

Patterns of Colonization With *Ureaplasma urealyticum* During Neonatal Intensive Care Unit Hospitalizations of Very Low Birth Weight Infants and the Development of Chronic Lung Disease

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ABSTRACT. *Background.* *Ureaplasma urealyticum* and its association with chronic lung disease (CLD) of prematurity has remained a controversial topic. To address this question, we performed a longitudinal study using culture and polymerase chain reaction to detect *U urealyticum* in the respiratory tract of very low birth-weight infants throughout their neonatal intensive care unit hospitalizations.

Methods. We screened 125 infants weighing <1500 g and/or <32 weeks' gestational age over a 12-month period, collecting endotracheal, nasopharyngeal, and throat specimens on days of age 1, 3, 7, and weekly thereafter. CLD was defined as dependency on supplemental oxygen at 28 days and at 36 weeks' postconceptional age.

Results. Forty infants (32%) had 1 or more positive specimens by culture or polymerase chain reaction. We identified 3 patterns of *U urealyticum* colonization: persistently positive ($n = 18$), early transient ($n = 14$), and late acquisition ($n = 8$). We compared the rates of CLD in each of the 3 colonized groups with the rate of CLD in the noncolonized group. We found a significantly higher rate of CLD at 28 days of age (odds ratio: 8.7; 95% confidence interval: 3.3, 23) and at 36 weeks' postconception (odds ratio: 38.5, 95% confidence interval: 4.0, 374) only for infants with persistently positive colonization.

Conclusions. This study demonstrates that the risk of developing CLD varies with the pattern of *U urealyticum* colonization. Only the persistently positive colonization pattern, which accounted for 45% of the *U urealyticum*-positive infants, was associated with a significantly increased risk of development of CLD. *Pediatrics* 2002; 110(4). URL: <http://www.pediatrics.org/cgi/content/full/110/4/e45>; bronchopulmonary dysplasia, chronic lung disease, premature infant, polymerase chain reaction, *Ureaplasma urealyticum*, very low birth weight infant.

ABBREVIATIONS. CLD, chronic lung disease; PDA, patent ductus arteriosus; PCR, polymerase chain reaction; VLBW, very low birth weight; NICU, neonatal intensive care unit; OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio.

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Chronic lung disease (CLD) of prematurity has multifactorial etiologies including hyperoxia,¹ barotrauma,² surfactant deficiency,³ nutritional deficiencies,^{4,5} fluid overload,^{6,7} patent ductus arteriosus (PDA),⁸ inflammation of the lungs,⁹ and infection.^{10–12} With respect to infection, several microorganisms have been implicated; *Chlamydia trachomatis*, cytomegalovirus, adenovirus, and *Ureaplasma urealyticum*.

Controversy regarding the association between *U urealyticum* colonization and CLD has persisted despite numerous studies. The association between *U urealyticum* respiratory tract colonization and the development of CLD was first reported by 3 independent research groups in 1988.^{13–15} Subsequently, >30 studies have addressed this issue with conflicting results. Most of these studies have confirmed this association,^{16–27} whereas others have not.^{28–34}

The majority of investigators screened for *U urealyticum* colonization only during the first week of life using culture exclusively. Few studies were longitudinal; these varied widely in terms of number and duration of time between samplings. Recent studies have used polymerase chain reaction (PCR) in addition to culture. No longitudinal studies using both PCR and culture for the detection of *U urealyticum* colonization of very low birth weight (VLBW) infants throughout their neonatal intensive care unit (NICU) hospitalizations have been reported.

The primary objective of this study was to determine the natural history of *U urealyticum* colonization of VLBW infants throughout their NICU hospitalizations with respect to the development of CLD using both culture and PCR.

MATERIALS AND METHODS

The study was approved by the Department of Pediatrics and Columbia University Institutional Review Boards.

All VLBW infants consecutively admitted to Children's Hospital of New York between March 1999 and April 2000 were eligible for the study. Exclusion criteria were admission after 2 weeks of life, lethal anomalies, congenital heart disease, and congenital pulmonary disorders other than pulmonary disease due to prematurity. Infants who died or were transferred by 14 days of life were excluded from the final analyses. Gestational age was established based in order of priority by obstetrical estimates using early ultrasound (<17 weeks), last menstrual period or Ballard assessment. Clinical courses of mothers and infants were followed by ongoing chart abstraction until discharge or 36 weeks' postconceptional age.

