Radiographic Changes Associated with Tracheal Isolation of *Ureaplasma Urealyticum* from Neonates

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Recent studies show an association between the presence of *Ureaplasma urealyticum* in tracheal aspirates and bronchopulmonary dysplasia. We hypothesized that among infants with birth weights ≤1,250 g and respiratory disease, those with *U. urealyticum* in their tracheal aspirates would have radiographic evidence of more-severe pulmonary disease more often than would those without this organism. A total of 292 low-birth-weight infants who had endotracheal aspirate cultured within 7 days of birth were enrolled. The radiographic outcome variables were pneumonia, early severe bronchopulmonary dysplasia (precocious), and chronic lung disease. Microorganisms were isolated from 128 infants (44%); *U. urealyticum* was isolated from 44 (15%). Pneumonia was significantly more common in infants with than without *U. urealyticum* (30% vs. 16%, $P = .03$). *U. urealyticum* also was associated with precocious bronchopulmonary dysplasia independent of prematurity, race, and sex (odds ratio, 2.2; $P < .05$). Tracheal isolation of *U. urealyticum* within 7 days of birth is associated with pneumonia and precocious bronchopulmonary dysplasia.

Bronchopulmonary dysplasia is a debilitating pulmonary disease that is limited primarily to preterm infants [1]. Approximately 1,300 new cases were identified annually in the United States in the early 1980s, at a projected cost of at least $25 million for caring for these infants during their first year [2]. The approximate incidence of this disease at the end of the decade was 7,000 new cases per year, with a concomitant increase in costs [3]. Even though the associated mortality is now decreasing, up to one-third of these infants die before their first birthday [4]. One-half of those who survive may have pulmonary dysfunction throughout their life [5]. The number of new cases each year is likely to increase as the number of very premature infants who survive increases.

Bronchopulmonary dysplasia usually results from the treatment of an acute lung disease [6]. Although advances in therapy have been important in the reduction of morbidity and mortality, the key to the treatment of bronchopulmonary dysplasia is prevention. Hyaline membrane disease, patent ductus arteriosus, and pneumonia are diseases or conditions associated with bronchopulmonary dysplasia [6–9]. Pneumonia is particularly troublesome to the clinician because of the difficulty in diagnosis. We undertook a study of the role of microorganisms in the respiratory tracts of neonates and found that the isolation of *Ureaplasma urealyticum* from the trachea of infants who weighed ≤1,000 g at birth and had an oxygen requirement was associated with bronchopulmonary dysplasia [10]. We did not evaluate chest radiographs of the infants in that study. Therefore, the purpose of this study was to correlate findings on chest radiographs, paying particular attention to pneumonic and chronic changes and to the results of culture of the initial tracheal aspirate obtained within 7 days of birth from low-birth-weight infants with respiratory disease.

Patients and Methods

Study Design

This study was conducted in a nonconcurrent, prospective manner [11]. Enrollment of infants was based on tracheal aspirate culture results obtained prospectively. At the conclusion of the entry period, all of the chest radiographs for each infant were obtained from the archives and examined for selected outcomes. We tested the hypothesis that the chest radiographic findings for those infants who had *U. urealyticum* isolated from their trachea would more frequently be consistent with pneumonia, severe chronic lung disease, or both than would radiographic findings for infants who did not have *U. urealyticum* isolated from their trachea. This study was approved by the institutional review board for human use at the University of Alabama at Birmingham (UAB).

Study Population

Infants who had respiratory disease, birth weights ≤2,500 g, a tracheal aspirate cultured for mycoplasmas and bacteria

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within 7 days of birth, and chest radiographs available for review and who were admitted to the Regional Newborn Intensive Care Unit at UAB from July 1985 to February 1989 were enrolled in this study. February 1989 was chosen as a stopping point for enrollment because a trial of a surfactant was beginning at that date.

Specimen Collection

Tracheal aspirates were obtained routinely as part of a work-up for sepsis when respiratory disease was present. The technique for collection of tracheal aspirates has been described in detail [10]. In brief, a neonate was intubated with an endotracheal tube, and tracheal secretions were aspirated with a suction catheter inserted through the endotracheal tube. If secretions were scant, 0.5–1.0 mL of sterile normal saline was ventilated into the tracheobronchial tree and aspiration was attempted again. The distal portion of the catheter was severed and flushed with 1.0 mL of sterile normal saline; 0.1–0.2 mL was placed in 0.9 mL of 10B media and sent to the Diagnostic Mycoplasma Laboratory at UAB, and the rest was sent to the UAB hospital clinical microbiology laboratory for routine aerobic bacterial culture. Serial 10-fold dilutions of the aliquot for culture of mycoplasmas were performed as soon as the sample arrived at the laboratory. Samples (20 μL) of each dilution were plated on A8 agar. Cultures were kept for 7 days before being discarded and reported as negative. Routine aerobic bacterial cultures were inoculated into brain-heart infusion (BHI) broth and plated on chocolate, blood, and MacConkey agar. The agar plates were discarded after 48 hours if they were negative. BHI broth was examined daily for turbidity, and samples were gram-stained on day 5 before being discarded as negative. No viral, chlamydial, and anaerobic bacterial cultures were prepared because of the extremely low yield in this population [10].

Data Collection

Demographic and clinical data were obtained from the medical records. Cultural data were obtained from the Diagnostic Mycoplasma Laboratory data base and from the reports of clinical laboratory data in the medical records.

Radiographic Analysis

On admission to the neonatal intensive care unit, each infant with respiratory disease had a chest radiograph taken as part of routine care. As a rule, chest radiographs were obtained daily during the first week while the infant had acute lung disease or was intubated for mechanical ventilation. Subsequent radiographs were ordered at the discretion of the physicians caring for the infant as clinical status dictated.

