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Detection of Ureaplasma DNA in Endotracheal Samples Is Associated With Bronchopulmonary Dysplasia After Adjustment for Multiple Risk Factors

TARAH T. COLAIZY, CYNTHIA D. MORRIS, JODI LAPIDUS, RONALD S. SKLAR, AND DE-ANN M. PILLERS

Department of Pediatrics [T.T.C.], University of Iowa, Carver College of Medicine, Iowa City, Iowa 52241; Departments of Medical Informatics [C.D.M.], Public Health and Preventative Medicine [J.L.], and Pediatrics [D.-A. M.P.], OR Health and Science University, Portland, Oregon 97239; Neonatology [R.S.S.], Kaiser Permanente Northwest, Portland, Oregon 97232

ABSTRACT: Microorganisms are hypothesized to contribute to the pathogenesis of bronchopulmonary dysplasia (BPD) in very low birth weight (VLBW) infants. This hypothesis remains controversial. We sought to determine whether endotracheal colonization with *Ureaplasma* sp., adenovirus, or *Chlamydia* sp. increases the risk of BPD. Intubated VLBW infants were included. Polymerase chain reaction (PCR) analysis was used to detect *Ureaplasma* sp., adenovirus, and *Chlamydia* sp. The outcome measure was BPD or death due to lung disease. Detection of microorganisms was compared between subjects with and without BPD. Logistic regression was used to control for covariates. Of 139 subjects, 33 (25%) screened positive for *Ureaplasma* sp., 22 of 136 (16%) were positive for adenovirus; eight of 133 (6%) were positive for *Chlamydia* sp. At 36 wk postmenstrual age, 14 patients had died, 68 (57%) had BPD. Detection of *Ureaplasma sp.* was associated with BPD or death ($p < 0.001$); adenovirus ($p = 0.52$) and *Chlamydia sp.* ($p = 0.33$) were not. Controlling confounding factors, the odds ratio for *Ureaplasma sp.* and BPD or death was 4.2 (95% CI 1.03, 17). In our population, detection of *Ureaplasma sp.,* but not adenovirus or *Chlamydia sp.* was associated with BPD or death due to lung disease. **(*Pediatr Res* 61: 578–583, 2007)**

BPD represents an ongoing challenge in neonatal care. BPD is a mixed obstructive/restrictive lung disease of preterm infants, with very low birth weight infants (VLBW, <1500 g) being at highest risk of significant morbidity and mortality (1,2). BPD has been attributed to the effects of treatment of infant respiratory distress syndrome (iRDS), particularly oxygen supplementation and mechanical ventilation (3). Despite routine use of surfactant, gentle ventilation techniques, and judicious use of oxygen therapy, BPD remains a major morbidity for VLBW infants. Infants with minimal exposure to mechanical ventilation are also affected by BPD, suggesting that other factors are involved in its pathogenesis (4,5).

The development of lung inflammation resulting in abnormal alveolar development may be a common pathway that links these risk factors (2). Infectious microorganisms in the pulmonary tree may contribute to such inflammation. *Ureaplasma* sp. are associated with both a pulmonary and a systemic inflammatory response (6–13). Pulmonary adenovirus has been linked with BPD, although no studies have investigated the inflammatory response in newborn infants (14). *Chlamydia* sp. have been serologically linked to BPD (15). However, studies performed during routine maternal screening yielded low numbers of colonized infants, and no association with BPD was demonstrated (16,17).

Maternal *Ureaplasma* sp. infection is associated with chorioamnionitis, an inflammatory disorder of the membranes that can contribute to premature birth (18). The risk of vertical transmission of the organism is inversely proportional to gestational age, as is the risk of BPD (19). Associations between neonatal pulmonary colonization with *Ureaplasma* sp. and development of BPD have been shown (16,20–28), but the association remains controversial (17,29–35). Study design limitations that could minimize the likelihood of detecting an association include small sample size, failure to consider estimated gestational age (EGA) or birth weight during statistical analysis, failure to account for other pathogens, and varying definitions of BPD.

We used PCR to detect *Ureaplasma* sp. PCR has a lower false-negative rate than clinical culture and can detect organisms when simultaneous cultures fail to do so (13,36,37), making it an ideal assay for fastidious organisms that are difficult to culture under routine clinical laboratory conditions.

We undertook a prospective cohort study of VLBW infants, screening them for the presence of *Ureaplasma* sp., adenovirus, and *Chlamydia* sp. in the lungs using PCR on endotracheal aspirates and following them for the development of BPD. We identified potential confounding variables and adjusted for them in our analysis. Our goal was to determine the contribution of pulmonary detection of these microorganisms to the development of BPD.

