increased risk of CP8 and neonatal encephalopathy9 associated with maternal infection among normal birth weight infants make it tempting to assume a similar association among those born preterm.

Studies that have directly investigated the association between intrauterine infection and CP among prematurely born children have had inconsistent results; most report increased risk of CP among exposed infants,2,6,7,10-19 but others report no association.1,20-24 Results are also inconsistent with regard to an association between intrauterine infection and white matter damage on neonatal neuroimaging,20,23-36 a neonatal finding often associated with later-diagnosed CP in pre-
mature infants. The inconsistencies among studies are no doubt attributable in part to differences in populations and methods, including whether and how potential confounding factors, particularly gestational age and preeclampsia, are considered. A further complication is that studies of premature infants lack a “normal” control group for evaluation of risk factors because preterm birth itself is abnormal.

We report a retrospective case-control study focusing on a relatively homogeneous subgroup of very preterm infants at high risk for CP. This relatively large birth cohort approximates a population-based sample, with the exception of excluding births in level 1 hospitals. Within a study population previously selected for evaluation of tocolytic treatment, we sought to examine several indicators of intrauterine infection to determine if infants who were exposed to intrauterine infection were at greater risk for CP than those without such exposure.

METHODS

SUBJECTS

The study population was singleton children born between January 1, 1988, and December 31, 1994, with gestational ages less than 32 weeks (as verified by antenatal records) and birth weights less than 1990 g, who were delivered in 1 of the 22 hospitals offering level 2 or level 3 neonatal intensive care in the San Francisco Bay Area or the Northern or Central San Joaquin Valley of California and who survived to age 2 years. Children who met these criteria were initially identified from the live birth population of 7978 infants with birth weights less than 1500 g or from 1500 g to 1999 g and gestational ages less than 33 weeks.

Initial Identification of Cases and Controls

Children with possible CP were identified through linkage with the electronic client records of 2 state agencies known to enroll virtually all eligible children without regard to financial or citizenship status. Full medical record review was conducted to verify the presence of CP and characterize CP subtype. We defined CP as a chronic disability of central nervous system origin that was characterized by aberrant control of movement or posture, appeared early in life, and was not the result of a progressive disease. Children included as cases had mild (no functional impairment), moderate (some functional ability in the most-affected limb, although assistive devices may be used), or severe (no functional ability in the most-affected limb) congenital spastic CP. Because the intent was to assess risk associated with intrapartum factors, children were excluded if their neurologic condition was determined in the absence of preeclampsia, pregnancy-induced hypertension, or a severe condition that would determine the management of the delivery. (Prior cesarean delivery or breech presentation were not sufficient grounds for exclusion from this group.) “Tocolytic-eligible” women included those for whom delivery occurred more than 3 hours after admission in the absence of preeclampsia, pregnancy-induced hypertension, or a severe systemic disorder or previous operation that would determine the management of the delivery. Although controls were initially sampled in a ratio of 2 per case, a disproportionate number of control women were assigned to the tocolytic-ineligible group because preeclampsia was more common among them than among case women. Comprehensive data abstraction was concentrated on tocolytic-eligible mothers for whom multiple indicators of infection were obtained. For the tocolytic-ineligible group, a limited set of clinical data was abstracted to permit evaluation of clinical diagnoses of definite or suspected intrauterine infection. This data collection strategy was based on an a priori decision to include both tocolytic-eligible and tocolytic-ineligible women in analyses of the association of clinical diagnoses of intrauterine infection and congenital spastic CP. All completed abstraction forms were reviewed by a trained nurse abstractor.