For purposes of this study, chest radiographs were retrieved from the archives after the last infant was enrolled in this study. A pediatric radiologist (G.T.O.) reviewed the chest radiographs without the benefit of clinical history. The radiographs from ~60 infants were interpreted during each session. Toward the end of the radiographic reading process, radiographs from ~1% of the infants were reinterpreted without the radiologist's knowledge. No discrepancies were found between the first and second interpretations. In addition, ~10% of the radiographic interpretations for this study were checked against the original written account of the clinical interpretations. Again, no discrepancies were found.

The primary radiographic outcomes were chronic lung disease, which was defined as the presence of any grade of bronchopulmonary dysplasia at the end of the fourth postnatal week; precocious dysplastic changes, which were defined as radiographic findings consistent with grade III or IV bronchopulmonary dysplasia but that occurred during the second or early third postnatal week; and pneumonia, which was defined as the presence of one or more of the following: (1) radiating peripheral streakiness, (2) coarse patchy parenchymal infiltrates or small diffuse nodules, (3) subtle hazy or nodular basilar infiltrates, or (4) diffuse granularity indistinguishable from hyaline membrane disease but with better-than-expected aeration (figures 1 and 2) [12, 13].

Radiographs also were reviewed for the following ad hoc outcome variables: hyaline membrane disease, transient tachypnea of the newborn, pulmonary interstitial emphysema, pneumothorax, and atelectasis [13, 14].

Data Analysis

Infants were placed in subgroups according to the identification of the organisms isolated from their initial tracheal aspirate. Those with *U. urealyticum* isolated from their trachea were grouped together regardless of the other organisms isolated. All infants who had organisms other than *U. urealyticum* isolated were grouped in the bacteria subgroup. This subgroup included infants who had bacteria or mycoplasmas other than *U. urealyticum*. Infants who had negative cultures or cultures negative for mycoplasmas and no bacterial cultures ($n = 4$) were placed in the negative subgroup.

Categorical data were analyzed by the χ^2 test for independence or by Fisher's exact test where appropriate. Continuous data were analyzed by the analysis of variance technique, and intergroup differences were analyzed by Duncan's multiple-range test. Multiple logistic regression was used to determine the strength of the association of the cultural data and the primary radiographic outcomes while holding the effects of demographic influences constant. The level of significance was set at $P < .05$ for demographic, clinical, and primary outcome variables and at $P < .01$ for other ad hoc outcome variables. For completeness, all P values of $<.05$ are presented in the tables and figures.

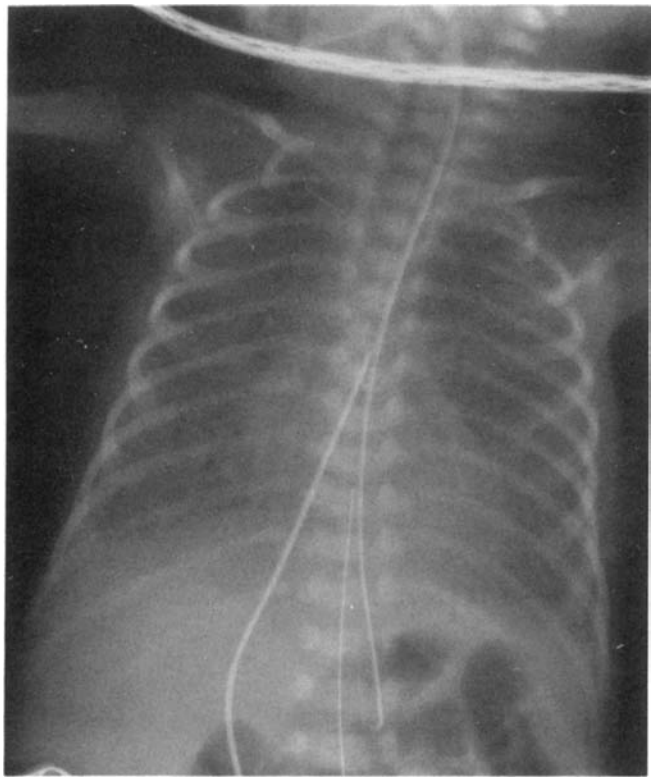

Figure 1. Chest radiograph of an infant showing many features consistent with pneumonia. The lung fields are hazy with some streaky densities. The diaphragmatic excursion is good, signifying good compliance, as opposed to the small lung fields seen with poorer compliance, as in hyaline membrane disease.

Results

Study Population

A total of 314 infants were eligible for this study. Four infants were excluded because of congenital heart disease or meconium aspiration syndrome. For 18 of the remaining 310 infants eligible for this study, no radiographs were available for review, and thus 292 infants were enrolled in this study. The mean birth weight and mean gestational age for the infants who were enrolled were 1,210 g (SD ± 478 g) and 29 weeks (SD ± 3.0 weeks), respectively; 48% of the infants were white, and 56% were boys. No significant differences between the birth weights, gestational age, race, sex, percentage of Apgar scores <7 at 1 minute or 5 minutes, or survival of enrolled infants and those who did not have radiographs available were detected. Thus, no obvious selection biases associated with the inclusion of only those infants with radiographs available were detected.

Cultural Data

The mean age of infants when the initial tracheal aspirate was obtained was 1.3 days (SD ± 1.5 days; median, 1.0 day; 95th percentile, 4 days). No organisms were isolated from 66% (192) of the 292 infants, whereas *U. urealyticum* was isolated from 15% (45) of the infants and bacteria were isolated from 19% (55) (table 1). *U. urealyticum* was the organism isolated most frequently and was in pure culture 71% of the time (32 of 45). Bacteria were isolated in pure culture 67% (37 of 55) of the time. *Mycoplasma hominis*, which was isolated from 8% (22) of the infants, was the second most commonly isolated organism. Group B β-hemolytic *Streptococcus* was isolated from eight infants (3%); in two, it was isolated in combination with *U. urealyticum*.