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Correspondence: De-Ann M. Pillers, M.D., Ph.D., Department of Pediatrics, NRC-5, OR Health & Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239; e-mail: pillersd@ohsu.edu

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Abbreviations: BPD, bronchopulmonary dysplasia; **EGA,** estimated gestational age; **PMA,** postmenstrual age; **VLBW,** very low birth weight

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METHODS

Study design, inclusion/exclusion criteria. We designed a prospective cohort study of the contribution of endotracheal detection of infectious microorganisms to the development of BPD in VLBW infants. The Institutional Review Board of Oregon Health and Science University (OHSU) approved the protocol and granted a waiver of patient consent, as the study samples were deemed medical waste. Samples were coded to preserve anonymity during analysis.

All VLBW infants admitted to OHSU Hospital before 72 h of age who required intubation and mechanical ventilation were eligible for study entry. Patients were excluded if they had congenital anomalies, congenital heart disease, or chromosomal anomalies. Endotracheal aspirates were obtained within the first 72 h of life. Specimens were screened for *Ureaplasma* sp., adenovirus, and *Chlamydia* sp. by PCR. Clinical data regarding known risk factors for chronic lung disease were collected.

Outcome definitions. We used a common definition of BPD: need for supplemental oxygen at 36 wk postmenstrual age (PMA) (1). Infants met this criteria if they had a room air SpO_2 of <88% by bedside pulse oximetry (Spacelabs Medical, Issaquah, WA).

A combined outcome of death due to lung disease or BPD at 36 wk PMA was also modeled. For infants who died before 36 wk PMA, whose death was due to respiratory failure, pneumothorax, pulmonary hemorrhage, iRDS, or pulmonary hypertension, were included in the death-due-to-lung-disease group. These patients were added to the survivors with BPD at 36 wk PMA to form the combined outcome group.

Clinical data collection. Respiratory course, comorbid conditions, and preexisting demographic risks for BPD were considered as potential confounding variables. Total white blood cell counts were collected at birth as a measure of systemic inflammation. Clinical data were abstracted from hospital charts after hospital discharge.

To keep study personnel blinded, samples were coded anonymously. Clinical data collection was completed before PCR results were decoded to prevent bias.

Sample collection. Endotracheal aspirates were obtained using in-line suction sets and sterile suction traps (Busse Hospital Disposable, Hauppauge, NY) as part of routine clinical care. Up to 1 mL of sterile nonbacteriostatic saline was instilled into the trachea, with a resultant 0.5 to 1.0 mL sample obtained. Samples were stored at −80°C.

Sample preparation. Three hundred microliters of aspirate was centrifuged at 14,000 × *g* for 20 min. The pellet was incubated at 60°C for 60 min with 50 μL of solution A (10 mM Tris HCl, pH 8.3, 100 mM KCl, 2.5 mM $MgCl_2$) and 100 μL of solution B (1% Tween 20, 10 mM Tris HCl, 1% Triton X, 2.5 mM $MgCl_2$, 120 μg/mL proteinase K). It was heated at 95°C for 10 min to inactivate the proteinase K, quenched on ice, and stored at −20°C until PCR analysis.

Primers. Primers used for detection of *Ureaplasma* sp., *Chlamydia* sp., and adenovirus were synthesized and purified by Invitrogen (Carslbad, CA), and are shown in Table 1.

PCR screening for microorganisms. Primers designed and validated in neonatal endotracheal aspirate samples by Blanchard and colleagues (38) were used to amplify a 429-bp fragment of the urease gene common to all serotypes of *Ureaplasma* sp. The Ampliwax (Perkin-Elmer ABI)-mediated hot-start PCR technique was performed in a 50-μL reaction mix. The lower suspension contained 2.5 μL of each primer (U4 and U5, 10 μmol), 1 μL deoxyribonucleoside triphosphate and distilled and deionized H_2O (ddH2O) to 20-μL volume and was sealed with a 50-μL Ampliwax gem melted at 72°C. The upper suspension contained 10× PCR buffer (5 μL), 25 mM $MgCl_2$ (6 μL), 1U *Taq* polymerase, and ddH20 to 25-μL volume. Last, and in a different location from where the PCR reaction mixes were assembled (to minimize potential contamination of reagents by PCR products), 5 μL of experimental sample was added. Positive and negative controls were used. A GenAmp 2400 DNA thermal cycler (Perkin-Elmer-Cetus) was used for 40 cycles of 20 s at 95°C for denaturation, 60 s at 62°C for primer annealing, and 30 s at 72°C for extension.

Chlamydia sp. and adenovirus are also amenable to PCR detection (14). The Ampliwax hot-start PCR technique described above was used, substituting appropriate primers, and adjusting PCR protocols as noted below.

A 330-bp fragment of the hexon gene of adenovirus was amplified using nested PCR with ADH-01 and ADH-02 primers (14). These primers detect at least 10 subtypes of adenovirus, including 1, 2, 3, 5, 6, 7, 18, 31, 41, and 50. The protocol for the primary reaction was 5 min of incubation at 94°C, followed by 40 cycles of 45 s at 94°C, 45 s at 64°C, and 45°s at 72°C. Two microliters of the primary PCR product was used in the secondary PCR reaction, with the ADH-11 and ADH-22 primers.

