ORIGINAL ARTICLES

Role of *Ureaplasma urealyticum* in lung disease of prematurity

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Abstract

***Aim*—To examine the role of *Ureaplasma urealyticum* colonisation or infection in neonatal lung disease.**

***Methods*—Endotracheal aspirates from ventilated infants less than 28 weeks of gestation were cultured for *U urealyticum* and outcomes compared in infants with positive and negative cultures.**

***Results*—*U urealyticum* was isolated from aspirates of 39 of 143 (27%) infants. Respiratory distress syndrome (RDS) occurred significantly less often in colonised, than in non-colonised infants (p=0.002). Multivariate logistic regression analysis showed that in singleton infants, ureaplasma colonisation was the only independent (negative) predictor of RDS (OR 0.36; p=0.02). Both gestational age (OR 0.46; p=0.006) and isolation of *U urealyticum* (OR 3.0; p=0.05) were independent predictors of chronic lung disease (CLD), as defined by requirement for supplemental oxygen at 36 weeks of gestational age. Multiple gestation was also a major independent predictor of RDS and CLD.**

***Conclusions*—Colonisation or infection with ureaplasma apparently protects premature infants against the development of RDS (suggesting intrauterine infection). However, in singleton infants, it predisposes to development of CLD, independently of gestational age. Treatment of affected infants after birth is unlikely to significantly improve the outcome and methods are required to identify and treat the women with intrauterine ureaplasmal infection, before preterm delivery occurs.**

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Keywords: *Ureaplasma urealyticum*; hyaline membrane disease; chronic neonatal lung disease; intrauterine infection

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Although the causes of preterm delivery are still poorly understood, survival of premature infants has improved progressively over the past 30 years because of advances in neonatal intensive care. However, survivors remain at risk from chronic lung disease (CLD).[1 2] The pathogenesis of CLD is poorly understood, but iatrogenic factors, including endotracheal intubation, high peak inspiratory pressures, and high oxygen concentrations during mechanical ventilation, are important.[3 4] The use of antepartum steroids and exogenous surfactant has significantly decreased the incidence and severity of respiratory distress syndrome (RDS). The reduced need for ventilatory support—and consequently its potentially adverse effects—and other improvements in neonatal management, have been reflected in a decrease in the incidence of CLD. However, although administration of exogenous surfactant to infants with RDS has improved the immediate outcome, it has not consistently reduced the incidence of CLD.[5]

Ureaplasma urealyticum is a commensal organism in the lower genital tracts of 40–80% of women. Isolation of ureaplasmas from the placenta or amniotic fluid is consistently associated with preterm delivery, histological evidence of chorioamnionitis, and postpartum endometritis.[6–8] However, prospective studies have shown neither an association between vaginal colonisation and premature delivery, nor any reduction in prematurity resulting from treatment of colonised women with erythromycin.[9 10] Presumably, any adverse effects associated with ureaplasma colonisation are confined to a subset of colonised women with intrauterine infection.

Upper respiratory colonisation with *U urealyticum* in premature neonates is often associated with clinical, radiological, or laboratory evidence of respiratory infection or frank pneumonia.[11–16] An infective and inflammatory basis for CLD has been proposed[17–18]and, in particular, neonatal colonisation or infection with *U urealyticum* has been linked to the development of CLD.[11 19–22] However, some studies have failed to confirm an association and a causal association remains unproved and controversial.[23–26] Differences in results between studies can, in part, be attributed to variability in study design, the characteristics and size of patient cohorts, and the quality of laboratory and statistical methods.

This prospective cohort study aimed to examine the association between *U urealyticum* colonisation and respiratory disease in premature infants most at risk of both; optimal

specimens and culture methods and three different definitions of CLD were used. A newly developed polymerase chain reaction (PCR) was used to determine the biotype and serotypes of *U urealyticum* isolates.

Methods

Westmead and Royal Prince Alfred Hospitals are both teaching hospitals of the University of Sydney. They provide tertiary obstetric and perinatal referral services. The study was approved by the institutional committees of both hospitals.

The study was confined to infants of less than 28 weeks of gestational age. A preliminary study at Westmead Hospital showed that *U urealyticum* was isolated from only one of 53 (2%) infants of 28–32 weeks of gestation, compared with 14 of 60 (23%) of those of less than 28 weeks gestation (unpublished data). The risk of CLD is also significantly higher in infants of less than 28 weeks gestation.[2]

Consecutive infants admitted to neonatal intensive care at Westmead Hospital, who were less than 28 weeks of gestational age and required ventilation, were enrolled during two periods October 1993 to October 1994 and January 1995 to November 1996 (total of 34 months). Consecutive infants admitted to neonatal intensive care at Royal Prince Alfred Hospital who fulfilled the same criteria were enrolled over 16 months from February 1996 to June 1997.