Multiple indicators of maternal infection for women in the tocolytic-eligible group included clinical diagnoses documented in the medical record, individual signs or symptoms of infection as recorded during the admission for delivery up to 24 hours postpartum, culture data, inpatient treatment with anti-infective medications, and placental pathological findings. Copies of all available placental pathological reports were reviewed and coded by one of us (R.W.R.), an expert placental pathologist blinded to case status. Laboratory reports from blood,

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Final Determination of Case and Control Status

In premature children, neurologic status is commonly uncertain during the first year or two of life. Thus, we sought to verify later neurologic status through review of pediatric hospital and service agency records available until a minimum of age 4 years. Three children for whom an early diagnosis of CP was considered questionable were reclassified as controls, and 9 children initially sampled as controls were reclassified as having CP. Fifteen children sampled for the control group were identified as having a neurologic abnormality other than CP; these children were retained in the study but excluded from some analyses. From the larger study population of 7978 singleton survivors to age 2 years with birth weights less than 1500 g or from 1500 g to 1999 g and gestational ages less than 33 weeks, we identified 263 children with CP who had birth weights less than 1999 g and gestational ages less than 32 weeks (prevalence, 33/1000 live births). Further details of case and control ascertainment are provided elsewhere.
urine, or placental cultures were reviewed and coded by one of us (R.E.W.), an infectious disease specialist blinded to case status. Bacteria and viruses that commonly cause neonatal sepsis and death were classified as group 1, including group B streptococci, *Escherichia coli*, *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Listeria monocytogenes*, *Bacteroides fragilis*, *Pseudomonas aeruginosa*, and herpes simplex virus. Classified as group 2 were organisms that are frequent commensals and contaminants but may cause disease and organisms that often cause subclinical disease and are not detected unless expressly sought. Included in group 2 were *Mycoplasma hominis*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, or γ streptococci, *enterococci*, *coagulase-negative staphylococci*, lactobacilli, *Gardnerella vaginalis*, *Haemophilus* species, yeast, anaerobic organisms, and normal/mixed flora. 

Gestational age was abstracted based on measurements in the mothers' medical records that were known before delivery, and priority was given to dates established early in pregnancy and to ultrasonography performed before 19 weeks' gestational age. Children initially selected based on birth weight or gestational age data from birth certificates were excluded from the analysis if antenatally recorded gestational age in the mother's medical record was 32 weeks or more or the birth weight recorded in the newborn's medical record was 1999 g or more. Maternal self-identified race/ethnicity was as recorded on the infant's birth certificate.

The study was conducted with approval from the California Committee for the Protection of Human Subjects.

### STATISTICAL ANALYSIS

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional multiple logistic regression models (SAS statistical software; SAS Institute, Cary, NC) to estimate the relative risk between children with CP and control subjects with regard to measures of intrauterine infection; ORs were considered to represent a statistically significant association if the CIs did not include 1.0. All models included birth weight in 250-g strata and gestational age as a continuous variable. Gestational age was also evaluated with inclusion of a squared term and as a categorical variable using 2-week intervals. Because no substantial differences were found between these models with regard to the association of intrauterine infection and CP, results are reported only for models with gestational age as a continuous variable. Separate analyses were conducted for tocolytic-eligible women and for the entire study population. With the sample sizes available for analysis, this study has statistical power of more than 0.90 to observe a relative risk of 2.0 (1-sided α = .05) if the prevalence of intrauterine infection in the control population is 55%.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Children With CP (n = 170)</th>
<th>Controls (n = 270)</th>
<th>Adjusted Odds Ratio* (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, No. (%) of subjects</td>
<td>108 (64)</td>
<td>154 (57)</td>
<td>1.3 (0.9-2.0)</td>
</tr>
<tr>
<td>Gestational age, mean (SD), median, wk</td>
<td>27 (2.3), 27</td>
<td>27.3 (2.4), 27</td>
<td>0.83 (0.70-0.97)</td>
</tr>
<tr>
<td>Maternal age, mean (SD), median, y</td>
<td>27.6 (6.8), 28</td>
<td>26.7 (6.3), 27</td>
<td>1.0 (0.99-1.1)</td>
</tr>
<tr>
<td>Maternal race/ethnicity, No. (%) of subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>80 (47.1)</td>
<td>88 (32.6)</td>
<td>Reference</td>
</tr>
<tr>
<td>Hispanic</td>
<td>35 (20.6)</td>
<td>69 (25.6)</td>
<td>0.56 (0.33-0.91)</td>
</tr>
<tr>
<td>Black</td>
<td>39 (22.9)</td>
<td>86 (32.6)</td>
<td>0.49 (0.29-0.79)</td>
</tr>
<tr>
<td>Asian</td>
<td>10 (5.9)</td>
<td>11 (4.1)</td>
<td>0.99 (0.39-2.5)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (3.6)</td>
<td>10 (3.7)</td>
<td>0.54 (0.37-3.5)</td>
</tr>
<tr>
<td>Not recorded</td>
<td>1 (0.6)</td>
<td>6 (2.2)</td>
<td>...</td>
</tr>
</tbody>
</table>

*Adjusted for 250-g birth weight strata used to sample controls. Ellipses indicate not applicable.