A 609-bp fragment of *Chlamydia* (*trachomatis*, *psittaci*, *pneumoniae*) (16S rRNA gene) was amplified with a single PCR reaction: 5-min incubation at 94°C, followed by 40 cycles of 45 s at 94°C, 45 s at 64 C, 45 s at 72°C.

Positive and negative controls. Positive controls consisted of stock isolates of *Ureaplasma* sp. obtained from ATCC (Manassas, VA), and samples of *Chlamydia trachomatis* and adenovirus obtained from the OHSU Department of Microbiology. Negative controls consisted of pH 7.5 TE buffer. Samples were tested at least twice to ensure reliable results.

Analysis of PCR products. Amplified products were analyzed by electrophoresis through a 2% SeaKem LE agarose gel (Cambrex) at 60 V for 3 h, stained with 0.5 μg/mL ethidium bromide (Sigma Chemical Co., St. Louis, MO), and visualized with ultraviolet light.

Data analysis. Univariate analyses, including χ^2, Fisher's exact test, *t* test, and simple linear regression were used to identify variables associated with development of BPD. To determine the contribution of the microorganisms to BPD after adjustment for other risk factors, logistic regression models were used. Variables found to be significantly associated with the development of BPD ($p < 0.25$) were used to build two logistic regression models with incidence of BPD at 36 wk PMA, and the combined outcome of BPD at 36 wk PMA or death due to lung disease, as the dependent variables. All initial variables of interest ($p < 0.25$) were entered and removed and reassessed using methods described by Hosmer and Lemeshow (39). Interaction terms were also explored based on clinical relevance.

Analysis was performed with SPSS 11.0 for Macintosh, and the SAS package, version 8.0, for Windows.

RESULTS

The cohort included 139 VLBW infants born from January 1, 1998, to December 31, 2002, and included 79% (139/177) of eligible patients. Those not included were likely patients who were ventilated for less than 48 h. Sample collection was not performed on weekends; therefore, patients born on weekends who were ventilated for short periods of time were not included. This group of patients should not differ systematically from included patients.

Demographic information regarding the cohort as well as data regarding neonatal outcomes can be found in Table 2. Chorioamnionitis was defined by an obstetric clinical diagno-

Table 1. *PCR primers*

Organism	Name	Sequence	Size of PCR product	Gene of origin	Reference
Ureaplasma urealyticum	U5	5′-CAATCTGCTCGTGAAGTATTAC-3′	429 bp	Urease	Blanchard *et al.* (38), 1993
	U4	5′-ACGACGTCCATAAGCAACT-3′			
Adenovirus					
First step	ADH1	5′-ACTACAAYATTGGCTACCAGG-3′	440 bp	Hexon	Couroucli *et al.* (14), 2000
	ADH2	5′-CAAAACATAAAGAAGKGTGGGC-3′			
Second step	ADH11	5′-AACTTCCAGCCCATGAGCMG-3′	330 bp		
	ADH12	5′-CTCAAAAGTCATGTCBAGCGC-3′			
Chlamydia *sp.*	CHLAM01	5′-ACACTCGCAAGGGTGAAACTC-3′	609 bp	16S	Couroucli *et al.* (14), 2000
	CHLAM02	5′-CGACTTCATCCYAGTCATCAG-3′			

(Y = C + T, K = T + G, M = A + C, B = T + C + G, H = A + T + C, R = A + G).

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Table 2. *Study population (n = 139) demographics*

Sex (male)	59% (83/139)
Race	
White	58% (85/139)
Hispanic	33% (44/139)
African-American	4% (5/139)
Asian/Pacific Islander	4% (5/139)
Mean EGA, wk	26.8 (±1.9)
Mean birth weight, g	941 (±257)
Deaths before 36 wk PMA	14
Deaths due to lung disease	8
Transfers before 36 wk PMA	4
Prenatal steroids	80% (108/135)
Prenatal antibiotics	72% (97/135)
Surfactant	93% (129/139)
PDA	66% (88/133)
Days of supplemental oxygen(median)	60 (±35)
Days of mechanical ventilation(median)	14 (±18)
Hospital days (median)	81 (±26)
ROP stage 3 or 4	22% (31/119)
IVH grade III or IV	14% (18/129)
NEC	7% (10/139)
Sepsis	37% (51/139)
BPD at 36 wk PMA	57% (69/121)
Ureaplasma detection	24% (34/139)
Adenovirus detection	16% (22/136)
Chlamydia sp. detection	6% (8/133)

PDA, patent ductus arteriosus.