Demographic and Clinical Data

The greater immaturity of infants who had *U. urealyticum* isolated from their trachea compared to infants who had bacteria or other mycoplasmas or no organisms isolated is reflected by the significantly lower mean birth weight and gestational age, the higher percentage with 5-minute Apgar scores of <7, and the greater percentage receiving mechanical ventilation for any reason (table 2). These differences were not significant when the infants were subgrouped by birth weight.

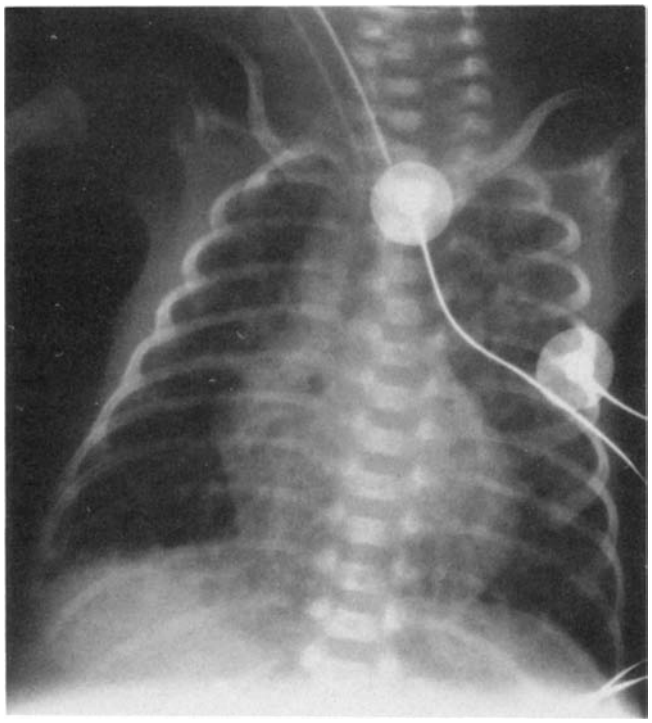

Figure 2. Chest radiograph of a 9-day-old infant with precocious dysplastic changes. The lung fields have areas of variable aeration. Patchy areas of atelectasis, or early scarring, are noted along with cystic emphysematous areas. The lungs appear overaerated, a finding that correlates clinically with air trapping. This radiograph is consistent with grade III bronchopulmonary dysplasia, but these changes are noted much earlier than is usual.

Table 1. Results of tracheal aspirate culture for 292 low-birth-weight infants.

Microorganism isolated	No. of infants (%)
None	192 (66)
Any microorganism	100 (34)
*Ureaplasma urealyticum**	45 (15)
Other bacteria†	55 (19)
Mycoplasma hominis	22 (8)
Group B *Streptococcus*	6 (2)
Staphylococcus epidermidis	7 (2)
Coagulase-negative *Staphylococcus*	9 (3)
Streptococcus, viridans group	3 (1)
Escherichia coli	1 (1)
Enterococcus	2 (1)
Propionibacterium acnes	4 (1)
Other‡	8 (3)

* Includes 13 infants with bacteria isolated concurrently (group B *Streptococcus*, 2; *M. hominis*, 2; *Staphylococcus epidermidis*, 2; *Streptococcus*, viridans group, 2; coagulase-negative *Staphylococcus*, 2; and one each of *Candida albicans*, *P. acnes*, *Staphylococcus aureus*, and *Peptostreptococcus* species).

† Total for individual microorganisms and "other bacteria" total will not agree because of multiple isolates in some cultures.

‡ Includes one each of *Bacillus* species, *Bacteroides* species, *Candida albicans*, diphtheroid species, *Proteus mirabilus*, *Pseudomonas* species, *S. aureus*, and *Peptostreptococcus* species.

Primary Radiographic Outcomes

Pneumonia. Radiographic evidence of pneumonia was twice as common in *U. urealyticum*–positive infants than in *U. urealyticum*–negative infants (30% vs. 16%, $P = .03$) (figure 3). When comparisons were made by individual culture groups, pneumonia was over three times more common in infants in the *U. urealyticum* group than in those in the bacteria group ($P = .01$) and tended to be more common than in the negative group ($P = .08$) (figure 4). Similar relationships were found when infants weighing ≤1,250 g at birth were examined (figure 4). *U. urealyticum* was associated with pneumonia when the data were analyzed by multiple logistic regression (odds ratio, 3.2; $P = .05$) but did not reach statistical significance. Conversely, tracheal isolation of bacteria was not associated with radiographic evidence of pneumonia.

To determine if the increased incidence of pneumonia in infants with *U. urealyticum* might be due to the inclusion of infants who also had bacteria isolated from their trachea, we examined the percentage of infants in the *U. urealyticum* group with and without bacteria. In the *U. urealyticum* group, infants with bacteria present had a lower incidence of pneumonia than did those without bacteria (23% vs. 32%, respectively, $P > .05$). Thus, the inclusion of those infants who had *U. urealyticum* in pure culture with those infants who had *U. urealyticum* isolated with other bacteria did not skew the results toward a higher incidence of pneumonia. In fact, the opposite was true. Statistical significance could not be achieved because of the small sample size and lack of power.

Precocious dysplastic changes. Precocious dysplastic changes were significantly more common in infants with *U. urealyticum* isolated than in those without this isolate ($P = .01$) (figure 3). When comparisons were made by individual culture groups, the percentage of infants with precocious dysplastic changes was significantly higher in infants with *U.*

Table 2. Demographic and clinical data for infants with different results of tracheal aspirate culture.