Endotracheal (if intubated), nasopharyngeal and throat specimens were collected on days of age 1, 3, and 7, and weekly thereafter through to discharge or transfer. All specimens were

processed by culture and PCR. Study investigators processing the specimens were blinded to the identities of the infants.

Culture Methods

Tracheal aspirates were obtained during routine suctioning after instillation of 1 mL normal saline and collected into tracheal suction traps (Sherwood Services, Chicopee, MA). Nasopharyngeal and throat specimens were obtained using mini-tip culturettes (BBL, Starks, MD). Specimens were inoculated onto A7 agar plates (Remel, Lenexa, Kansas) and into tubes containing 2 mL of 10B broth (Remel) at the bedside, transported to the laboratory and incubated at 37°C. A7 plates were incubated in 5% CO₂. Plates and broths were observed for 7 days. Broths exhibiting a color change were subcultured onto A-7 agar plates. *U urealyticum* were identified as morphotypical golden-brown colonies on days 1, 2, 5, and 7 of incubation.

PCR Methods

After 24 hours, 250 µL of inoculated broths were removed, frozen at -20°C, and batched for PCR processing.³⁵ Broths were thawed and centrifuged at 12 000 g at 4°C for 20 minutes. The supernatant was discarded; the pellet was resuspended in 50 µL solution A (TRIS HCL pH 8.3 10 mM, KCL 100 mM, MgCl₂ 2.5 mM) and an equal volume of solution B (TRIS HCL 8.3 20 mM, Tween 2%, MgCl₂ 5 mM, Trition-X 2%, Proteinase K [GIBCO, Gaithersburg, MD, 0.5 mg/mL], Proteinase K buffer [TRIS HCL 7.5 10 mM, CaCl₂ 20 mM, Glycerol 50%]) and incubated at 60°C for 60 minutes then heated to 100°C for 10 minutes. Primers used were the U5 sense (5'-CAATCTGCTCGTGAAGTATTAC-3') and U4 antisense (5'-ACGACGTCCATAAGCAACT-3') of the urease structural genes. One positive (*U urealyticum*, ATCC, Manassas, VA) and one negative control (10B broth) were included in each PCR batch. Twenty two and a half microliters of Taq supermix (GIBCO), 2.5 µL of sample, and 5 µL of water were added to each reaction. A thermal cycler was used to process samples through 41 cycles of denaturation at 94°C for 2 minutes and 20 seconds, primer annealing at 62°C for 60 seconds, and extension at 72°C for 60 seconds. Amplified products were analyzed by electrophoresis with 2% agarose gels containing ethidium bromide and the 429 base pair DNA fragments were visualized by ultraviolet fluorescence.

Data Analyses

Analysis of preliminary data suggested that the rate of CLD among *U urealyticum* positive infants was approximately twice that of *U urealyticum* negative infants. Using a baseline CLD rate of 25% in the noncolonized group and the assumption that the number of *U urealyticum* positive and negative infants would be similar, we calculated that an overall sample size of 132 infants would be needed to have 80% power to detect a significant difference between CLD rates with *U urealyticum* exposure. Data analyses were performed using SPLUS, version 4.5 (Mathsoft, Inc, Seattle, WA). The primary outcome measures were oxygen dependency at 28 days of age and oxygen dependency at 36 weeks' postconceptional age. Other outcome measures included radiographic diagnosis of CLD and length of hospital stay.

In univariate analyses, colonization status was treated as a single variable with 4 nominal categories (persistently positive, early transient, late acquisition, and negative patterns). We examined the possible association between colonization status and maternal and infant characteristics (presented as binary or continuous variables) using χ^2 test, Fisher exact test, or 1-way analysis of variance F test. In addition, we separately compared the rates of maternal and infant characteristics for each positive colonization pattern with their rates in the negative colonization pattern. Results are reported as odds ratios (ORs) with 95% confidence intervals (95% CIs).