Table 3. *Bronchopulmonary dysplasia at 36 weeks PMA, univariate analyses*

	BPD 36 wk (69/121)	No BPD 36 wk (52/121)	*p*
Sex (male)	57% (39/69)	57% (30/52)	1.0
Race			
Caucasian	58% (40/69)	61% (32/52)	
Hispanic	33% (23/69)	51% (18/52)	0.61
African-American	4% (3/69)	2% (1/52)	
Asian/Pacific Islander	4% (3/69)	4% (2/52)	
Mean EGA, wk	26.3 (±1.6)	27.7 (±1.9)	<0.001
Mean birth weight, g	872 (±219)	1070 (±227)	<0.001
Prenatal steroids	82% (57/69)	79% (38/52)	0.60
Surfactant	99% (68/69)	85% (44/52)	0.004
Chorioamnionitis	14% (10/69)	21% (11/52)	0.47
PDA	77% (52/69)	53% (27/52)	0.01
Days of supplemental oxygen(median)	81 (±21)	30 (±20)	<0.001
Days of mechanical ventilation(median)	24 (±20)	7 (±12)	<0.001
Hospital days (median)	95 (±22)	67 (±22)	<0.001
ROP stage 3 or 4	36% (25/69)	8% (4/52)	<0.001
IVH grade III or IV	16% (11/69)	6% (3/52)	0.43
Necrotizing enterocolitis	7% (5/69)	9% (5/52)	0.74
Sepsis	44% (30/69)	27% (13/52)	0.07
Ureaplasma sp.	37% (26/69)	8% (4/52)	<0.001
Adenovirus	16% (11/69)	12% (6/52)	0.52
Chlamydia sp.	9% (6/69)	4% (2/52)	0.33

ROP, retinopathy of prematurity; IVH, intraventricular hemorrhage.

sis recorded in the mother's chart consisting of fever, elevated white blood cell count, and uterine tenderness (Tables 3 and 5).

Twenty-four percent (34/139) of patients were positive for *Ureaplasma* sp. detection by PCR, and 16% (22/136) were positive for adenovirus, consistent with published values (14,17,33,40). *Chlamydia* sp. were rarely detected (6%, 8/133) (Table 2). There were no racial differences in detection rates for any organism (*Ureaplasma* sp., $p = 0.3$; adenovirus $p = 0.2$; *Chlamydia* sp., $p = 0.9$). There was no relationship between total white blood cell count at birth and detection status for any organism (*Ureaplasma* sp., $p = 0.9$; adenovirus, $p = 0.9$; *Chlamydia* sp., $p = 0.6$). There was no relationship between total white blood cell count at birth and the outcome, BPD, or death ($p = 0.7$).

At 36 wk PMA, 121 patients were still in the cohort. Of the remainder, 14 had died, and four had been transferred to other institutions. The proportions of transferred infants with *Ureaplasma* sp. (25%) and adenovirus (25%) detected were similar to those retained in the cohort.

At 36 wk PMA, 57% of the population (69/121) met the criteria for BPD. Fifty-nine percent of the patients had either been diagnosed with BPD or had died from lung disease before 36 wk PMA (76/130). Incidence of death or BPD was similar in all racial groups.

BPD at 36 wk PMA. Several factors emerged as significant predictors of BPD at 36 wk PMA. Smaller and less gestationally mature infants were more likely to develop BPD, as were those who required longer courses of mechanical ventilation. The median number of days of ventilation was 24 in the BPD group *versus* 7 in the unaffected group. Each day of mechanical ventilation increased the risk of BPD by 8%. These infants were also more likely to be treated for PDA. Sepsis was more prevalent; 44% of patients in the BPD group had at least one episode, in contrast to 27% of the unaffected group. BPD-affected infants also had significantly higher incidence of stage 3 or 4 ROP. *Ureaplasma* sp. detection was significantly associated with BPD at 36 wk, whereas adenovirus and *Chlamydia* sp. were not (Table 3).

The logistic model created for the 36 wk PMA outcome included EGA, PDA, sepsis, *Ureaplasma* sp. colonization, number of days of ventilation, and the interaction of days of ventilation and EGA. The interaction of days of ventilation with EGA was a significant predictor of BPD at 36 wk PMA. Days of ventilation were increasingly important as risk factors for BPD as babies became more gestationally mature. The OR for each additional ventilator day at 25 wk EGA was 1.05, indicating that each day on the ventilator increased risk by 5%. At 26 wk EGA, the additional risk was 8% per day, and at 28 wk EGA, it was 14% per day. The odds ratios (ORs) for these gestational ages are found in Table 4.

BPD at 36 wk PMA or death due to lung disease. Individual predictors of BPD or death due to lung disease were similar to those for BPD at 36 wk PMA (Table 5).

