Pulmonary Morbidity in Infancy after Exposure to Chorioamnionitis in Late Preterm Infants

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Abstract

Rationale: Chorioamnionitis is an important cause of preterm birth, but its impact on postnatal outcomes is understudied.

Objectives: To evaluate whether fetal exposure to inflammation is associated with adverse pulmonary outcomes at 6 to 12 months' chronological age in infants born moderate to late preterm.

Methods: Infants born between 32 and 36 weeks' gestational age were prospectively recruited (N = 184). Chorioamnionitis was diagnosed by placenta and umbilical cord histology. Select cytokines were measured in samples of cord blood. Validated pulmonary questionnaires were administered (n = 184), and infant pulmonary function testing was performed (n = 69) between 6 and 12 months' chronological age by the raised volume rapid thoracoabdominal compression technique.

Measurements and Main Results: A total of 25% of participants had chorioamnionitis. Although infant pulmonary function testing variables were lower in infants born preterm compared with historical normative data for term infants, there were no differences between infants with chorioamnionitis

(n = 20) and those without (n = 49). Boys and black infants had lower infant pulmonary function testing measurements than girls and white infants, respectively. Chorioamnionitis exposure was associated independently with wheeze (odds ratio [OR], 2.08) and respiratory-related physician visits (OR, 3.18) in the first year of life. Infants exposed to severe chorioamnionitis had increased levels of cord blood IL-6 and greater pulmonary morbidity at age 6 to 12 months than those exposed to mild chorioamnionitis. Elevated IL-6 was associated with significantly more respiratory problems (OR, 3.23).

Conclusions: In infants born moderate or late preterm, elevated cord blood IL-6 and exposure to histologically identified chorioamnionitis was associated with respiratory morbidity during infancy without significant changes in infant pulmonary function testing measurements. Black compared with white and boy compared with girl infants had lower infant pulmonary function testing measurements and worse pulmonary outcomes.

Keywords: fetal inflammation; wheeze; asthma; infant pulmonary function test; fetal programming

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Diminished lung function during infancy after normal term birth predicts poor lung function up to 22 years later (1, 2). These findings imply that factors affecting lung growth and/or function during fetal life may have a lasting impact on lung function later in life. One important fetal factor is prematurity. Of the roughly 11% of all deliveries born prematurely, 15% are born at less than 32 weeks' gestation, and 85% are moderate (32–33 wk) or late (34–36 wk) preterm infants (3, 4). Moderate and late preterm infants have a higher morbidity and mortality than term infants (5, 6).

Although the association of early preterm birth (<32 wk) with later development of wheeze is well established (7), the association of moderate or late preterm birth with subsequent wheezing is reported in some studies (8, 9) but not others (10, 11). A limitation of these studies is that the findings were based on retrospective review of databases. Furthermore, it is not clear if prematurity alone, conditions predisposing to prematurity, or comorbidities confer a risk for later development of respiratory disease, particularly wheezing.

About 20 to 30% of late preterm infants and more than 50% of infants less than 30 weeks' gestation at birth are exposed to chorioamnionitis, defined as inflammatory cell infiltration of fetal membranes (12). Thus, chorioamnionitis is a common fetal exposure that may in part explain the adverse respiratory outcomes of prematurity. Indeed, both prospective and retrospective studies found an independent association of chorioamnionitis in premature infants with subsequent wheezing (13, 14). In animal models, chorioamnionitis can disrupt alveolar and pulmonary vascular development and modulate fetal/neonatal immune responses (15). Thus, chorioamnionitis and prematurity can compromise future lung function both via effects on lung development and via fetal inflammation.

There are few previous studies examining the relationship between chorioamnionitis and lung function. Prendergast and colleagues (16), and Jones and colleagues (17) measured lung function in a cohort of preterm infants born at 23 to 36 weeks either in the neonatal period or in the first year of life. After adjusting for prematurity, neither study found differences in lung function between preterm infants with and without chorioamnionitis. However, neither study

included clinical follow up for correlation of lung function with pulmonary morbidity or biochemical determination of fetal inflammation.

To understand pulmonary outcomes, we measured lung function and collected clinical data during early infancy (6–12 mo postnatal age) in moderate/late-preterm infants with and without fetal exposure to chorioamnionitis. We postulated that exposure to chorioamnionitis would result in lower infant pulmonary function tests (iPFT). To further define the inflammation present in chorioamnionitis, we measured cord blood cytokines (particularly IL-6), which have been validated as biomarkers of fetal inflammation (18).

Methods

The study was approved by the institutional review boards of Cincinnati Children's Hospital and Good Samaritan Hospital. A detailed description of the methods is presented in the online supplement.