	All infants (n = 292)				Infants ≤1,250 g (n = 177)			
Variable	Negative	*U. ureolyticum*	Bacteria	P*	Negative	*U. urealyticum*	Bacteria	P*
No. (%)	192 (66)	45 (15)	55 (19)		120 (68)	34 (19)	23 (13)	
Birth weight in g (range)	1,215 (505–2,490)	1,062† (510–2,400)	1,314† (604–2,410)	.05	900 (505–1,250)	860 (510–1,240)	840 (604–1,250)	NS
Gestational age in weeks (range)	29 (22–38)	28† (23–36)	30† (22–38)	.05	27 (22–32)	27 (23–31)	27 (23–31)	NS
Male	55	62	55	NS	52	68	52	NS
White	52	44	36	NS	47	44	39	NS
Apgar score at 1 min <7	63	78	63	NS	76	79	96	NS
Apgar score at 5 min <7	30‡	53‡	24‡	.01	39	56	52	NS
Survived hospital	76	71	87	NS	69	65	74	NS
Oxygen required for 28 days	21	29	22	NS	27	38	48	NS
Ventilation	75	87	65	NS	87	91	91	NS
Apnea	56	60	44	NS	67	71	65	NS

NOTE. Values in table represent percentages unless otherwise noted.

* Probability of difference within groups. NS = not significant.

† Significantly different from *U. urealyticum* group at $P < .05$.

‡ Significantly different from *U. urealyticum* group at $P < .01$.

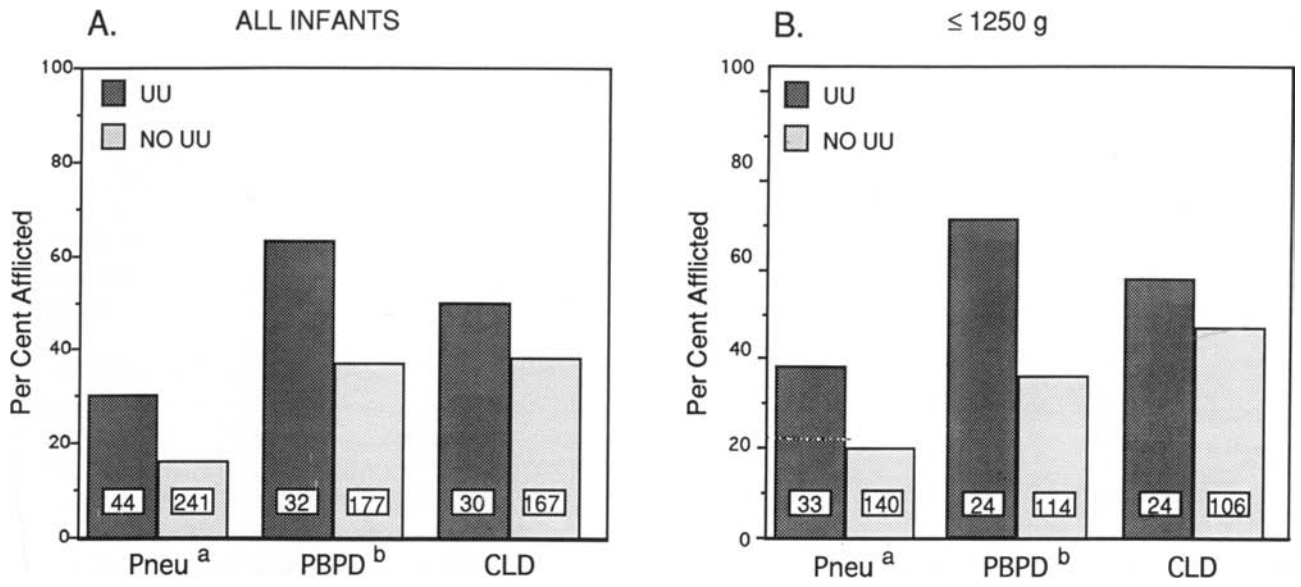

Figure 3. Percentage of infants with primary radiographic outcomes grouped according to whether *Ureaplasma urealyticum* (UU) was present or absent. Pneu = radiographic evidence of pneumonia; PBPD = precocious dysplastic changes; CLD = chronic lung disease. All infants are depicted in A, and those with birth weights ≤1,250 g are depicted in B. Significant differences (by χ^2) are indicated by *a* ($P < .05$) and *b* ($P < .01$). The number in each bar represents the number of infants included in the denominator.

urealyticum isolated than in those with negative cultures (63% vs. 36%, $P = .005$) but was not significantly higher than in those with bacteria isolated (63% vs. 41%, $P > .05$) (figure 4). The same associations were present when only infants with birth weights ≤1,250 g were included in the comparisons (figure 4). When multiple logistic regression analysis was used to control for the influences of prematurity (birth weight), race, and sex, precocious dysplastic changes were still significantly associated with the presence of *U. urealyticum* ($P < .05$; odds ratio, 2.2). Infants who died before they were 14 days old were excluded from this analysis because no radiograph was available for review. No significant differences were found between culture groups in the percentage of infants who died before 14 days of age (*U. urealyticum*, 29%; bacteria, 13%; and negative, 24%; $P = .11$). As expected, birth weight was inversely associated with precocious dysplastic changes ($P < .0001$). No association was found between gender and precocious dysplastic changes, whereas