The model for the combined outcome of BPD at 36 wk PMA or death from lung disease was identical to that for BPD at 36 wk PMA, although it was arrived at by an independent model-building process. Significant predictors of BPD included *Ureaplasma* sp. colonization and the interaction of ventilation with gestational age. The OR for *Ureaplasma* sp. colonization and BPD at 36 wk PMA or death due to lung disease was 4.2 (95% CI: 1.03–17.0), indicating a fourfold

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Table 4. *ORs for risk factors for BPD at 36 wk PMA*

Factor	OR	95% CI	*p*
PDA	1.77	0.66–4.77	0.26
Sepsis	0.65	0.23–1.88	0.43
Ureaplasma colonization	2.85	0.8–10.0	0.10
EGA 25 wk, each day of mechanical ventilation	1.05	0.99–1.1	0.053
EGA 26 wk, each day of mechanical ventilation	1.08	1.03–1.13	0.003
EGA 28 wk, each day of mechanical ventilation	1.14	1.06–.24	0.0008

CI, confidence interval.

Table 5. *Bronchopulmonary dysplasia at 36 wk PMA or death due to lung disease, univariate analyses*

	Death or BPD at 36 wk (76/130)	No death or BPD at 36 wk (54/130)	*p*
Sex (male)	60% (45/76)	56% (30/54)	0.72
Mean EGA, wk	26.2 (±1.5)	27.6 (±1.9)	<0.001
Mean BW, g	854 (±212)	1055 (±240)	<0.001
Prenatal steroids	82% (60/74)	73% (42/52)	0.97
Surfactant	99% (74/75)	85% (45/53)	0.003
Chorioamnionitis	15% (11/76)	19% (10/54)	0.63
PDA	73% (54/74)	53% (27/51)	0.02
Days of supplemental oxygen(median)	78 (±30)	30 (±20)	<0.001
Days of mechanical ventilation(median)	23 (±20)	8 (±12)	<0.001
Hospital days (median)	95 (±22)	67 (±22)	<0.001
ROP stage 3 or 4	36% (27/76)	8% (4/54)	<0.001
Necrotizing enterocolitis	7% (5/76)	9% (5/54)	0.74
Sepsis	41% (30/74)	27% (13/48)	0.13
Ureaplasma sp.	38% (29/76)	6% (3/53)	<0.001
Adenovirus	17% (13/75)	12% (6/51)	0.39
Chlamydia sp.	8% (6/74)	4% (2/50)	0.36

Table 6. *ORs for risk factors for BPD at 36 wk PMA or death due to lung disease*

Factor	OR	95% CI	*p*
PDA	1.59	0.6–4.22	0.35
Sepsis	0.70	0.24–2.03	0.51
Ureaplasma colonization	4.23	1.03–17.0	0.04
EGA 25 wk, each day of mechanical ventilation	1.02	0.97–1.06	0.42
EGA 26 wk, each day of mechanical ventilation	1.05	1.004–1.1	0.03
EGA 28 wk, each day of mechanical ventilation	1.12	1.04–1.21	0.003

increase in risk of BPD or death in *Ureaplasma* sp.–colonized infants after statistical adjustment for the effects of PDA, sepsis, EGA, and days of ventilation. Ventilation became increasingly important as gestational age at birth increased. At 25 wk EGA, the risk increased 2% per day, 5% per day at 26 wk, and 12% per day at 28 wk (Table 6).

DISCUSSION

Endotracheal detection of *Ureaplasma* sp. emerged as an important predictor for the combined outcome of BPD or death due to lung disease in our population (OR = 4.2) after statistical adjustment for the effects of gestational age, PDA, sepsis, and days of ventilation.

More than 20 candidate variables were investigated as potential confounders of the association between *Ureaplasma* sp. and BPD. Our study is the first to use PCR methods statistically corrected for duration of mechanical ventilation, an important risk factor for BPD. By using the most currently useful definition of BPD, a very sensitive detection method, and adjusting for several of the most significant risk factors for BPD, this study improves on the previous literature.

The relationship between BPD and *Ureaplasma* sp. has been investigated for >20 y. Most publications in the presurfactant era clearly demonstrated an association; a meta-analysis of 18 publications between 1988 and 1994 demonstrated an increased risk of BPD in colonized infants (OR = 1.72) (41).

Since surfactant has come into common usage, the data conflict. Studies in infants with mean birth weights of 827–1000 g demonstrated OR of 2.0–3.8 (24,26–28), yet in studies of larger infants (975–1300 g) there was no association detected between *Ureaplasma* sp. and BPD (14,33,35). This ambiguity may be related to the difference in size, as smaller, more gestationally immature infants are at higher risk of BPD. A population with a lower prevalence of BPD would require a larger sample size to detect any effect of the organism. Our population had a high prevalence BPD, increasing our ability to detect differences in BPD between colonized and uncolonized infants.

A recent meta-analysis determined that *Ureaplasma* sp. colonization is associated with BPD at 36 wk PMA (OR = 1.62, 95% CI: 1.1–2.3) (42). Our study differs from many of the studies in the analysis due to use of PCR and adjustment for known confounders.