Recruitment of Study Participants

Women with preterm delivery between 32⁰ and 36⁶ weeks' gestation were prospectively consented from 2009 to 2012 under institutional review board approved protocols. Preterm infants were followed through the course of their initial hospital stay. After discharge from the hospital, the parents were reapproached for consent for the pulmonary follow-up study, consisting of a respiratory health questionnaire and iPFT. All parents who consented to the health questionnaire were offered iPFT. Mother and infant demographics were collected by interview and chart review during labor and delivery, and study data were managed using REDCap electronic data capture tools (19). Maternal and infant race data were obtained by self-report of the mother.

Diagnosis of Chorioamnionitis and Fetal Inflammation

Sections of chorioamnion, umbilical cord, and placental tissues were scored in a blinded fashion for histological chorioamnionitis on the basis of Redline's criteria (20). In this classification scheme, the grade of chorioamnionitis refers to the qualitative assessment of the degree of neutrophil infiltration in the fetal membranes. The staging of chorioamnionitis is based

on the tissue plane of neutrophil infiltration in the fetal membranes. Stage 1 refers to subchorionic or decidual neutrophil infiltration, and stage 2 refers to invasion of the fibrous chorion or amnion. Stage 3 changes denote necrotizing changes in the amniotic epithelium. Funisitis was defined as neutrophilic infiltration surrounding the umbilical cord vessels or within Wharton jelly.

We defined mild chorioamnionitis as stage 1 grade 1 chorioamnionitis (20). Severe chorioamnionitis was defined as greater than stage 1 or greater than grade 1 chorioamnionitis or the presence of funisitis. Cord blood was collected by cannulating the umbilical vein after cord clamping while the placenta was still attached to the uterus to maximize blood collection. This technique results in a mixed fetal arteriovenous sample. Cytokine/chemokine concentrations in cord blood were determined by Luminex using MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel (Millipore, Billerica, MA). Concentrations were calculated from standard curves using recombinant proteins.

Infant Pulmonary Function Testing

iPFT was performed between 6 and 12 months of age on a subset of enrolled infants whose parents consented to the iPFT. There were no other exclusions for the iPFT from the larger cohort. Infants were screened for respiratory illness within 3 weeks before the test. If any respiratory symptoms were present within 3 weeks before the testing date, the iPFT was rescheduled for when the infant was well.

Testing was performed on the nSpire Infant Pulmonary Lab system (Longmont, CO) using the raised-volume rapid thoracoabdominal compression technique and infant plethysmography according to the American Thoracic Society/European Respiratory Society guidelines (21, 22). After sedation with chloral hydrate (100 mg/kg), plethysmography was performed followed by measurement of forced expiratory flows. Testing was repeated after administration of bronchodilator (albuterol) via metered dose inhaler. All practitioners performing and interpreting iPFT were blinded to the chorioamnionitis exposure status. Lung function measurements were initially performed and interpreted by K.M.M. and subsequently over-read for quality and interpretation by S.D.D. and her team.

Respiratory Health Questionnaire

A breathing outcomes respiratory health questionnaire specifically designed for infants was administered for information about respiratory symptoms, respiratory medications, and healthcare use at age 6 to 12 months and 18 to 24 months (23). The questionnaire was administered by trained nurses either by telephone, or, for those performing iPFT, in a face-to-face encounter. Study nurses were also blinded to the chorioamnionitis status of participants.

Statistical Analysis

For demographic variables we used the Wilcoxon rank-sum test to compare continuous measures and the chi-square test or Fisher exact test for categorical measures. To assess for the independent effects of chorioamnionitis on iPFT and pulmonary survey outcomes, multivariable analysis of covariance models (for continuous outcomes) or logistic regression models (for dichotomous outcomes) were applied.

Our modeling approach for confounders was to include predictors, if in univariate analysis the predictor was significant at $P \leq 0.1$ for the outcome of interest. In our final modeling for adjustments, sex, race, and gestational age were included as initial covariates. In a preterm sheep model of chorioamnionitis induced by intraamniotic injection of LPS in one of the twin amniotic sacs, fetal inflammation did not transfer from one twin to the other (24). However, to account for possible nonindependence of twins due to genetic factors (25) or possible maternal-fetal transfer of inflammation to the other twin when one of the twins had inflammation, all models with significant associations were reanalyzed adding twin as a random effect.

We used the linear mixed-effect model for the estimation of a covariance (correlation) component between twins, within the overall variance structure to assess for nonindependence of outcomes in twins (26). Furthermore, we also included respiratory support in the neonatal intensive care unit (NICU) as a potential confounder even though respiratory support did not meet the univariate predictor threshold of $P \leq 0.1$, because neonatal respiratory support is known to be a factor in later pulmonary morbidity. All models were assessed for assumptions of normality and constant variance. Significance was set *a priori* at $\alpha = 0.05$.