Gestational age was determined by the last normal menstrual period and ultrasound examination before 20 weeks of gestation.

Endotracheal aspirates (ETA) were collected aseptically on the first and fourth days of life and, if the infant was still being ventilated, on day 28 of life (at Westmead Hospital only). The first ETA was collected immediately before administration of surfactant, if indicated, for treatment of hyaline membrane disease (HMD).

After instillation of sterile isotonic saline (0.3 ml) into the endotracheal tube, the infant was ventilated for 10 breaths. Using an appropriately sized catheter, the trachea was suctioned at a point 0.5 cm beyond the tip of the endotracheal tube; after another 10 ventilator breaths suctioning was repeated with a new catheter. ETA were stored at 4°C in a sterile container and processed within 24 hours of collection (average 15 hours), or transported by courier in ureaplasma culture broth to the laboratory and processed immediately.

LABORATORY METHODS

Cultures

Semi-quantitative cultures for genital mycoplasmas were performed using three 10-fold dilutions of the aspirate in ureaplasma broth, containing urea, neutral red indicator, and penicillin. Each dilution was plated on to A8 agar and cultured for 7 days at 35°C in 5% CO_2. If colour change occurred, broths were subcultured on to A8 agar plates, which were examined daily for 7 days for the presence of typical mycoplasma or ureaplasma colonies. Growth of *U urealyticum* was graded as scant, moderate, or heavy if the highest dilution at which it was detected was 10^{-1}, 10^{-2}, or 10^{-3}, respectively.

ETAs from infants at Westmead Hospital were also cultured aerobically for bacteria on horse blood agar for 48 hours in 5% CO_2. *Chlamydia trachomatis* infection is rare in our population (<1%; unpublished data) and cultures were not performed routinely.

Polymerase chain reaction (PCR)

Immediately after colour change had occurred, positive broth cultures (10^{-1} dilution), and a selection of negative cultures after one week's incubation, were stored at −70°C for PCR. After thawing, 0.5 ml of each culture was harvested by centrifugation at 14000 × *g* for 20 minutes. DNA was isolated, as described before.[27]

The methods used for identification, biotyping, and serotyping of *U urealyticum* have been described before.[27] Briefly, primers UMS-125 and UMA226 were used, initially to detect *U urealyticum* and distinguish biovars 1 and 2. Specimens in which *U urealyticum* serovar 1 was detected were reamplified to identify serovars using primers UMS-125 and UMA269 for serovars 3/14, UMS-125 and UMA 269' for serovars 1 and 6 and UMA 54 and UMA 269' for serovar 6.

CLINICAL DATA COLLECTION AND DEFINITIONS

Demographic and clinical data were obtained from the neonatal intensive care clinical databases and individual patients' medical records. Threatened premature labour (TPL) was defined as spontaneous uterine contractions that did not result in the delivery of the fetus during that episode. Pregnancy induced hypertension (PIH) was defined as hypertension first detected during pregnancy with a diastolic blood pressure of 90 mm Hg or more on at least two occasions separated by 6 hours. Antepartum haemorrhage (APH) was defined as clinically significant bleeding from the birth canal after week 20 of pregnancy. Premature rupture of membranes (PROM) was that occurring at any time before delivery. Antenatal steroids (two doses of betamethasone, 24 hours apart) were administered if preterm delivery was anticipated at less than 34 weeks of gestation. Mothers were recorded as having had steroids if they had received at least one dose before delivery. At Westmead Hospital, erythromycin and metronidazole, and at Royal Prince Alfred Hospital, amoxycillin, were normally administered to patients presenting with PROM or clinical manifestations of sepsis. Antibiotics were given at the discretion of the obstetrician.

RDS was diagnosed in ventilated infants with a requirement for supplemental oxygen of more that 40%, to maintain arterial oxygen tension above 60 mm Hg, and radiological changes consistent with HMD (bilateral fine reticular pattern). Surfactant was given for treatment of persistent RDS. Three different definitions of CLD were used: (a) the need for supplemental oxygen at 28 days of age; (b) the

need for supplemental oxygen at 28 days of age with radiological changes consistent with CLD[28]; and (c) the need for supplemental oxygen at 36 weeks postconceptional age. The latter is now regarded as the best predictor of long term outcome in very low birthweight infants.[29] Neonatal sepsis was defined by a positive blood culture.

Differences between groups were compared using Fisher's exact test, the χ^2 test, or the unpaired Student's *t* test, as appropriate. Logistic regression analysis, both univariate and multivariate, was used to test for associations between potential risk factors and the outcome of interest—either HMD or CLD(c). Odds ratios (OR) and their 95% confidence intervals (CI) were used to quantify the degree of association.