We constructed a set of multiple logistic regression models for each of the 2 primary outcomes. Independent variables in these models included binary measures of birth weight (<750 g), gestational age (<26 weeks), gender, presence of symptomatic PDA (defined as murmur and/or bounding pulses with echocardiogram confirmation), inborn (vs outborn), mode of delivery, antenatal steroid treatment, sepsis (defined as symptomatic infant with positive blood culture), surfactant administration, and persistently positive *U urealyticum* colonization pattern. Nonsignifi-

cant terms were removed one by one from the models by backward elimination. In multivariate analyses, colonization status was treated as a binary variable (persistent colonization vs a combination of the other 3 categories). The strength of association between predictor and outcome variables in these models is reported as an adjusted odds ratio (AOR) with 95% CI.

RESULTS

One hundred fifty-nine infants weighing <1500 g and/or <32 weeks' gestational age were consecutively admitted within the first 2 weeks of life to the NICU during the study period. Five infants had congenital heart disease, 1 infant had an undefined neuropathy, 15 infants expired, and 13 infants were transferred to other institutions within the first 2 weeks of life. The remaining 125 infants were followed until discharge, transfer, or death. One infant in the persistently positive category and 4 infants in negative group expired beyond 2 weeks of life. Of the infants in the negative group who had recovered from acute lung disease, 3 infants died and 2 were transferred by 28 days of age, and 2 additional infants were transferred by 36 weeks' postconceptional age.

A total of 3720 specimens (1860 culture and 1860 PCR) were collected (mean: 29.8 and range: 6–92 per infant). Thirty-two percent (40/125) of study infants had 1 or more positive specimens by culture or PCR for *U urealyticum*. PCR identified 100% of all colonized infants versus 57.5% identified by culture. The greater sensitivity of PCR versus culture was most apparent beyond 21 days of age.³⁶

Three patterns of infant colonization were identified: Persistently positive colonization ($n = 18$) defined as having positive specimens by culture or PCR throughout their hospitalization, early transient colonization ($n = 14$) defined as having at least 1 specimen positive by culture or PCR at ≤ 21 days of age with all subsequent specimens negative and late acquisition ($n = 8$) defined as negative cultures and PCR specimens until day of age 21 with subsequent positive specimens. Eighty-five infants had all culture and PCR specimens negative for *U urealyticum*.

The overall incidences of CLD at 28 days and at 36 weeks' postconceptional age were 24.2% (29/120) and 6.8% (8/118), respectively. Comparison of *U urealyticum* colonization compared with the negative group was significantly different for CLD at 36 weeks' postconceptional age ($P < .003$). The incidences of CLD at 28 days and 36 weeks' postconceptional age for infants in each of the colonization patterns and culture-negative group are presented in Table 1. CLD at 28 days and 36 weeks' postconceptional age occurred significantly more often only in the persistently positive group ($P < .0001$ and $P < .0001$, respectively). Infants with persistently positive colonization also had significantly higher incidences of radiographic diagnoses of CLD ($P < .002$) and mean length of hospital stay measured in days ($P < .004$).

Table 2 displays measures of association between infant characteristics and each pattern of *U urealyticum* colonization. We found significant differences in mean birth weight, mean gestational age, birth weight distribution, gender, inborn (vs outborn),

TABLE 1. Outcome Data and Colonization Patterns of *Ureaplasma urealyticum* in Preterm VLBW Infants

Outcome*	Persistently Positive N = 18 n/N (%) OR (95% CI)	Early Transient N = 14 n/N (%) OR (95% CI)	Late Acquisition N = 8 n/N (%) OR (95% CI)	Negative N = 85 n/N (%) Reference	P Value†
CLD at 28 d of age	12/18 (67) 8.7 (3.3, 23)‡	1/14 (7) 0.33 (.02, 5.6)	1/8 (12.5) 0.62 (.04, 10.5)	15/80 (19)	<.0001
CLD at 36 wk postconceptional age	6/18 (33) 38.5 (4.0, 74)‡	0/14 (0) —	1/8 (12.5) 11.0 (0.4, 330)	1/78 (1.2)	<.0001
Radiographic diagnosis of CLD	9/17 (53) 6.1 (2.1, 8.4)‡	0/14 (0) —	2/8 (25) 1.8 (0.2, 14.1)	13/84 (15)	.002
Mean length of stay¶ d ± standard error (range)	78.8 ± 1.62§ (40–108)	49.5 ± 2.69 (16–108)	68.4 ± 4.43 (28–110)	50.5 ± 0.43 (19–121)	.004

* One infant in persistently positive category and 4 infants in negative group expired beyond 2 weeks of life. Of the infants in the negative group who had recovered from acute lung disease, 3 infants died and 2 were transferred by 28 days of age, and 2 additional infants were transferred by 36 weeks' postconceptional age.