All statistical analyses were performed using SAS 9.3 software (Cary, NC).

Results

Clinical and Epidemiological Variables of the Cohort

The study cohort ($N = 184$) included 123 singletons, 58 twins (29 sets), and 3 triplets (1 set) born to 153 mothers. Only one twin had a monochorionic monoamniotic placenta, and the rest had a diamniotic architecture. Chorioamnionitis was diagnosed histologically in 25% of placentae. Of the cases with chorioamnionitis, 39% had severe chorioamnionitis (10% of the cohort). Compared with those with no chorioamnionitis, mothers with chorioamnionitis were more likely to have public insurance, less likely to have completed high school, and more likely to deliver vaginally (Table 1). Only 13% of women with histologic chorioamnionitis had clinical signs of chorioamnionitis. There were no differences between the two groups in the length of rupture of membranes before delivery or antenatal use of antibiotics or steroids.

The median gestation at birth was 35 weeks, with infants in the chorioamnionitis group skewed slightly toward lower gestational age than the no chorioamnionitis group. The birth weights and the sex distribution between the two groups were similar (Table 2). Infants with chorioamnionitis were 2.5 times more likely

to be black. There were no cases of pneumonia or sepsis during the initial hospital stay. Although infants with chorioamnionitis were more than twice as likely as the infants with no chorioamnionitis to receive some form of respiratory support, differences between groups for the length of stay and the need for respiratory support were not significant after adjusting for the degree of prematurity (data not shown). None of the infants was prescribed home oxygen or respiratory medications at initial discharge from the birth hospital.

Infant Pulmonary Function Testing Measurements

iPFT measurements were performed in 70 of 184 (38%) participants based on parental consent. The participants versus nonparticipants in the iPFT were similar in gestational age at birth, birth weight, frequency of histologic chorioamnionitis, length of stay in the NICU, and rates of emergency room visits or hospitalization for respiratory symptoms during the first year of life (see Table E1 in the online supplement). Only 1 out of 70 iPFT measurements by the raised-volume rapid thoracoabdominal compression RTC technique and two plethysmography measurements did not meet research acceptability standards (22) and were therefore excluded from analysis. There were no adverse events associated with either the sedation or the iPFT procedure.

Table 1. Antenatal information of mothers of enrolled infants

	Chorio ($n = 46$)	No Chorio ($n = 138$)	P Value
Maternal demographics			
Maternal age, yr	28 (23–32)	28 (24–33)	0.78
Medicaid	55	32	0.03
Completed high school	29	54	0.01
Delivery data			
One or more signs of clinical chorio	13	7	0.21
Duration of PPRM, h*	11.7 (2.9–31.4)	9.8 (6.7–16.3)	0.64
PPROM ≥ 72 h*	18	7	0.22
Vaginal delivery	73	52	0.01
Medications before delivery			
Antibiotics	61	60	0.91
Antenatal steroids	40	33	0.45
Reason for delivery			
Spontaneous preterm labor†	89	76	0.14
Medical indication	4	28	<0.0001

Definition of abbreviations: chorio = acute chorioamnionitis, IQR = interquartile range, PPRM = preterm premature rupture of membranes.

Data are presented as median (IQR) or %. Boldface type indicates significance at $P \leq 0.05$.

* $N = 76$.

† $N = 127$.

Table 2. Demographic and clinical information of study infants

	Chorio (n = 46)	No Chorio (n = 138)	P Value
Female	56	50	0.49
Gestational age, wk	34.9 (33.3–36.3)	35.6 (34.6–36.3)	0.03
Birth weight, g	2,440 (1,975–2,835)	2,550 (2,161–2,855)	0.49
Race*			
White	59	77	0.004
Black	39	14	
Other	2	9	
Delivery data			
1-min Apgar < 7	26	13	0.04
5-min Apgar < 7	4	0	0.06
Need for supplemental oxygen or resuscitation	96	95	0.84
Postnatal diagnoses or interventions			
RDS	26	11	0.02
Pneumonia or sepsis	0	0	1
Need for supplemental oxygen but no respiratory support	4	2	0.60
CPAP	37	15	0.003
Mechanical ventilation	9	2	0.04
Any respiratory support†	39	15	0.001
Hospitalization LOS at birth, d	8 (2–17)	4 (2–9)	0.053
Clinical information for the iPFT cohort	Chorio (n = 20)	No chorio (n = 49)	
Gestational age at birth, wk	35.7 (33.6–36.3)	35.6 (34.9–36.3)	0.20
Postmenstrual age at iPFT, wk	69.4 (67.1–74.0)	73.4 (68.3–80.0)	0.10
Weight at iPFT, kg	8.5 (7.7–9.3)	8.4 (8.0–8.8)	0.82
Length at iPFT, cm	69.8 (68.1–71.5)	70.1 (69.0–71.3)	0.73

Definition of abbreviations: chorio = acute chorioamnionitis; CPAP = continuous positive airway pressure; iPFT = infant pulmonary function test; IQR = interquartile range; LOS = length of stay; RDS = respiratory distress syndrome.