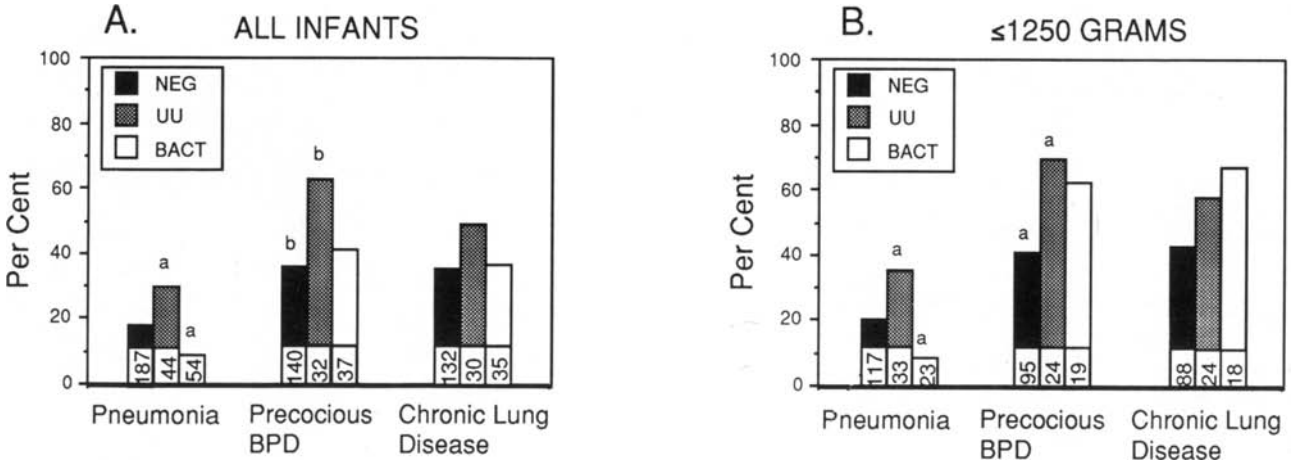

Figure 4. Percentage of infants with primary radiographic outcomes, by culture groups. BPD = dysplastic changes. In different outcome groups, the number of infants in each culture group varied because of death; therefore, the denominator of each group is presented on the corresponding bar. All infants are depicted in A, and those with birth weights ≤1,250 g are depicted in B. All infants who had *Ureaplasma urealyticum* (UU) isolated from their initial tracheal aspirate, whether or not other bacteria also were isolated, are included in the UU group. Significant differences (by χ^2) are indicated by *a* ($P < .01$) and *b* ($P < .05$).

among infants with *U. urealyticum* isolated from their trachea, white infants were twice as likely as black infants to have precocious dysplastic changes ($P < .02$).

The contribution of other bacteria to the incidence of precocious dysplastic changes in the *U. urealyticum* group was examined. The incidence of precocious dysplastic changes was lower in infants whose tracheal aspirates yielded both *U. urealyticum* and bacteria than in infants with pure cultures of *U. urealyticum* (54% vs. 68%, respectively; $P > .05$). Logistic regression analysis detected a small interaction between *U. urealyticum* and other bacteria. When this interaction was accounted for in the logistic regression procedure, the level of significance dropped slightly ($P = .055$; odds ratio, 2.2). Thus, the inclusion of infants who had pure cultures of *U. urealyticum* with those infants who had *U. urealyticum* isolated with other bacteria did not skew the results toward a higher incidence of precocious dysplastic changes.

Chronic lung disease. The radiographic diagnosis of chronic lung disease tended to be more common in the *U. urealyticum*–positive group than in the *U. urealyticum*–negative group (50% vs. 38%, respectively), although statistical significance was not achieved ($P > .05$). In addition, chronic lung disease was not associated with the isolation of any individual microbe, although the direction of the interaction between *U. urealyticum* and bronchopulmonary dysplasia was the same as that with *U. urealyticum* and precocious dysplastic changes (figure 4). Grouping infants who had pure cultures of *U. urealyticum* with infants who had mixed cultures of *U. urealyticum* with other bacteria did not increase the incidence of chronic lung disease in this group (50% vs. 50%, $P > .05$).

The incidence of chronic lung disease appeared higher in infants in the group with bacteria isolated who weighed ≤1,250 g at birth as compared with infants in either the *U. urealyticum* group or the culture-negative group, although statistical significance was not achieved ($P > .05$) (figure 4). Nine different organisms were isolated from the infants in the bacteria group. *Mycoplasma hominis* accounted for the majority of the organisms (9 of 18) isolated from these tiny infants and for most of the cases of chronic lung disease (6 of 12) in the bacteria group.

Evidence for Disease Progression

Twice as many infants with pneumonia (25 of 51) than infants without pneumonia (58 of 234) developed precocious dysplastic changes (49% vs. 25%, $P < .001$). The odds ratio for the development of precocious dysplastic changes when pneumonia was present was 2.5 ($P = .01$, multiple logistic regression). The likelihood of development of precocious dysplastic changes in the different subgroups was examined. More infants with pneumonia and *U. urealyticum* developed precocious dysplastic changes (69%, 9 of 13) as compared with infants with pneumonia and bacteria (40%, 2 of 5) or infants with pneumonia and negative cultures (42%, 14 of 33) ($P > .05$). A similar preponderance of precocious dysplastic changes was found in infants who did not have pneumonia (data not shown). Only trends could be noted because of the small number of infants in each category. It is striking, however, that 85% of the infants with precocious dysplastic changes (11 of 13) developed bronchopulmonary dysplasia whereas only 9% of the infants without these changes (2 of 20) developed bronchopulmonary dysplasia ($P < .0001$).

Ad Hoc Outcomes

No significant differences at $P < .01$ were detected for the ad hoc outcome variables (figure 5).

Discussion

The purpose of this study was to describe the radiographic changes associated with isolation of *U. urealyticum* from the trachea of low-birth-weight infants and not to describe clinical outcomes. Our data show that low-birth-weight infants with respiratory distress who have *U. urealyticum* isolated from their trachea within 7 days of birth are likely to show evidence of pneumonia and precocious dysplastic changes on chest radiographs. Precocious dysplastic changes are independent of prematurity, sex, and race. Conversely, bacteria are not associated with these radiographic changes. The association of *U. urealyticum* and precocious dysplastic changes are confined mainly to the low-birth-weight group, the group at most risk for chronic lung disease.