† P value based on global χ^2 test or Fisher exact test on 3 degrees of freedom, or on 1-way analysis of variance F test (for length of stay).

‡ P value <.05 when 95% CI for OR excludes 1.

§ P < .05 by post-hoc Dunnett test, using negative *U urealyticum* colonization status as the reference level (control).

|| Radiographs were obtained within a 7-day window from the CLD definitions (day of life 28 and 36 weeks' postconceptional age). The radiographs were read independently by pediatric radiologists who were blinded to the *U urealyticum* colonization patterns. Radiographic results were not available for 1 infant in the persistently positive and 1 infant in the negative categories.

¶ Infants transferred to other institutions were not included in the analysis.

need for delivery room resuscitation, low Apgar scores at 5 minutes of life, and need for conventional ventilation among infants with persistently positive *U urealyticum* colonization compared with infants with negative colonization. Early transient and late acquisition colonization rates compared with those for infants in the negative colonization group did not differ.

Maternal characteristics considered possible risk factors for *U urealyticum* colonization were also analyzed. In addition, although this was not part of the study design, 71 placentas were examined for clinical indications: 52 from the negative group, 6 from early transient, 4 from late acquisition, and 9 from persistently positive colonization patterns. Compared with the pattern of negative colonization, persistently positive *U urealyticum* colonization was significantly associated with histologic evidence of placental chorioamnionitis (OR: 19.0; 95% CI: 5.64, 64.0), clinical chorioamnionitis defined as maternal evidence of infection including fever, leukocytosis, maternal and fetal tachycardia, uterine tenderness or malodorous vaginal discharge (OR: 7.40; 95% CI: 1.03, 53.2), prolonged rupture of membranes defined as rupture of membranes for >24 hours (OR: 13.0; 95% CI: 4.16, 40.4), premature rupture of membranes defined as rupture of membranes before the onset of labor (OR: 19.2; 95% CI: 7.10, 52.1), and preterm labor (OR: 34.6; 95% CI: 15.2, 78.9). There was no significant association between these factors and the other 2 *U urealyticum*-positive colonization patterns.

Table 3 displays ORs for the strength of association between the CLD outcomes (at 28 days of age and 36 weeks' postconceptional age) and risk factors for the development of CLD. Birth weight <750 g, gestational age <26 weeks, gender, PDA, inborn (vs outborn), sepsis defined as a symptomatic infant prompting a work up, and yielding a positive blood culture, surfactant administration, and persistently positive *U urealyticum* colonization were significantly associated with CLD at 28 days of age. Risk factors significantly associated with CLD at 36 weeks'

postconceptional age were birth weight <750 g, gestational age <26 weeks, PDA, surfactant administration, and persistently positive *U urealyticum* colonization.

Table 3 displays the adjusted ORs and CIs for factors associated with CLD in the 2 multiple logistic regression models (at 28 days of age and 36 weeks' postconceptional age) produced after stepwise removal of nonsignificant predictor terms. In the first model, birth weight <750 g (AOR: 5.03; 95% CI: 1.29, 19.7), gestational age <26 weeks (AOR: 5.58; 95% CI: 1.48, 21), gender (AOR: 4.03; 95% CI: 1.08, 15.1), PDA (AOR: 14.1; 95% CI: 2.97, 67), and persistently positive *U urealyticum* colonization (AOR: 5.33; 95% CI: 1.05, 27) were associated with CLD at 28 days of age. The adjusted ORs for each of these terms corresponds in magnitude to the unadjusted OR. In the second model only 2 variables, sepsis (AOR: 10.3; 95% CI: 1.53, 69) and persistently positive colonization (AOR: 33.6; 95% CI: 4.78, 237), were associated with CLD at 36 weeks' postconceptional age. The AOR for persistently positive colonization in this model is similar in magnitude to the unadjusted OR (OR: 24.5; 95% CI: 4.44, 135). The wide CIs for these ORs is most likely attributable to the small number of infants (8, of whom 6 had persistently positive colonization) with CLD at 36 weeks' postconceptional age in our sample.