### RESULTS

**Tocolytic-Eligible Women**

The population of singleton children born to tocolytic-eligible women included 170 children with spastic CP and 270 controls. Among children with CP, 91 (54%) had spastic diplegia, 45 (26%) had spastic quadriplegia, and 29 (17%) had hemiplegia; in 5 (3%) the CP subtype could not be classified. Mild CP was present in 54 children with CP (32%), moderate CP in 61 (36%), severe in 51 (30%), and severity was unknown in 4 (2%).

Maternal age and child's sex were not associated with CP (*Table 1*), nor was the year of birth (data not shown). Mothers of children with CP were more often white and fewer were Hispanic or black compared with control mothers. Children with CP were slightly younger in completed weeks of gestational age than were control children.

**Intrauterine Infection**

In women without preeclampsia and with at least 3 hours between admission and delivery, a clinical diagnosis of chorioamnionitis was recorded for similar proportions of mothers of children with CP and controls (OR, 0.98; 95% CI, 0.65-1.5) (*Table 2*). None of the clinical signs or symptoms of infection recognized before birth were observed significantly more frequently in children with CP than in control children. Maternal fever greater than 37.7°C during the admission for delivery or up to 24 hours post partum was observed in half of mothers of children with CP and 41% of control mothers (OR, 1.4; 95% CI, 0.95-2.1); raising the cutoff point to higher than 38°C changed the OR to 1.3 (95% CI, 0.84-1.9). Incidence of maternal temperature exceeding 37.7°C together with any 2 of the clinical signs of uterine tenderness, fetal tachycardia, leukocytosis, or foul vaginal discharge was not different between the case and control groups. The administration of antibiotics prior to delivery and purulent amniotic fluid were not associated with risk of CP.

Placental pathological reports were available for 75% of children with CP and 76% of controls. Histologic evidence of acute placental inflammation was present in more than 70% of children with CP and control children whose
placentas were examined and was not associated with CP risk (Table 2). Histologic evidence of a placental fetal inflammation was similar for children with CP and controls (45% vs 43%). Placental cultures were reported for 33% of mothers of children with CP and 29% of control mothers. Identification of group 1 organisms (see “Methods” section) in placental cultures (alone or in combination with other organisms) was associated with doubling of risk of CP, a difference that approached statistical significance (OR, 2.1; 95% CI, 0.9-4.9). Correlations among the individual markers, and of each with histologic evidence of fetal inflammation, the factor sometimes noted to be most tightly related to CP risk, were modest (ie, r<0.50).

Limiting consideration to children with moderate or severe CP and neurologically normal controls did not substantially alter these findings, nor did consideration of spastic diplegia alone. A placental pathological report was available for 23 of 29 children with hemiplegia; of this subgroup, 22 children (96%) were reported to have acute placental inflammation (OR, 7.5; 95% CI, 1.4-138).

DIFFERENCES BY RACE/ETHNICITY

The association between intrauterine infection and CP was observed to differ by self-identified maternal race/ethnicity. Among children with CP, infection indicators were found with approximately equal or greater frequency in children born to white women compared with children born to women of other races/ethnicities (ie, Hispanic, black, Asian, or other) (Table 3). However, among control children, infection indicators were significantly less frequent in white children compared with children of other races/ethnicities. As a result of these case and control differences, operating in opposite directions, indicators of infection were significantly associated with increased risk of CP among white children but not among children of other races/ethnicities. Among children born to Hispanic women, a consistent but nonsignificant decrease in risk was observed for different measures of infection. Among children born to black or Asian mothers, there was no consistency across infection measures (data not shown).