Another potential explanation for the controversy may be the modality used to detect *Ureaplasma* sp. Relying on a single clinical culture may misclassify up to 40% of positive patients as negative (13,17,37,43). PCR is more sensitive than culture and can detect both low numbers of organisms and dead organisms (36,37,44). Sensitivities for PCR detection of *Ureaplasma* range from 90% to 100% in neonatal endotracheal aspirate samples. Contemporaneous cultures of the same specimens yield detection sensitivities of 40%–91% (36,37). Although PCR detection does not differentiate between colonization and infection, this will only underestimate any association, as infected infants are presumably at greater risk of complications. Unfortunately, it is impossible to clearly determine which patients are incidentally colonized with these organisms and which have true pathologic infection, regardless of detection method.

We also investigated the contributions of adenovirus and *Chlamydia* sp. detection to BPD. Adenovirus was first reported in association with BPD by Couroucli *et al.* (14) in a population with EGA <30 wk by PCR . They found no association between *Ureaplasma* sp. and BPD. The majority of adenovirus isolates were type 5, a group C adenovirus that has been detected in neonatal myocarditis and pediatric respiratory infection. No clinical data such as gestational age, birth weight, and neonatal diagnoses were reported.

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Prosch *et al.* (40) screened VLBW infants for adenovirus by both PCR and culture techniques. Their cohort was similar to ours in terms of clinical and demographic parameters. They detected adenovirus at a similar rate to that of Couroucli *et al.* (14), but did not demonstrate an association between adenovirus and BPD. The adenovirus isolates detected by Prosch *et al.* were predominantly type 2, another group C adenovirus that is associated with respiratory disease.

We detected adenovirus at a rate similar to both groups (14,40) and also demonstrated no association with BPD. Our isolates were not sequenced. Our sample size and prevalence of adenovirus were similar to those of Couroucli *et al.* and was adequate to have reproduced this finding. That we did not suggests that their population is different in some important way from the very immature, high-risk population we studied or that their association was due to chance. The role of adenovirus in BPD is still uncertain. *Chlamydia* sp. were uncommon in our population (eight patients) and not associated with BPD.

Like all epidemiologic studies, ours contained potential limitations. Our study was limited to intubated VLBW infants. We obtained samples on 79% of eligible infants. The excluded patients were likely intubated for shorter durations; however, no data were collected to support this assertion. If intubated for a shorter duration, they would represent a group at lower risk of BPD, and if included, might lead to a shift in the OR of our logistic models toward 1. Our population consisted of a subset of the total who were smaller, younger, and, in general, at higher risk of BPD. This is a potential source of selection bias. Our findings can only be generalized to intubated VLBW infants.

Infants who never require mechanical ventilation can still develop BPD. With mechanical ventilation being used more sparingly, other risk factors for BPD become more important to understand. Given the sample size and the large effect of mechanical ventilation on the risk of BPD in our population, there was no way to adequately assess the effects of other risk factors, such as sepsis, PDA, and maternal chorioamnionitis. Therefore, our study cannot be generalized to the entire VLBW population, only those who have been mechanically ventilated.

We clearly demonstrated that after accounting for other common risk factors for BPD, the presence of *Ureaplasma* predisposed intubated VLBW infants to BPD or death due to lung disease. *Ureaplasma* is an independent risk factor for BPD in this population. The mechanism for this increased risk is not known, but could be due to inflammation arising before birth due to intrauterine exposure. Indeed, our sample collection in the first 72 h of life reflects such antenatal or perinatal exposure to *Ureaplasma*. We did not have amniotic fluid samples or other maternal data with which to determine timing or chronicity of *Ureaplasma* exposure. We collected total white blood cell counts at birth for all subjects and found no association between elevated white blood cell count and *Ureaplasma* colonization. White blood cell counts are a crude estimate of systemic inflammation, and we did not collect other data such as pulmonary cytokine levels to assess the level of pulmonary inflammation, as others have done (11,12).

Future studies should be aimed at combining epidemiologic studies with contemporaneous measurement of inflammatory cytokines to determine whether *Ureaplasma* sp. is causing an inflammatory response in neonates who are then followed for the development of BPD. Species and serovar ascertainment would also be helpful in such studies to identify any varying pathogenicity of the *Ureaplasma* sp. Finally, no large prospective studies of antibiotic treatment of *Ureaplasma* sp., starting in the first days of life, exist. Such a trial could contribute much to the understanding of the impact of this microbe in this vulnerable population. Understanding the pathogenesis of BPD is essential to the development of interventions to optimize the health and long-term well-being of our tiniest patients.