DISCUSSION

This prospective longitudinal study demonstrates that the pattern of persistently positive *U urealyticum* colonization is associated with CLD at 28 days and 36 weeks' postconceptional age. Neither early transient colonization nor late acquisition of *U urealyticum* was associated with CLD. Recognition that different patterns of *U urealyticum* colonization exist and have different risks of CLD may help clarify the conflicting earlier reports regarding the association between *U urealyticum* and CLD.

Although this work demonstrates a strong associ-

TABLE 2. Patient Characteristics by *Ureaplasma urealyticum* Colonization Pattern in Preterm VLBW Infants

Characteristics†	Persistently Positive N = 18	Early Transient N = 14	Late Acquisition N = 8	Negative N = 85	P Values*
Continuous Variables	Mean ± Standard Error, Range	Mean ± Standard Error, Range	Mean ± Standard Error, Range	Mean ± Standard Error, Range	
Categorical Variables	Number (%) OR (95% CI)	Number (%) OR (95% CI)	Number (%) OR (% CI)	Number (%) Reference	
Birth weight (g)	755 ± 9.0† (530–1170)	1192 ± 19.9 (705–1360)	1068 ± 25.1 (900–1580)	1028 ± 3.6 (510–1350)	<.001
Gestational age (wk)	25.2 ± 0.10† (23–29)	29.4 ± 0.15 (25–32)	28.0 ± 0.28 (25–32)	28.2 ± 0.03 (23–35)	<.001
Birth weight distribution (g)					
<750	10 (56) 3.58 (1.31, 9.8)	1 (7) 0.22 (0.01, 3.7)	0 (0) —	22 (26)	.004
750–999	7 (39) 2.37 (0.74, 7.6)	2 (14) 0.62 (0.08, 4.7)	1 (12) 0.53 (0.03, 8.9)	18 (21)	.33
≥1000	1 (6) 0.05 (0, 0.86)	11 (79) 3.26 (1.28, 8.3)	7 (88) 6.22 (2.01, 19.3)	45 (53)	<.001
Male gender	10 (56) 0.96 (0.4, 2.6)	2 (14)‡ 0.13 (0.02, 0.96)	4 (50) 0.77 (0.2, 3.3)	48 (56)	.03
Vaginal delivery	10 (56)‡ 4.1 (1.5, 11.2)	4 (29) 1.3 (0.3, 5.7)	3 (37.5) 2.0 (0.4, 10.4)	20 (24)	.06
Inborn	12 (67)‡ 0.15 (0.05, 0.48)	14 (100) 2.1 (0.7, 6.5)	6 (75)‡ 0.23 (0.06, 0.93)	79 (93)	.003
Multiple birth	5 (28) 0.98 (0.3, 3.7)	7 (50) 2.5 (0.8, 8.0)	1 (12.5) 0.36 (0.02, 6.0)	24 (28)	.26
Resuscitation at delivery	15 (83)‡ 11.4 (4.8, 27)	4 (29) 0.91 (0.2, 3.9)	1 (12.5) 0.32 (.02, 5.4)	26 (31)	<.001
Apgar score <3, 1 min	7 (39) 3.1 (0.9, 10)	2 (14) 0.82 (0.1, 6.3)	0 (0) —	14 (17)	.07
Apgar score <6, 5 min	6 (33) 6.4 (1.6, 26)	0 (0) —	1 (12.5) 1.8 (0.1, 33)	6 (7)	.006
Conventional ventilation	14 (78) 5.5 (2.3, 13)	3 (21) 0.4 (0.08, 2.3)	4 (50) 1.6 (0.4, 6.7)	33 (39)	.006
High-frequency oscillatory ventilation	0 (0) —	0 (0) —	1 (12.5) 3.9 (0.19, 79)	3 (4)	.34
Surfactant administration	4 (22) 1.6 (0.4, 7.0)	1 (7) 0.4 (0.02, 7.1)	2 (25) 1.8 (0.2, 14)	13 (15)	.55
Pneumothorax	2 (11) 1.7 (0.2, 13)	0 (0) —	0 (0) —	6 (7)	.52
Late postnatal steroid use	1 (6) 5.0 (0.2, 150)	0 (0) —	0 (0) —	1 (1)	.56
Any intraventricular hemorrhage	2 (11) 1.5 (0.3, 6.5)	0 (0) —	2 (25) 1.7 (0.2, 13)	14 (17)	.23
Necrotizing enterocolitis	2 (11) 1.6 (0.2, 13)	1 (7) 0.97 (0.05, 18)	3 (38)* 7.6 (1.3, 46)	6 (7)	.08
Any episode of sepsis	5 (28) 1.4 (0.4, 5.4)	4 (29) 1.5 (0.3, 6.5)	4 (50) 3.7 (0.8, 16)	18 (21)	.30
Retinopathy of prematurity	5 (28) 2.4 (0.6, 9.7)	2 (14) 1.1 (0.15, 8.9)	1 (12.5) 0.9 (0.05, 15)	11 (13)	.50
Patent ductus arteriosus	12 (67)‡ 2.5 (1.0, 6.2)	4 (29) 0.6 (0.1, 2.4)	4 (50) 1.2 (0.3, 5.3)	37 (44)	.22
Death beyond 2 weeks of life	1 (6) 0.9 (0.05, 17)	0 (0) —	0 (0) —	4 (5)	1.0