The designation *precocious dysplastic changes* was used to describe findings on radiographs consistent with severe bronchopulmonary dysplasia that occur at or near the end of the second postnatal week. In reality, this is the progression described in the original paper by Northway et al. on bronchopulmonary dysplasia [15]. Since that description around 25 years ago, the progression through the stages of bronchopulmonary dysplasia has slowed down [2]. It is now customary to find that the hazy appearance of stage II disease lasts for several weeks and that stages III and IV disease occur much later if at all [2]. Thus, we chose to use precocious dysplastic changes to describe the more rapid progression to severe disease typical of the original description of bronchopulmonary dysplasia but atypical for the disease progression seen today.

The demonstration of an association between *U. urealyticum* and dysplastic changes in this study is in accordance with previous reports. We described 200 neonates with birth weights ≤2,500 g and respiratory disease who had *U. urealyticum* isolated from their trachea [10]. Those with birth weights ≤1.0 kg were more likely to develop bronchopulmonary dysplasia, as defined by their requirement for continuous oxygen support until 28 days of age. Sánchez and Regan studied 111 infants at Columbia University with birth

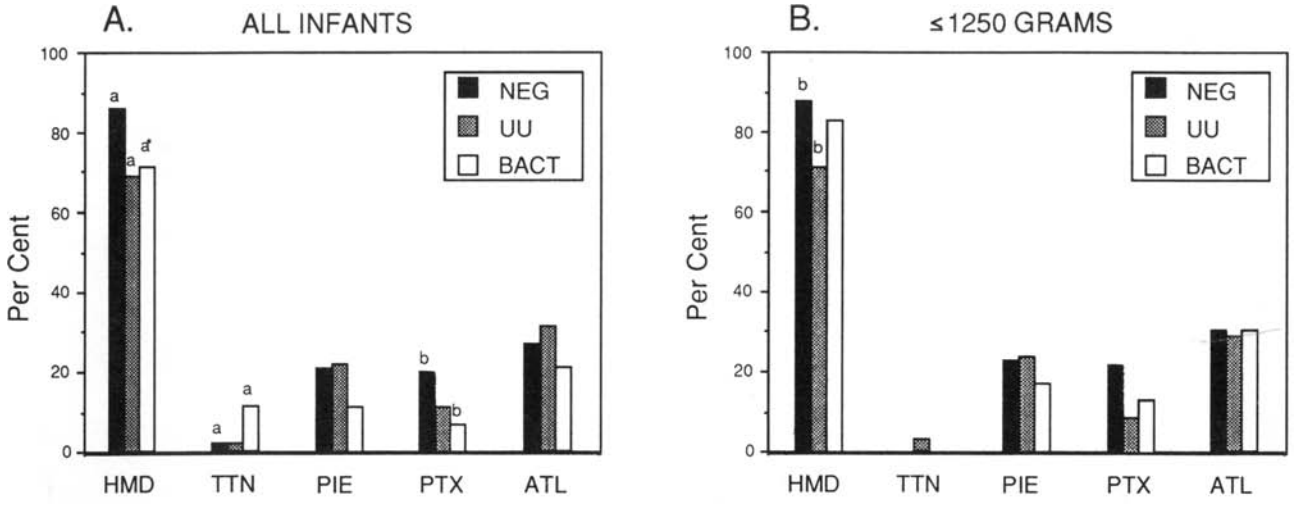

Figure 5. Percentage of infants with ad hoc radiographic outcomes, by culture group. HMD = hyaline membrane disease; TTN = transient tachypnea of the newborn; PIE = pulmonary interstitial emphysema; PTX = pneumothorax; ATL = atelectasis; and UU = *Ureaplasma urealyticum*. All infants are depicted in A, and those with birth weights ≤1,250 g are depicted in B. The number of infants in each culture group varied little by outcome group (data not shown but available). Significant differences (by χ^2) are indicated by *a* ($P < .01$) and *b* ($P < .05$).

weights <2.0 kg admitted to a newborn intensive care unit and demonstrated that infants colonized with *U. urealyticum* had a significantly higher incidence of bronchopulmonary dysplasia than did those without such colonization (30% vs. 8%) [16]. In their studies, they used both abnormal radiographs and oxygen requirement at 30 days of age as the factors determining bronchopulmonary dysplasia. Wang et al. studied 107 infants at McMaster University who weighed <1,250 g at birth and found that colonization with *U. urealyticum* produced a relative risk of 3.4 for the development of bronchopulmonary dysplasia [17]. This effect was found to be independent of prematurity and respiratory support. Bronchopulmonary dysplasia was defined by an abnormal radiograph and abnormal arterial blood gas results at 28 days of age or by the radiologist's opinion, based on the last available radiograph for an infant who died before the age of 28 days, that the infant would have bronchopulmonary dysplasia as defined by the investigators' criteria. That these studies performed at three different centers with disparate patient populations obtained similar results attests to the generalizability of these results.

The lack of association of bacteria with radiographic evidence of pneumonia is in accordance with the findings of previous studies. Sherman et al. demonstrated a correlation between the isolation of bacteria from the trachea within 8 hours of birth and subsequent bacteremia but none for radiographic evidence of pneumonia [18]. Ablow et al. demonstrated that radiographic findings of infection with group B streptococci were often indistinguishable from those for the respiratory distress syndrome [19, 20].

The most likely explanation for why bacteria were not associated with precocious dysplastic changes is the insufficient power of our study to detect a difference at the level of $P < .05$. This assumption is supported by our finding that the direction of the association between tracheal isolation of bacteria and precocious dysplastic changes is the same as that seen with tracheal isolation of *U. urealyticum* (figure 4). A second explanation is that tracheal isolation of bacteria might reflect colonization after birth rather than true infection. Support for this speculation is given in the report by Harris et al., who reported that 43% of infants with respiratory disease were colonized with bacteria at the time of intubation [21]. Unfortunately, Harris et al. combined the results for nasopharyngeal and tracheal aspirates. Additional support is provided by Lau and Hey, who reported that bacteria are commonly isolated from the respiratory tract of infants with pulmonary disease and are not specific for pneumonia [22]. Differences in the significance of tracheal isolation of *U. urealyticum* and that of bacteria might also be explained by the mode of transmission. The major mode of transmission of *U. urealyticum* is vertically from mother to offspring, whereas transmission of bacteria may be either vertical or nosocomial [23].