Data presented as median (IQR) or %. Boldface type indicates significance at $P \leq 0.05$.

*All subjects were non-Hispanic.

†Respiratory support includes administration of surfactant, or CPAP or mechanical ventilator use.

At the time of iPFT, the median postmenstrual age of infants was 72 weeks (Table 2). Forced expiratory volume in 0.5 seconds ($FEV_{0.5}$), FVC, $FEV_{0.5}/FVC$, and forced expiratory flow at 75% of FVC (FEF_{75}) were skewed lower for the entire preterm cohort compared with historical normative data for term infants ($P < 0.0001$) (27) (Figure 1).

Subgroup analysis revealed no differences in any of the iPFT variables between infants exposed to chorioamnionitis ($n = 20$) and those who were not exposed ($n = 49$). However, there was a trend toward lower FEF_{75} in the chorioamnionitis versus no exposure group. Compared with girls ($n = 34$), boys ($n = 35$) had lower $FEV_{0.5}$ ($P = 0.004$), $FEV_{0.5}/FVC$ ($P = 0.03$), and FEF_{75} ($P = 0.001$). There were no sex differences in total lung capacity ($P = 0.25$) or functional residual capacity ($P = 0.31$) (Table 3).

Compared with white infants ($n = 40$), black infants ($n = 22$) had lower $FEV_{0.5}$ ($P = 0.0006$), FVC ($P = 0.007$), and FEF_{75}

($P = 0.03$) values, but $FEV_{0.5}/FVC$ ratios were similar ($P = 0.31$) (Figure 1). Compared with white infants, black infants had lower total lung capacity ($P = 0.01$), but functional residual capacity was not different ($P = 0.48$) (Table 3). Of note, significantly more black infants than white infants had low z scores (less than -1.645) for FEF_{75} (59% vs. 23%, respectively; $P = 0.007$), and 49% of boys versus 18% of girls had z scores less than -1.645 ($P = 0.01$) (Figure 1).

After adding twin status as a potential confounder, the sole change in the associations was for $FEV_{0.5}/FVC$ boy versus girl, where the P value was 0.07 after the correction. Adding respiratory support in the NICU as a potential confounder in the models did not change the result (data not shown).

In this iPFT cohort ($n = 69$), only 16 infants had a chest radiograph at birth. Abnormal chest imaging was present at birth in 69% ($n = 11$) of infants, but there

was no correlation between radiographic findings at birth and iPFT variables at 6 to 12 months' postnatal age.

Positive bronchodilator response was detected in ~29% of infants and did not vary based on chorioamnionitis status, race, or sex. We did not find any correlation between the degree of prematurity and bronchodilator response in this cohort.

Respiratory Disease Burden

We modeled the impact of chorioamnionitis on respiratory health by both univariate and multivariate analysis after adjusting for the confounders of race, sex, twin status, and gestational age at birth. Participants with chorioamnionitis were more likely to have physician visits for respiratory problems (odds ratio [OR], 3.18; confidence interval [CI], 1.45–7.0) than participants without chorioamnionitis (Table 4). Adding respiratory support in the NICU as a potential confounder in the unadjusted or the adjusted models did not change the OR or the CI of the associations (data not shown).

Because 33% of our cohort were born in multiple deliveries (mostly twins), we performed additional statistical analyses to assess for possible nonindependence of twins. Using the linear mixed model including twin as a random effect, we determined that twin status did not significantly confound the associations of chorioamnionitis with adverse pulmonary outcome. Indeed, the twins as a group had lower rates of chorioamnionitis and corresponding lower rates of wheeze or physician visits for respiratory causes in the first year than the singleton population (Table 5). The difference in the rates of chorioamnionitis in the twins versus singletons is most likely due to different reasons for preterm delivery (multiple gestation rather than intrauterine inflammation).

In a subgroup that was followed up at 18 to 24 months of age (76 of 184 infants), increase in caregiver-reported wheeze for the chorioamnionitis group persisted (adjusted OR, 4.30; CI, 1.18–15.65). Despite the increased respiratory morbidity in the infants exposed to chorioamnionitis, the hospitalization rates in the first and second year were similar between the two groups.