ENTIRE STUDY POPULATION

The entire study population (tocolytic-eligible and tocolytic-ineligible combined) included 269 children with mild to severe spastic CP and 510 control children. In this population, a clinical diagnosis of definite or suspected intrauterine infection was observed for 24% of children with CP and 19% of control children, yielding an OR of 1.3 (95% CI, 0.92-1.9; controlled for 250-g birth weight stratum). The association between definite or suspected intrauterine infection and CP varied by maternal race/ethnicity (whites: OR, 3.0; 95% CI, 1.6-5.6; other races/ethnicities: OR, 0.81; 95% CI, 0.50-1.3).

The risk of CP increases dramatically with decreasing gestational age, as does the presence of intrauterine infection. Whether exposure to intrauterine infection is causally related to long-term motor disability in very preterm infants is uncertain. We sought to investigate whether intrauterine infection has an independent association with CP by evaluating prematurely born infants of similar gestational age and multiple measures of intrauterine infection. We found that neither clinical nor histologic indicators of intrauterine infection were consistently or significantly associated with spastic CP, nor with the spastic diplegia subtype, among infants with gestational ages less than 32 weeks who were born to women without preeclampsia or delivered soon after admission to a level 2 or 3 facility. In the total study population, not restricted by absence of preeclampsia or time from admission to delivery, there was also no association between a clinical diagnosis of definite or suspected intrauterine infection and CP risk.

Although not predicted by prior hypothesis, we observed a significant interaction between measures of intrauterine infection and self-identified maternal race/ethnicity with regard to risk of CP. Among whites, the increased risk of CP associated with intrauterine infection was substantial, ranging from 2-fold to 4-fold, depending on the measure evaluated. Among children of other races/ethnicities, there was no increase in risk of CP associated with intrauterine infection, and there was a statistically significant decrease in risk for some infection measures. This negative association was most consistent among Hispanics.

This study is considerably larger than others that have sought to address the association of infection and CP in premature infants. Other strengths include its population-
Table 3. Indicators of Maternal Infection and Risk of Spastic Cerebral Palsy (CP) in Singleton Children by Race/Ethnicity

<table>
<thead>
<tr>
<th>Indicator</th>
<th>White†</th>
<th>Other Race/Ethnicity‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With CP (n = 80)</td>
<td>Controls (n = 88)</td>
</tr>
<tr>
<td>Chorioamnionitis, clinical diagnosis</td>
<td>30 (38)</td>
<td>19 (22)</td>
</tr>
<tr>
<td>Maternal temperature ≥37.7°C</td>
<td>45 (56)</td>
<td>29 (33)</td>
</tr>
<tr>
<td>Maternal fever and ≥2 clinical signs§</td>
<td>14 (18)</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Acute placental inflammation</td>
<td></td>
<td>47 (78)</td>
</tr>
<tr>
<td>Placental fetal inflammation</td>
<td>29 (48)</td>
<td>12 (20)</td>
</tr>
</tbody>
</table>

*Data are presented as the number (percentage) of subjects unless otherwise indicated.†Race/ethnicity is according to maternal self-identification; white indicates white, non-Hispanic; other race/ethnicity, black, Hispanic, Asian, and other.
‡Adjusted for 250-g birth weight strata and gestational age in weeks.
§Clinical signs are uterine tenderness, fetal tachycardia, white blood cell count >15,000/µL, and/or foul vaginal discharge.
|Data are presented as the percentage of subjects with placental pathological reports.

Based on the above, we sought to control for the effects of gestational age on estimates of association. A further consequence of sampling controls within birth weight strata was oversampling of black women and undersampling of white women among controls because of differential birth-weight-for-gestational-age distributions. Analyses were conducted separately for strata of white subjects and subjects of other races/ethnicities, and within-strata analyses were statistically controlled for gestational age, minimizing the confounding associated with those factors.

A potential limitation of this study is the exclusion from the tocolytic-eligible group of women delivering less than 3 hours after admission. Women with fulminant or severe intrauterine infection might deliver soon after admission, and their infants might also be at higher risk of CP; thus, their exclusion could lead to underestimation of the true association between intrauterine infection and risk of CP by removing a subgroup at high risk. A priori recognition of this possibility led us to examine clinically recognized or suspected intrauterine infection in the entire study population, without consideration of time between admission and delivery. The analysis of clinical diagnoses of definite or suspected intrauterine infection in the entire study population also failed to yield a significant association between exposure to intrauterine infection and risk of CP when women of all racial/ethnic groups were combined.