* P value based on global χ^2 test or Fisher exact test on 3 degrees of freedom, or on 1-way analysis of variance F test (for continuous variables).

† P < .05 by post-hoc Dunnett test, using negative *U urealyticum* colonization status as the reference level (control).

‡ P value < .05 when 95% CI for OR excludes 1.

§ Delivery room resuscitation defined as need for positive pressure ventilation with bag and mask or intubation with positive pressure ventilation. All other infants were placed immediately onto nasal continuous positive airway pressure.

Use of conventional ventilation defined as being required at any time during hospitalization. Sepsis defined as a symptomatic infant prompting a work up and yielding a positive blood culture. The organisms identified from blood cultures included: 1 *Escherichia coli*, 1 group D *Streptococcus*, 1 Group B *Streptococcus*, 15 *Staphylococcus epidermidis*, 5 *Staphylococcus aureus*, 3 *Staphylococcus hominis*, 2 *Klebsiella pneumoniae*, 3 *Enterobacter cloacae*, 1 *Acinetobacter*, and 4 *Candida*. Retinopathy of prematurity defined as stage 1 or greater during screening examination at any age during hospitalization. Patent ductus arteriosus defined as a symptomatic infant with a murmur and/or bounding pulses with echocardiographic confirmation.

ation between persistently positive *U urealyticum* colonization and CLD, it does not establish a causal relationship. It is possible that persistent *U urealyticum* colonization is a marker of other multifactorial factors that lead to CLD. Persistently colonized in-

fants were smaller, younger, and sicker than the culture-negative, early transient, and late acquisition groups. However, controlling for these factors in the multivariate analyses, this association between persistently positive colonization and CLD was similar

TABLE 3. Risk Factors for Development of CLD in Preterm VLBW Infants

Variable	Univariate Analysis		Multivariate Analysis	
	Oxygen Dependency at 28 Days	Oxygen Dependency at 36 Weeks	Oxygen Dependency at 28 Days	Oxygen Dependency at 36 Weeks
	OR (95% CI)	OR (95% CI)	AOR (95% CI)	AOR (95% CI)
Birth weight <750 g	7.38 (2.89, 18.9)*	6.67 (1.48, 30.1)*	5.03 (1.29, 19.7)*	NS
Gestational age <26 wk	13.3 (4.89, 35.9)*	7.06 (1.56, 31.9)*	5.58 (1.48, 21)*	NS
Male gender	2.83 (1.16, 6.89)*	1.73 (0.39, 7.59)	4.03 (1.08, 15.1)*	NS
Patent ductus arteriosus	17.9 (5.01, 64)*	9.23 (1.10, 77.6)*	14.1 (2.97, 67)*	NS
Inborn	0.27 (.08, .93)*	0.24 (.04, 1.36)	NS	NS
Vaginal delivery	1.12 (0.45, 2.79)	2.55 (0.60, 10.8)	NS	NS
Antenatal steroids	3.66 (0.80, 16.8)	0.67 (0.13, 3.55)	NS	NS
Any episode of sepsis	3.03 (1.22, 7.52)*	4.11 (0.88, 19.1)	NS	10.3 (1.53, 69)*
Surfactant administration	3.24 (1.13, 9.26)*	24.5 (4.44, 135)*	NS	NS
Persistently positive <i>U urealyticum</i> colonization	10.0 (3.30, 30.3)*	24.5 (4.44, 135)*	5.33 (1.05, 27)*	33.6 (4.78, 237)*

NS indicates not significant.