Among the respiratory symptoms analyzed, black infants were twice as likely as white infants to have been diagnosed with bronchitis/bronchiolitis or pneumonia

Figure 1. Infant pulmonary function test (iPFT) measurements. iPFTs were performed using the raised-volume rapid thoracoabdominal compression technique in moderate/late preterm infants at a chronological age of 6 to 12 months. Box plot for each variable shows the median and interquartile range. The whiskers for each variable depict the 5th percentile to 95th percentile range. Dashed reference lines represent approximate z score ranges (± 1.645), and the percentage values for each variable are derived from normative data from healthy, full-term infants (27). Median values for all subjects ($N = 69$) were significantly different from predicted values for forced expiratory volume in 0.5 sec (FEV0.5), FVC, and forced expiratory flow at 75% FVC (FEF75) (signed-rank test, $P < 0.0001$). Boys ($n = 35$) had lower FEV0.5, FEV/FVC, and FEF75 than girls ($n = 34$). Black infants ($n = 22$) had lower FEV0.5, FVC, and FEF75 than white infants ($n = 40$). Chorioamnionitis (Chorio) did not impact iPFT variables ($n = 20$ for chorioamnionitis, $n = 49$ for no chorioamnionitis) ($*P < 0.05$ between the comparators). After adding twin status as a potential confounder, the only change in the associations was for FEV/FVC boy versus girl, where the P value was 0.07 after the correction.

(Table 6). Similarly, boys were 1.7 times more likely than girls to have physician visits for respiratory problems.

It should be noted that the exposure to cigarette smoking and family history of asthma or atopic conditions was similar in the groups stratified by chorioamnionitis exposure, sex, or race (Tables 4 and 6).

Severity of Prenatal Inflammation and Respiratory Outcomes

Severity of prenatal inflammation was assessed by measurement of cord blood cytokines implicated in fetal inflammation after chorioamnionitis (15) and by histologic grading of severity of

chorioamnionitis. Compared with infants with no chorioamnionitis or mild chorioamnionitis, cases with severe chorioamnionitis had increased cord blood IL-6, IL-8, and granulocyte colony stimulating factor (G-CSF) (Figure E1), but monocyte chemoattractant protein -1 (MCP-1) levels were similar between the groups (data not shown). We used the cutoff for cord blood IL-6 (>11 pg/ml), because this threshold is a well-defined biomarker of fetal inflammation (18).

Compared with infants with low cord blood IL-6 levels, those with elevated cord blood IL-6 were more likely to have physician-diagnosed bronchitis,

bronchiolitis, or pneumonia by 6 to 12 months (OR, 3.23; CI, 1.21–8.64), and there was a tendency for caregivers to report more wheeze (OR, 2.17; $P = 0.09$) (Table 7). There were no differences in pulmonary symptoms on the basis of high versus low cord blood IL-8 or G-CSF levels at birth (Tables E2 and E3).

Analyzing outcomes by severity of chorioamnionitis, the incidence of caregiver-reported wheeze was increased in cases exposed to severe chorioamnionitis but not mild chorioamnionitis (Figure 2). Physician visits for respiratory problems were higher for infants exposed to either mild or severe chorioamnionitis, and there was a

Table 3. Lung volumes in preterm infants at 6 to 12 months

	Race*			Sex			Chorioamnionitis Diagnosis		
	White (n = 40)	Black (n = 22)	P Value	Male (n = 35)	Female (n = 34)	P Value	Chorio (n = 20)	No Chorio (n = 49)	P Value
TLC, % predicted	88 (88)[†]	80 (81)[†]	0.01 (0.047)[†]	83.5	87.0	0.25	86	84	0.44
FRCpleth, % predicted	89	86	0.48	90.6	86.4	0.31	91	88	0.60

Definition of abbreviations: chorio = acute chorioamnionitis; FRCpleth = functional residual capacity by plethysmography; TLC = total lung capacity. Percent predicted is based on historical normative data from term infants. Boldface type indicates significance at $P \leq 0.05$.

*Data from mixed-race infants not included.

[†]Results after reanalyzing the data for twins as a random effect.

trend toward higher rates of being diagnosed with bronchitis/bronchiolitis or pneumonia in infants exposed to mild (OR, 1.77; CI, 0.64–4.89) or severe chorioamnionitis (OR, 2.52; CI, 0.76–5.52). Interestingly, there were no differences in iPFT variables for severe versus mild chorioamnionitis or high versus low cord blood IL-6 (data not shown), suggesting a dichotomy between respiratory morbidity (Figure 2) and lung function response to prenatal inflammation.