The statistical package SPSS for Windows, version 6.01, was used and a 5% level of significance was used throughout the analysis.

Results

One or more ETA cultures were collected from 113 infants at Westmead Hospital and 35 infants at Royal Prince Alfred Hospital, who fulfilled the study criteria. Aerobic bacteria (*Escherichia coli* in four patients and *Klebsiella pneumoniae* in one) were isolated (on HBA and/or in ureaplasma broth) from aspirates of two infants at Westmead and three at Royal Prince Alfred Hospital. These infants were excluded from further analysis. *Mycoplasma hominis* was not isolated from any ETA cultures.

Demographic and clinical details of the remaining infants from each hospital are shown in table 1. The only significant difference between the two units was in the rates of caesarean section. As this was not apparently related to any of the relevant outcomes, the data from both hospitals were combined. Around 30% of infants were twins or triplets.

Predictably, there was a high incidence of RDS and more than 70% of infants were treated with exogenous surfactant; 85% of mothers had been given steroids before delivery and, of these, two thirds had been given a full course. Antibiotics had been prescribed for 73 (59%) mothers in the week before delivery, including 52 (71% of those who received antibiotics or 42% of all mothers) who were given erythromycin alone or with other antibiotics. The duration of antibiotic treatment was not recorded.

U urealyticum was isolated on at least one occasion from 39 (27%) infants; of these, 38 had an ETA cultured on the first and or fourth day of life and 13 on day 28. Positive results were obtained from 25 of 36 (69.4%) infants on day 1, and 27 of 32 (84.4%) on day 4, and nine of 13 (69.2%) on day 28. Four infants were culture positive only on day 28, including three who had had one or more previous negative cultures. There was no difference in the proportion of infants whose mothers had been given antibiotics before delivery, between those with negative cultures on the first day of life who subsequently tested positive (8/10), and those whose cultures were positive on day 1 (22 /25). Cultures collected on day 4 were significantly more likely to have moderate to heavy growth (19/27; 70.3%) compared with those taken on the first (10/25; 40%; p <0.05) or 28th day (2/9; 22.2%; p=0.02).

Oxygen dependence at 28 days was common; it was present in 89% of survivors, of whom 60% also had radiological changes. Oxygen dependency was still present at 36 weeks postconceptional age—CLD (c)—in 34% of surviving infants.

Selected data for all mothers (table 2) and all infants (table 3) and for mothers and infants of singleton births only (table 4) were compared according to whether *U urealyticum* cultures were positive or negative.

Mothers of infants who were colonised with *U urealyticum* were significantly more likely to have been given antibiotics before delivery (p=0.002 for both singleton and multiple births). Mothers of colonised infants, overall,

Table 1 Comparison of infants admitted to neonatal intensive care units at Westmead and King George V Hospitals

	Westmead	*King George V*
Mothers n=	95	28
Antenatal steroids*	78 (82%)	27 (96%)
Antenatal antibiotics†	54 (57%)	19 (68%)
Caesarean delivery	30 (32%)	17 (61%)**
PROM‡ >24 hours	28 (30%)	7 (25%)
Infants (total): n=	111	32
Singleton births	76 (68%)	23 (72%)
F:M ratio	1.11	1.00
Mean birthweight (g)	827 (181)	850 (155)
Range	490–1275	590–1160
Mean gestation: weeks	25.8 (1.1)	25.8–0.9
Range	24–27	24–27
Neonatal sepsis††	38 (40%)	8 (29%)
HMD	84 (76%)	25 (78%)
Surfactant	79 (71%)	23 (72%)
Chronic lung disease (c)¶	28 (25%)	10 (31%)
Deaths§	28 (25%)	4 (13%)
U urealyticum isolated	28 (25%)	11 (34%)

**p<0.05; Caesarean section rates was the only significant difference between the two units.
*Steroids given to mother for fetal lung maturation if delivery anticipated before 34 weeks gestation.
†Antibiotics given to mother before (within 1 week of) delivery.
‡PROM, premature rupture of the membranes.
††Neonatal sepsis defined by a positive blood culture.
¶Requirement for supplemental oxygen at 36 weeks postconceptional age (see text for definitions).
§Deaths that occurred before 36 weeks postconceptional age (2 additional infants died subsequently).