Our procedures for sampling controls within 250-g birth weight strata resulted in a slightly, but significantly, lower gestational age distribution for children with CP compared with controls. This suggests some oversampling of controls who were small for their gestational age. By including terms for gestational age in multivariate models, we sought to control for the effects of specific genetic polymorphisms associated with intrauterine infection and CP is consistent with data from an earlier California study, but inconsistent with many other reported investigations. A meta-analysis of multiple studies concluded that clinical or histologic chorioamnionitis is associated with CP in preterm infants. However, many of the studies, including those on which the meta-analysis was based, were relatively small, some included gestationally older infants, and several may have involved largely white populations. Many studies did not have rigorous control for gestational age or preeclampsia, and criteria for CP differ among studies. Other studies relied on cytokine measurements in biological samples rather than clinical or histologic indicators and thus may not be comparable.

A search of the literature did not reveal other analyses of intrauterine infection and CP within racial or ethnic groups. However, results similar to ours have been reported in a recent study of chronic lung disease among infants exposed to clinical chorioamnionitis, ie, an increased risk for white infants and a nonsignificant protective effect for infants of other races/ethnicities. It has also been reported that in prematurely born white infants, perinatal mortality associated with premature rupture of membranes or amnionitis was higher than in black infants of similar birth weight.

Racial categories are imperfect simplifications of highly complex social and biological interactions, and recent discussions have emphasized the potential misuse of such categories in etiological investigations. Our use of self-identified maternal race/ethnicity provided an imperfect summary indicator of socioeconomic characteristics, medical care, and variation in the distribution of certain genetic polymorphisms associated with...
The risk of CP increases dramatically with decreasing gestational age and is particularly high among very premature infants. The hypothesis that this increased risk is caused by exposure to intrauterine infection or the fetal response to it has received support from some, but not all, prior studies. Limitations in these studies, including insufficient control for confounding by gestational age and the presence of preeclampsia, render interpretation difficult.

We report data from a large case-control study with rigorous control for gestational age and preeclampsia and multiple indicators of intrauterine infection abstracted from maternal medical records. We found that neither clinical nor histologic indicators of intrauterine infection were associated with total spastic CP or spastic diplegia. We also observed an approximate doubling of risk for infants exposed to intrauterine infection born to white women, whereas no association was noted for infants born to women of other races/ethnicities. This observation was not predicted by prior hypothesis and warrants consideration in future studies.

We thank the Children's Medical Services Branch, California Department of Health Services, the local California Children's Services programs, and the California Department of Developmental Services and local-regional centers for their cooperation. We also thank Jennifer Hoogstrate Barrett, MFA, for her careful data management.

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What This Study Adds

The inflammatory response that may be related to CP risk in the presence of intrauterine infection. Future studies of intrauterine infection and CP should describe the racial composition of their study populations. If verified, our unanticipated observation that CP risk associated with exposure to intrauterine infection varied by maternal race warrants further investigation in studies that can begin to untangle some of the complex underlying relationships for which the white vs nonwhite distinction is only a crude approximation.

The lack of a unitary, valid, and reliable gold standard for clinical diagnosis of intrauterine infection and the absence of adequate placental pathological examinations, even for many preterm deliveries, greatly hinders research in this area and requires examination of multiple measures (clinical, histologic, and microbiological) to seek potentially causal relationships. Inflammatory cytokines are frequently postulated to be pivotal in a causal pathway between intrauterine infection and fetal brain damage, but evidence is not consistent with regard to this hypothesis. We found that in a subgroup of the premature infants described here, inflammatory cytokines in neonatal blood were not associated with risk of CP, overall or among white subjects or subjects of other races/ethnicities. The lack of observed association between inflammatory cytokines in neonatal blood in the first days of life in infants of less than 32 weeks gestational age is in contrast with our report of a substantial association among term and near-term infants. Further investigation that includes biological sampling and consideration of race, ethnicity, and socioeconomic factors is clearly needed to elucidate pathways to CP both for term and preterm children.

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