Discussion

In this study designed to understand the contributions of prematurity and its associated comorbidities to respiratory outcomes in moderate and late preterm infants, we found that participants exposed to chorioamnionitis had more respiratory morbidity for up to 2 years after birth than

infants not exposed to chorioamnionitis. Furthermore, infants with severe chorioamnionitis had greater respiratory morbidity than those exposed to mild chorioamnionitis. In addition, cord blood proinflammatory cytokines G-CSF, IL-6, and IL-8 were selectively increased in infants exposed to severe chorioamnionitis. IL-6 levels at birth correlated with respiratory morbidity at 6 to 12 months of age. However, there were no significant differences in iPFT variables between participants with or without chorioamnionitis.

These novel findings support the hypothesis that chorioamnionitis increases the incidence of pulmonary morbidity during infancy. The association of respiratory morbidity but not iPFT measurements with elevated cord blood IL-6 supports altered immunologic mechanisms rather than altered structural lung development as potential mechanisms mediating these symptoms.

The findings of our study are significant, because moderate and late preterm infants account for 9% of all births in the United States (5, 6), and chorioamnionitis is implicated as a causative factor in at least 25% of preterm deliveries (28). We elected to study late-preterm infants (32–36 wk gestation at birth) because infants less than 32 weeks of gestation are often exposed to prolonged mechanical ventilation and postnatal supplemental oxygen and are at increased risk for bronchopulmonary dysplasia, all of which could significantly confound the associations between fetal exposures and subsequent adverse pulmonary health (29). The immediate postnatal course in our cohort was relatively uncomplicated: only 20% needed some form of respiratory support in the NICU, the median length of initial stay was 5 days, and none needed home oxygen use or respiratory medications at neonatal discharge.

Table 4. Pulmonary health questionnaire at 6 to 12 months of age

	Chorio (%) (n = 46)	No Chorio (%) (n = 138)	Unadjusted OR (CI)	Adjusted OR (CI)*
Prenatal exposure to cigarette smoking	18	15	1.22 (0.50–3.03)	1.03 (0.38–2.79)
Family history of asthma or atopic conditions	53	47	1.30 (0.66–2.57)	1.18 (0.56–2.48)
Caregiver report of wheezy symptoms	50	34	1.98 (1.00–3.92)	2.08 (0.99–4.40)
			1.99 (0.93–4.27) [†]	
Doctor visit for respiratory problems	49	25	2.82 (1.39–5.69)	3.18 (1.45–7.00)
			2.81 (1.29–6.11)[†]	3.20 (1.32–7.73)[†]
Emergency room visit for respiratory problems [‡]	50	53	0.87 (0.29–2.57)	1.05 (0.33–3.38)
Physician-diagnosed bronchitis, bronchiolitis, or pneumonia	30	16	2.31 (1.06–5.04)	2.02 (0.85–4.8)
			2.36 (1.01–5.49)[†]	
Respiratory medication prescription	9	6	1.55 (0.44–5.45)	1.15 (0.27–4.87)
Hospitalization	15	14	1.12 (0.44–2.89)	1.39 (0.50–3.88)

Definition of abbreviations: chorio = acute chorioamnionitis; CI = confidence interval; OR = odds ratio.

Boldface type indicates significance at $P \leq 0.05$.

*ORs were adjusted for race, sex, and gestational age.

[†]Results after accounting for twins as a random effect.

[‡]N = 63.

Table 5. Comparison of twins versus singletons for outcomes at 6 to 12 months

Patients	Chorio Positive % (n)	Wheeze Positive % (n)	Doctor Visit, Yes % (n)
Singletons (n = 123)	30.1 (37)	45.5 (56)	39.9 (49)
Twins (individuals, n = 58)	15.5 (9)*	20.7 (12)*	12.1 (7)*
Both in pair [†] (pairs, n = 29)	6.9 (2)	13.8 (4)	6.9 (2)
One in pair [†] (pairs, n = 29)	17.2 (5)	13.8 (4)	10.3 (3)
Neither in pair [†] (pairs, n = 29)	75.9 (22)	72.4 (21)	82.8 (24)
Triplets (individuals, n = 3)	0 (0)	33.3 (1)	33.3 (1)

Definition of abbreviation: chorio = acute chorioamnionitis.

*Proportions significantly different ($P < 0.05$) from singletons by chi-square test.

[†]% (n) based on number of pairs.