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REFERENCES

1. Bancalari E, Claure N, Sosenko IR 2003 Bronchopulmonary dysplasia: changes in pathogenesis, epidemiology and definition. Semin Neonatol 8:63–71
2. Jobe AH, Bancalari E 2001 Bronchopulmonary dysplasia. Am J Respir Crit Care Med 163:1723–1729
3. Northway WH Jr, Rosan RC, Porter DY 1967 Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. N Engl J Med 276:357–368
4. Charafeddine L, D'Angio CT, Phelps DL 1999 Atypical chronic lung disease patterns in neonates. Pediatrics 103:759–765
5. Bancalari E, Gonzalez A 2000 Clinical course and lung function abnormalities during development of neonatal chronic lung disease. In: Bland RD, Coalson, JJ (eds) Chronic Lung Disease in Early Infancy. Marcel Dekker, New York, pp 41–64.
6. Ohlsson A, Wang E, Vearncombe M 1993 Leukocyte counts and colonization with *Ureaplasma urealyticum* in preterm neonates. Clin Infect Dis 17:S144–S147
7. Panero A, Pacifico L, Rossi N, Roggini M, Chiesa C 1995 *Ureaplasma urealyticum* as a cause of pneumonia in preterm infants: analysis of the white cell response. Arch Dis Child Fetal Neonatal Ed 73:F37–F40
8. Groneck P, Goetze-Speer B, Speer CP 1996 Inflammatory bronchopulmonary response of preterm infants with microbial colonisation of the airways at birth. Arch Dis Child Fetal Neonatal Ed 74:F51–F55
9. Patterson AM, Taciak V, Lovchik J, Fox RE, Campbell AB, Viscardi RM 1998 *Ureaplasma urealyticum* respiratory tract colonization is associated with an increase in interleukin 1-beta and tumor necrosis factor alpha relative to interleukin 6 in tracheal aspirates of preterm infants. Pediatr Infect Dis J 17:321–328
10. Lyon A 2000 Chronic lung disease of prematurity. The role of intra-uterine infection. Eur J Pediatr 159:798–802
11. De Dooy JJ, Mahieu LM, Van Bever HP 2001 The role of inflammation in the development of chronic lung disease in neonates. Eur J Pediatr 160:457–463
12. Groneck P, Schmale J, Soditt V, Stutzer H, Gotze-Speer B, Speer CP 2001 Bronchoalveolar inflammation following airway infection in preterm infants with chronic lung disease. Pediatr Pulmonol 31:331–338
13. Colaizy TT, Kuforiji T, Sklar RS, Pillers DA 2003 PCR methods in clinical investigations of human ureaplasmas: a minireview. Mol Genet Metab 80:389–397
14. Couroucli XI, Welty SE, Ramsay PL, Wearden ME, Fuentes-Garcia FJ, Ni J, Jacobs TN, Towbin JA, Bowles NE 2000 Detection of microorganisms in the tracheal aspirates of preterm infants by polymerase chain reaction: association of adenovirus infection with bronchopulmonary dysplasia. Pediatr Res 47:225–232
15. Numazaki K, Chiba S, Kogawa K, Umetsu M, Motoya H, Nakao T 1986 Chronic respiratory disease in premature infants caused by *Chlamydia trachomatis*. J Clin Pathol 39:84–88
16. Garland SM, Bowman ED 1996 Role of *Ureaplasma urealyticum* and *Chlamydia trachomatis* in lung disease in low birth weight infants. Pathology 28:266–269
17. Da Silva O, Gregson D, Hammerberg O 1997 Role of *Ureaplasma urealyticum* and *Chlamydia trachomatis* in development of bronchopulmonary dysplasia in very low birth weight infants. Pediatr Infect Dis J 16:364–369
18. Abele-Horn M, Peters J, Genzel-Boroviczeny O, Wolff C, Zimmermann A, Gottschling W 1997 Vaginal *Ureaplasma urealyticum* colonization: influence on pregnancy outcome and neonatal morbidity. Infection 25:286–291
19. Sanchez PJ, Regan JA 1990 Vertical transmission of *Ureaplasma urealyticum* from mothers to preterm infants. Pediatr Infect Dis J 9:398–401