No study group showed a significant association with radiographic evidence of chronic lung disease. *M. hominis* was primarily responsible for the apparent increased incidence of chronic lung disease in the bacterial group. No other organism in the bacteria group seemed to be associated with chronic lung disease. In particular, virulent organisms such as group B streptococci were not associated with either early or late dysplastic changes. Because of small numbers of any one microbe and the resultant lack of statistical power, we cannot rule out an association between severe chronic lung disease and the presence of a particular microorganism. We

also used a liberal definition that did not differentiate severe changes from very mild lung disease; therefore, we may not have been able to detect long-term changes associated with infectious agents.

In this study, microorganisms were isolated from approximately one-third of the initial tracheal aspirates obtained from infants within 7 days of birth. Two-thirds of the organisms were mycoplasmas. *U. urealyticum* was significant in that it accounted for 44% of the organisms isolated and 15% overall. The microorganisms grouped into the bacteria subgroup were quite heterogenous. *M. hominis* was the most frequently isolated organism and group B *Streptococcus*, the second. The heterogeneity of the bacteria isolated and the frequency with which multiple isolates were obtained suggest that nosocomial colonization probably accounted for many of the positive bacterial cultures.

The results of this study combined with the results reported in the literature create a cohesive argument that *U. urealyticum* is a risk factor for, and not only associated with, bronchopulmonary dysplasia. In calves, *Ureaplasma diversum* causes a cuffing pneumonia characterized by alveolar and peribronchiolar mononuclear infiltration [24, 25]. We and others have shown that *U. urealyticum* causes pneumonia in human neonates that is characterized by both mononuclear and polymorphonuclear alveolar infiltration [26–28]. Rudd et al. fulfilled Koch's postulates by causing pneumonia in a newborn mouse by intranasal inoculation with *U. urealyticum* isolated from an infant with pneumonia [29]. We also have shown that supplemental oxygen increases lesion severity, delays clearance of the organism, and causes death in this mouse model of ureaplasmal pneumonia [30]. Reports by us and others have shown an association between *U. urealyticum* and bronchopulmonary dysplasia [10, 16, 17], and pneumonia caused by other microorganisms is associated with development of bronchopulmonary dysplasia [8, 9]. Finally, the events in the present study, i.e., the presence of *U. urealyticum* in the respiratory tract, the presence of radiographic evidence of pneumonia, the development of precocious dysplastic changes, and the subsequent development of bronchopulmonary dysplasia, have a sequential association. Thus, the data justify the pursuit of clinical trials designed to better define or alter this disease course.

Several aspects of this study are important. This study was performed before the routine administration of surfactant or steroids and the common administration of erythromycin. Therefore, the course of disease described here is unaffected by therapies known to alter the course of neonatal respiratory disease or by antibiotic therapy that may eradicate *U. urealyticum*. For these reasons, it would be difficult to repeat this study.

The conclusions of this study have been criticized on the grounds that *U. urealyticum* might promote subsequent bacterial colonization/infection of the respiratory tract in infants who have an increased incidence of precocious dysplastic changes and that it is the subsequent bacterial colonization/infection, not *U. urealyticum*, that is associated with precocious dysplastic changes. However, this is unlikely because dysplastic lung disease is associated with injury that occurs over time. Thus, the incidence of severe lung disease would be higher in infants who have bacteria isolated earlier, i.e., those in this study, than in infants with *U. urealyticum* isolated, and this was not the case. That logistic regression analysis indicated that infants within the *U. urealyticum* group who also had bacteria isolated did not experience a significantly higher incidence of precocious dysplastic changes than those with just *U. urealyticum* isolated argues against bacteria being a significant cause of disease. Third, even if bacterial colonization/infection was promoted by *U. urealyticum* and bacteria were associated with significant disease, it would be *U. urealyticum* that promotes this process and that is thus a potential target for therapies that alter the disease process.

Another criticism of this study is that the radiographs were not obtained at standardized times. We think this is a minor problem because the disease processes associated with our primary outcome variables are quite severe and the standard therapeutic technique is to follow these disease processes with frequent radiographic studies. In addition, these disease processes are a continuum, and radiographs taken even 3–4 days apart are extremely unlikely to overlook one of the primary disease outcomes.

In summary, these data show that the isolation of *U. urealyticum* from the trachea of premature neonates with respiratory disease within 7 days of birth identifies a group of infants likely to show radiographic evidence of pneumonia and to progress to severe lung disease by 2 weeks of age, as evidenced by radiographic developments. This finding is important since it demonstrates that severe pulmonary changes can take place rapidly in infants who have *U. urealyticum* isolated from their trachea. These data also suggest that therapy that reduces the risk or severity of bronchopulmonary dysplasia should be instituted as soon as possible because of the rapidity with which severe pulmonary disease develops in these infants. Therapeutic trials that focus on the elimination of bronchopulmonary dysplasia as the major outcome may be unrealistic, especially if sample sizes are small because of the rapidity with which these changes occur. A more realistic goal might be a reduction in the severity of chronic lung disease and/or a reduction in mortality.