* *P* value <.05 when 95% CI for OR excludes 1.

in magnitude to that obtained in the univariate analysis.

Although causality was not addressed in this study, a substantial body of literature including human, animal, and in vitro studies argues that *U urealyticum* may cause lung injury. At least 3 mechanisms of lung damage to the developing lung have been proposed.

Cassell et al¹⁴ first suggested that *U urealyticum* infection produces acute pulmonary inflammation with histologic evidence of bronchopneumonia. This finding was demonstrated in a murine model,³⁷ but not confirmed in human autopsy specimens.³⁸ A second hypothesis is that phospholipase A2, which is produced by *U urealyticum*,³⁹ causes inhibition of pulmonary surfactant,⁴⁰ thereby worsening acute respiratory disease and leading to CLD.

The most recently suggested mechanism is that proinflammatory cytokines found in tracheal aspirates of VLBW infants who are colonized with *U urealyticum* injure the lung, resulting in the development of CLD. Patterson et al⁴¹ found significantly higher levels of interleukin-1 β and tumor necrosis factor- α and significantly lower levels of interleukin-6 among infants colonized with *U urealyticum* on days 1 and 7 in comparison to noncolonized infants. Infants who ultimately developed CLD had significantly higher interleukin-1 β and interleukin-1 β :interleukin-6 ratios.

An unresolved question regarding the role of cytokines in the development of CLD is whether these cytokines are merely aspirated from amniotic fluid in a setting of chorioamnionitis or are produced by the infant in response to ongoing inflammatory stimuli.^{42–44} Several investigators have demonstrated that elevated cytokine levels are associated with *U urealyticum* colonization and that the highest levels of cytokines are induced in the presence of increased ambient oxygen concentrations.^{45,46} Li et al⁴⁷ have recently reported an in vitro study demonstrating that macrophages from tracheal aspirates of VLBW infants produce high levels of tumor necrosis factor- α and interleukin-6 when exposed to *U urealyticum*. We speculate that persistent colonization with

U urealyticum results in ongoing cytokine production leading to prolonged inflammation and lung injury.

Aside from persistent *U urealyticum* colonization, risk factors for oxygen dependency at 28 days and 36 weeks' postconceptional age differed. At 28 days of age these included lower birth weight, shorter gestation, and the presence of a symptomatic PDA, long considered "traditional" risk factors for CLD. At 36 weeks' postconceptional age the influence of these traditional risk factors disappeared.

One reason for the difference in risk factors for CLD at these 2 endpoints might be the long recovery period between 28 days chronological age and 36 weeks' postconceptional age experienced by most of the tiny infants with CLD in our sample.

Respiratory management at our center differs significantly from other NICUs.^{48,49} We use more nasal prong continuous positive airway pressure, less intubation, less surfactant administration, and less mechanical ventilation. In this setting of "gentler ventilation," only sepsis and persistently positive *U urealyticum* colonization emerged as significant risk factors for this outcome. Additional investigation of the association between CLD and *U urealyticum* colonization in different respiratory management settings would be valuable.

Our observations support the concept of the "new BPD,"⁵⁰ which unlike classic BPD is not thought to be primarily related to barotrauma and oxygen toxicity, but rather to ongoing injury that interferes with normal parenchymal development and alveolarization. We suggest that our findings of increased risk of CLD with long-term *U urealyticum* colonization are consistent with the concept of the "new BPD."

A clearer understanding of the factors that impact on lung development and disrupt normal alveolarization is needed. The interaction between *U urealyticum* colonization, phospholipase production and cytokine activity in the developing lung should be elucidated before large scale *U urealyticum* targeted treatment trials aimed at reducing the incidence of CLD are undertaken. The results of clinical trials of interventions to prevent CLD should include consid-

eration of persistent *U urealyticum* colonization in the pathogenesis of CLD.

CONCLUSION

This is the first study to demonstrate that different patterns of *U urealyticum* colonization relate to the development of CLD among VLBW infants. Although early transient and late acquisition colonization accounted for 55% of the *U urealyticum*-positive infants in our NICU population, only persistently positive colonization was associated with the development of CLD. Failure to distinguish between the patterns of *U urealyticum* colonization among VLBW infants may mask the association between *U urealyticum* colonization and CLD.

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