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20. Cassell GH, Waites KB, Crouse DT, Rudd PT, Canupp KC, Stagno S, Cutter GR 1988 Association of *Ureaplasma urealyticum* infection of the lower respiratory tract with chronic lung disease and death in very-low-birth-weight infants. Lancet 2:240–245
21. Horowitz S, Landau D, Shinwell ES, Zmora E, Dagan R 1992 Respiratory tract colonization with *Ureaplasma urealyticum* and bronchopulmonary dysplasia in neonates in southern Israel. Pediatr Infect Dis J 11:847–851
22. Payne NR, Steinberg SS, Ackerman P, Chrenka BA, Sane SM, Anderson KT, Fangman JJ 1993 New prospective studies of the association of *Ureaplasma urealyticum* colonization and chronic lung disease. Clin Infect Dis 17:S117–S121
23. Wang EE, Cassell GH, Sanchez PJ, Regan JA, Payne NR, Liu PP 1993 *Ureaplasma urealyticum* and chronic lung disease of prematurity: critical appraisal of the literature on causation. Clin Infect Dis 17:S112–S116
24. Iles R, Lyon A, Ross P, McIntosh N 1996 Infection with *Ureaplasma urealyticum* and *Mycoplasma hominis* and the development of chronic lung disease in preterm infants. Acta Paediatr 85:482–484
25. Pacifico L, Panero A, Roggini M, Rossi N, Bucci G, Chiesa C 1997 *Ureaplasma urealyticum* and pulmonary outcome in a neonatal intensive care population. Pediatr Infect Dis J 16:579–586
26. Abele-Horn M, Genzel-Boroviczeny O, Uhlig T, Zimmermann A, Peters J, Scholz M 1998 *Ureaplasma urealyticum* colonization and bronchopulmonary dysplasia: a comparative prospective multicentre study. Eur J Pediatr 157:1004–1011
27. Hannaford K, Todd DA, Jeffery H, John E, Blyth K, Gilbert GL 1999 Role of *Ureaplasma urealyticum* in lung disease of prematurity. Arch Dis Child Fetal Neonatal Ed 81:F162–F167
28. Perzigian RW, Adams JT, Weiner GM, Dipietro MA, Blythe LK, Pierson CL, Faix RG 1998 *Ureaplasma urealyticum* and chronic lung disease in very low birth weight infants during the exogenous surfactant era. Pediatr Infect Dis J 17:620–625
29. Smyth AR, Shaw NJ, Pratt BC, Weindling AM 1993 *Ureaplasma urealyticum* and chronic lung disease. Eur J Pediatr 152:931–932
30. Saxen H, Hakkarainen K, Pohjavuori M, Miettinen A 1993 Chronic lung disease of preterm infants in Finland is not associated with *Ureaplasma urealyticum* colonization. Acta Paediatr 82:198–201
31. Jonsson B, Karell AC, Ringertz S, Rylander M, Faxelius G 1994 Neonatal *Ureaplasma urealyticum* colonization and chronic lung disease. Acta Paediatr 83:927–930
32. Heggie AD, Jacobs MR, Butler VT, Baley JE, Boxerbaum B 1994 Frequency and significance of isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis* from cerebrospinal fluid and tracheal aspirate specimens from low birth weight infants. J Pediatr 124:956–961
33. Heggie AD, Bar-Shain D, Boxerbaum B, Fanaroff AA, O'Riordan MA, Robertson JA 2001 Identification and quantification of ureaplasmas colonizing the respiratory tract and assessment of their role in the development of chronic lung disease in preterm infants. Pediatr Infect Dis J 20:854–859
34. van Waarde WM, Brus F, Okken A, Kimpen JL 1997 *Ureaplasma urealyticum* colonization, prematurity and bronchopulmonary dysplasia. Eur Respir J 10:886–890
35. Ollikainen J, Korppi M, Heiskanen-Kosma T, Heinonen K 2001 Chronic lung disease of the newborn is not associated with *Ureaplasma urealyticum*. Pediatr Pulmonol 32:303–307
36. Cunliffe NA, Fergusson S, Davidson F, Lyon A, Ross PW 1996 Comparison of culture with the polymerase chain reaction for detection of *Ureaplasma urealyticum* in endotracheal aspirates of preterm infants. J Med Microbiol 45:27–30
37. Abele-Horn M, Wolff C, Dressel P, Zimmermann A, Vahlensieck W, Pfaff F, Ruckdeschel G 1996 Polymerase chain reaction versus culture for detection of *Ureaplasma urealyticum* and *Mycoplasma hominis* in the urogenital tract of adults and the respiratory tract of newborns. Eur J Clin Microbiol Infect Dis 15:595–598
38. Blanchard A, Hentschel J, Duffy L, Baldus K, Cassell GH 1993 Detection of *Ureaplasma urealyticum* by polymerase chain reaction in the urogenital tract of adults, in amniotic fluid, and in the respiratory tract of newborns. Clin Infect Dis 17:S148–S153
39. Hosmer DW Jr, Lemeshow S 2000 Applied Logistic Regression. John Wiley & Sons, New York
40. Prosch S, Lienicke U, Priemer C, Flunker G, Seidel WF, Kruger DH, Wauer RR 2002 Human adenovirus and human cytomegalovirus infections in preterm newborns: no association with bronchopulmonary dysplasia. Pediatr Res 52:219–224
41. Wang EE, Ohlsson A, Kellner JD 1995 Association of *Ureaplasma urealyticum* colonization with chronic lung disease of prematurity: results of a metaanalysis. J Pediatr 127:640–644
42. Schelonka RL, Katz B, Waites KB, Benjamin DK Jr 2005 Critical appraisal of the role of *Ureaplasma* in the development of bronchopulmonary dysplasia with meta-analytic techniques. Pediatr Infect Dis J 24:1033–1039.
43. Nelson S, Matlow A, Johnson G, Th'ng C, Dunn M, Quinn P 1998 Detection of *Ureaplasma urealyticum* in endotracheal tube aspirates from neonates by PCR. J Clin Microbiol 36:1236–1239
44. Teng K, Li M, Yu W, Li H, Shen D, Liu D 1994 Comparison of PCR with culture for detection of *Ureaplasma urealyticum* in clinical samples from patients with urogenital infections. J Clin Microbiol 32:2232–2234

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