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References

1. Hack M, Horbar JD, Malloy MH, Tyson JE, Wright E, Wright L. Very low birth weight outcomes of the National Institute of Child Health and Human Development Neonatal Network. Pediatrics **1991**; 87:587–97.
2. Bancalari E. Bronchopulmonary dysplasia. In: Milner AD, Martin RJ, eds. Neonatal and pediatric respiratory medicine. London: Butterworths, **1986**.
3. Northway WH Jr. Bronchopulmonary dysplasia: then and now. Arch Dis Child **1990**;65:1076–81.
4. Abman SH, Burchell MF, Schaffer MS, Rosenberg AA. Late sudden unexpected deaths in hospitalized infants with bronchopulmonary dysplasia. Am J Dis Child **1989**;143:815–9.
5. Bader D, Ramos AD, Lew CD, Platzker ACG, Stabile MW, Keens TG. Childhood sequelae of infant lung disease: exercise and pulmonary function abnormalities after bronchopulmonary dysplasia. J Pediatr **1987**;110:693–9.
6. O'Brodovich HM, Mellins RB. Bronchopulmonary dysplasia: unresolved neonatal acute lung injury. Am Rev Respir Dis **1985**;132:694–709.
7. Brown ER. Increased risk of bronchopulmonary dysplasia in infants with patent ductus arteriosus. J Pediatr **1979**;95:865–6.
8. Sawyer MH, Edwards DK, Spector SA. Cytomegalovirus infection and bronchopulmonary dysplasia in premature infants. Am J Dis Child **1987**;141:303–5.
9. Campognone P, Singer DB. Neonatal sepsis due to nontypable *Haemophilus influenzae*. Am J Dis Child **1986**;140:117–21.
10. Cassell GH, Waites KB, Crouse DT, et al. Association of *Ureaplasma urealyticum* infection of the lower respiratory tract with chronic lung disease and death in very-low-birth-weight infants. Lancet **1988**;2:240–4.
11. Riegelman RK. Studying a study and testing a test: how to read the medical literature. Boston: Little, Brown and Company, **1981**:64–5.
12. Friis B, Eiken M, Hornsleth A, Jensen A. Chest x-ray appearances in pneumonia and bronchiolitis: correlation to virological diagnosis and secretory bacterial findings Acta Paediatr Scand **1990**;79:219–25.
13. Edwards DK III. The radiology of bronchopulmonary dysplasia and its complications. In: Merritt TA, Northway WH Jr, Boynton BR, eds. Contemporary issues in fetal and neonatal medicine. Vol 4. Bronchopulmonary dysplasia. Boston: Blackwell Scientific Publications, **1988**:185–234.
14. Tudor J, Young L, Wigglesworth JS, Steiner RE. The value of radiology in the idiopathic respiratory distress syndrome: a radiological and pathological correlation study. Clin Radiol **1976**;27:65–75.
15. Northway WH Jr, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease: bronchopulmonary dysplasia. N Engl J Med **1967**;276:357–68.
16. Sánchez PJ, Regan JA. *Ureaplasma urealyticum* colonization and chronic lung disease in low birth weight infants. Pediatr Infect Dis J **1988**;7:542–6.
17. Wang EEL, Frayha H, Watts J, et al. Role of *Ureaplasma urealyticum* and other pathogens in the development of chronic lung disease of prematurity. Pediatr Infect Dis J **1988**;7:547–51.
18. Sherman MP, Goetzman BW, Ahlfors CE, Wennberg RP. Tracheal aspiration and its clinical correlates in the diagnosis of congenital pneumonia. Pediatrics **1980**;65:258–63.
19. Ablow RC, Gross I, Effmann EL, Uauy R, Driscoll S. The radiographic features of early onset group B streptococcal neonatal sepsis. Radiology **1977**;124:771–7.
20. Ablow RC, Driscoll SG, Effmann EL, et al. A comparison of early-onset group B streptococcal neonatal infection and the respiratory-distress syndrome of the newborn. N Engl J Med **1976**;294:65–70.
21. Harris H, Wirtschafter D, Cassady G. Endotracheal intubation and its relationship to bacterial colonization and systemic infection of newborn infants. Pediatrics **1976**;58:816–23.
22. Lau YL, Hey E. Sensitivity and specificity of daily tracheal aspirate cultures in predicting organisms causing bacteremia in ventilated neonates. Pediatr Infect Dis J **1991**;10:290–4.
23. Rempen A, Martius J, Hartmann AA, Wecker I. Transmission rate of *Ureaplasma urealyticum*, *Mycoplasma* spp., *Gardnerella vaginalis*, B-streptococci, *Candida* spp. and *Chlamydia trachomatis* from the mother to the newborn. Arch Gynecol Obstet **1987**;241:165–70.
24. Knudtson WU, Reed DE, Daniels G. Identification of mycoplasmatales in pneumonic calf lungs. Vet Microbiol **1986**;11:79–91.
25. Howard CJ, Gourlay RN, Thomas LH, Stott EJ. Induction of pneumonia in gnotobiotic calves following inoculation of *Mycoplasma dispar* and ureaplasmas (T-mycoplasmas). Res Vet Sci **1976**;21:227–31.
26. Waites KB, Crouse DT, Philips JB III, Canupp KC, Cassell GH. Ureaplasmal pneumonia and sepsis associated with persistent pulmonary hypertension of the newborn. Pediatrics **1989**;83:79–85.
27. Quinn PA, Gillan JE, Markestad T, et al. Intrauterine infection with *Ureaplasma urealyticum* as a cause of fatal neonatal pneumonia. Pediatr Infect Dis **1985**;4:538–43.
28. Brus F, van Waarde WM, Schoots C, Oetomo SB. Fatal ureaplasmal pneumonia and sepsis in a newborn infant. Eur J Pediatr **1991**;150:782–3.
29. Rudd PT, Cassell GH, Waites KB, Davis JK, Duffy LB. *Ureaplasma urealyticum* pneumonia: experimental production and demonstration of age-related susceptibility. Infect Immun **1989**;57:918–25.
30. Crouse DT, Cassell GH, Waites KB, Foster JM, Cassady G. Hyperoxia potentiates *Ureaplasma urealyticum* pneumonia in newborn mice. Infect Immun **1990**;58:3487–93.