In our study, 54% of moderate or late preterm infants exposed to chorioamnionitis and 26% without chorioamnionitis reported wheeze at 18 to 24 months of age. We were surprised at the magnitude of pulmonary morbidity in our study of the relatively “healthy” moderate or late preterm infants (32–36 wk at birth), because follow-up studies at 1 to 2 years for extremely low birth weight infants (ELBW) (<28 wk at birth) with prolonged NICU course demonstrated wheezing in 53% in the Hibbs and colleagues study (30) and 58% in the Stevens and colleagues study (23). Notably, the questionnaire used in our study was the same one used by Stevens and colleagues, lending validity to the comparisons. Although the magnitude of pulmonary morbidity was similar to the ELBW infants, our rehospitalization rate of 15% is much lower than the 35% (23) and 22% (30) reported for ELBW babies.

Our rehospitalization rate of 15% through the first year is similar to those

reported for late preterm infants and twice the rate for term infants (31). These results suggest that the severity of pulmonary morbidity was lower in the moderate or late preterm infants than in ELBW infants.

The increased risk for respiratory morbidity in cases exposed to chorioamnionitis persisted after adjustment for the cofounders of gestational age, sex, twin status, respiratory support in the neonatal period, and race. The associations could not be explained by exposure to environmental tobacco smoke or family history of asthma. Furthermore, pulmonary morbidity increased with worsening severity of chorioamnionitis. Collectively, the results suggest that chorioamnionitis and prematurity are contributors to childhood respiratory illness. Increased pulmonary morbidity with severe compared with mild chorioamnionitis supports a causal association.

Although boys and black infants had lower iPFT measurements than girls and white infants, respectively, our findings

demonstrate that within our sample size, exposure to chorioamnionitis did not affect iPFT variables. There was no difference in pulmonary function, even when comparing the subset of patients with severe chorioamnionitis or those with elevated cord blood IL-6. These findings suggest that marked alteration in lung development is an unlikely explanation for chorioamnionitis-induced pulmonary morbidity. However, a small effect of chorioamnionitis on lung function cannot be completely ruled out, because the proportion of infants with z score less than 2 for FEF₇₅ was 50% versus 15% for the groups exposure to chorioamnionitis versus no exposure, respectively ($P = 0.08$). Thus, this study may have been underpowered to uncover some of the differences.

Our findings are also consistent with previous findings of no significant effect of chorioamnionitis on iPFT variables in both early and late preterm infants at 6 to 12 months' age (16). Although Prendergast and colleagues (16) performed lung function studies before discharge from the birth hospitalization, we performed iPFT at about 9 months' postnatal age. However, in contrast to Jones and colleagues (17), who found a selective decrease in lung function in preterm girls with chorioamnionitis compared with girls without chorioamnionitis, we found that boys had lower lung function than girls. Our study did not have the power to demonstrate sex-specific differences in iPFT within the subgroup of chorioamnionitis.

Although chorioamnionitis exposure status did not affect iPFT measurements, the

Table 6. Pulmonary health questionnaire at 6 to 12 months of age

	Race			Sex		
	White* (%) (n = 133)	Black* (%) (n = 38)	OR (CI)	Male (%) (n = 89)	Female (%) (n = 93)	OR (CI)
Prenatal exposure to cigarette smoking	13	24	0.48 (0.19–1.20)	15	18	0.81 (0.36–1.82)
Family history of asthma or atopic conditions	45	63	0.48 (0.23–1.01)	51	47	1.19 (0.66–2.15)
Caregiver report of wheezy symptoms	35	50	0.53 (0.25–1.10)	44	32	1.67 (0.91–3.07)
Doctor visit for respiratory problems	31	35	0.82 (0.38–1.78)	40	24	2.13 (1.12–4.06)
Emergency room visit for respiratory problems [†]	50	58	0.73 (0.24–2.24)	62	38	2.63 (0.92–7.54)
Physician-diagnosed bronchitis, bronchiolitis, or pneumonia	16	34	0.36 (0.16–0.82)	25	15	1.85 (0.88–3.92)
Respiratory medication prescription	5	11	0.47 (0.13–1.72)	10	3	3.37 (0.88–13.02)
Rehospitalization since birth	15	11	1.50 (0.48–4.74)	18	11	1.81 (0.77–4.28)

Definition of abbreviation: CI = confidence interval; OR = odds ratio.

Boldface type indicates significance at $P \leq 0.05$.

*Data from mixed race infants not included.

[†]N = 63.

Table 7. Correlation of IL-6 on postnatal pulmonary health at 6 to 12 months of age

	Cord Blood IL-6 < 11 pg/ml (%) (n = 109)	Cord Blood IL-6 ≥ 11 pg/ml (%) (n = 25)	OR (CI) for High vs. Low IL-6
Caregiver report of wheezy symptoms	33	52	2.17 (0.89–5.27)
Doctor visit for respiratory problems	27	36	1.53 (0.60–3.88)
Emergency room visit for respiratory problems	50	33	0.50 (0.10–2.42)
Physician-diagnosed bronchitis, bronchiolitis, or pneumonia	15	36	3.23 (1.21–8.64)* 3.29 (1.10–9.88)[†]

Definition of abbreviation: CI = confidence interval; OR = odds ratio.

Boldface type indicates significance at $P \leq 0.05$.

* $P < 0.05$ between the two comparators.

[†]Results after accounting for twins as a random effect.

larger cohort of moderate or late preterm infants in our study had airflow limitation compared with normative historical term values. These findings are consistent with previous reports of moderate and late preterm infants having a persistent deficit in expiratory flow but normal lung volumes through 16 months of age (32, 33). Our findings of boys with airway obstruction compared with girls are consistent with prior reports (27, 34–36), and the finding of black infants with increased airway

obstruction compared with white infants also supports previous studies (37–39).

The responsiveness to bronchodilators in our study (29% of infants) is comparable to that reported by Goldstein and colleagues (40), who found response to albuterol in 20 to 25% of a small cohort of healthy infants and young children born at more than 36 weeks of age, and to those of Debley and colleagues, who reported a bronchodilator response in 24% of 76 infants with recurrent wheezing (41). Bronchodilator response in infants with bronchopulmonary dysplasia is

reported to be slightly higher at 35% (42). Although the mechanism for increased bronchodilator response is not known, prematurity and inflammatory responses may be potential explanations for the airway reactivity.

We found an association of increased cord blood IL-6, but not IL-8 or G-CSF levels, with physician-diagnosed respiratory disease at 6 to 12 months age. However, we did not do an exhaustive survey of cord blood cytokines. Cord blood IL-6 was elevated only in infants exposed to severe

Figure 2. Severity of fetal inflammation and subsequent pulmonary morbidity at 6 to 12 months. Forest plot showing the odds ratios and 95% confidence intervals for the following respiratory outcomes at 6 to 12 months of age by levels of chorioamnionitis (Chorio) severity: Compared with no chorio, the severe chorio group had more caregiver report of wheeziness, and both mild chorio and severe chorio had more doctors' visits for respiratory problems (Resp. MD visit). Emergency room visit for respiratory problems (Resp. ER visit), and diagnosis of bronchitis, bronchiolitis, or pneumonia (Bronchitis) were not different in participants with chorioamnionitis versus no chorioamnionitis.

chorioamnionitis, suggesting that the respiratory morbidity associated with chorioamnionitis exposure is mediated, at least in part, by inflammation. Our results are consistent with a recent report of reduced FEV_{0.5} at 1 month of age in term infants with elevated IL-6 measured at 6 months of age (43). Because infants exposed to mild chorioamnionitis in our study did not have elevated IL-6 levels, but had increased respiratory problems in the first year of life, factors other than elevated IL-6 are also implicated in the pathogenesis of respiratory morbidity. IL-6 is known to skew the development of T-cell precursors to the proinflammatory Th17/Th22 cells rather than the antiinflammatory T-regulatory cells (Tregs) (44, 45).

We found that in a subset of infants from our cohort, cord blood Tregs from preterm infants had lower suppression of allogeneic T cells than infants, and cases with severe chorioamnionitis had lower Treg suppressive effects than gestation-matched preterm infants without exposure to chorioamnionitis (46). Thus, prenatal exposure to chorioamnionitis may result in alterations of the infant immune system

via IL-6-mediated inflammation and inhibition of Treg function predisposing to later respiratory disease.

Strengths and Limitations

Strengths of our unique study are the follow up of a cohort of moderate/late preterm infants with the combination of comprehensive assessments of fetal inflammation, pulmonary function testing, and pulmonary morbidity during infancy.

Limitations of our study include potential selection bias, as the patients were recruited exclusively from an urban tertiary maternal-fetal care hospital. In addition, iPFT may not be sensitive enough to detect subtle changes of function in the infants exposed to chorioamnionitis, and we may be underpowered to uncover small differences. Another unavoidable drawback is that we could not obtain direct measurements of pulmonary inflammation in the neonatal period because most infants were not intubated.

Conclusions

Our findings support the hypothesis that exposure to inflammation may increase risk

for wheeze during infancy via immune modulation of the fetus. Biologically, these findings may represent a paradigm of fetal programming. The persistence of respiratory morbidity at 2-year follow up raises the possibility of long-term impact of chorioamnionitis on respiratory health and supports future studies to address this important question. ■

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