Table 2 Comparison of antenatal data between mothers of all infants with and without U urealyticum colonisation

	*Isolated**	*Not isolated*	*p Value*
Mothers n=	36*	89*	
TPL	22 (61%)	47 (52%)	
PIH	1 (3%)	11 (12%)	
APH	15 (42%)	20 (23%)	0.05
Steroids†	34 (94%)	74 (83%)	
Antibiotics‡	29 (81%)	44/88 (50%)	0.002
Caesarean delivery	11 (31%)	36 (40%)	
Spontaneous labour	30 (83%)	61 (69%)	
PROM <24 hours	22 (61%)	68 (76%)	
PROM 1–7 days	9 (25%)	10 (11%)	
PROM >7 days	5 (14%)	11 (12%)	

**U urealyticum* was isolated from 39 infants. Mothers of two sets of twins, with discrepant culture results, are included in both columns. "Isolated" column also includes a mother of twins and a mother of triplets, all five of whose infants were culture positive.
†Steroids given to mother before delivery for fetal lung maturation.
‡Antibiotics given to mother before (within 1 week of) delivery.

Table 3 Comparison of neonatal data between all infants with and without U urealyticum

	Isolated	*Not isolated*	*p Value*
N=	39 (27.3%)	104	
Hyaline membrane disease	22 (56%)	87 (84%)	0.002
Neonatal sepsis*	15 (39%)	31 (30%)	
Surfactant	20 (51%)	82 (79%)	0.002
Deaths	6 (15%)	26 (25%)	
CLD (a)†	31/34 (91%)	69/78 (89%)	
CLD (b)†	22/34 (65%)	38/78 (49%)	
CLD (c)†	15/34 (44%)	23/78 (30%)	
CLD (c) and deaths	21/39 (54%)	49/104 (47%)	

*As defined by a positive blood culture; clinical significance was not determined.
†CLD (a) are those still requiring supplemental oxygen at 28 days of life; CLD (b) are those still requiring supplemental oxygen at 28 days of life with an abnormal chest *x*-ray; CLD (c) are those still requiring supplemental oxygen at 36 weeks post-conceptional age (PCA); denominators are surviving infants.

were more likely to have had an antepartum haemorrhage ($p<0.05$) but this difference was not significant in the singleton only group.

Infants colonised with *U urealyticum* were significantly less likely to have RDS and to have been given surfactant ($p=0.002$). This difference was confined to singletons ($p=0.001$); proportions of infants of multiple births who had RDS were identical whether or not they were colonised with ureaplasmas (8/9 *vs* 31/35; 89% for both). Among singletons, the mortality was lower in colonised than in non-colonised infants, but the numbers were small and the difference was not significant (7% *vs* 23% among singletons; $p=0.09$). Despite the apparently protective effect of ureaplasma colonisation for HMD, significantly more colonised singleton infants developed CLD ($p=0.03$), as defined by supplemental oxygen requirement at 36 weeks of postconceptional age. However, among infants of multiple births almost identical proportions developed CLD, whether or not they were colonised with ureaplasmas (3/6 *vs* 13/25).

The independent risk factors for the development of RDS and CLD were identified by multivariate logistic regression analysis, using backward elimination. When all infants were included, the independent predictors of RDS were multiple gestation (positive association; OR 3.6; 95% CI 1.2–10.7); administration of antibiotics to the mother (negative association; OR 0.33; 95% CI 0.12–0.88); and a positive culture for *U urealyticum* (negative association; OR 0.36, 95% CI 0.15–0.85). For singleton infants, a positive culture for *U urealyticum* was the only independent (negative) predictor of RDS (OR 0.2, 95% CI, 0.08–0.5). Independent predictors of CLD (c), are shown in table 5. In singleton infants, gestational age and isolation of *U urealyticum* were both independent predictors of CLD (c).

Table 4 Comparison of selected data between singleton infants and their mothers with and without U urealyticum colonisation

	Isolated	*Not isolated*	*p Value*
N = mothers/infants	30 (30.3%)	69	
Antenatal steroids*	28 (80%)	55 (80%)	
Antibiotics†	24 (80%)	31 (45%)	0.002
Spontaneous labour	25 (83%)	44 (64%)	
Cesarean delivery	10 (33%)	30 (44%)	
PROM‡ <24 hours	18 (60%)	49 (71%)	
PROM 1–7 days	8 (27%)	10 (15%)	
PROM >7 days	4 (13%)	10 (15%)	
Mean birthweight, g (range)	850 (161) (620–1245)	825 (181) (490–1275)	
Mean gestation; weeks (range)	25.7 (1.3) (24–27)	25.8 (1.2) (24–27)	
HMD	14 (47%)	56 (81%)	0.001
Surfactant	12 (40%)	53 (77%)	0.001
Neonatal sepsis††	13 (43%)	22 (32%)	
Deaths	2 (7%)	16 (23%)	0.09
CLD (a)	25/28 (89%)	46/53 (87%)	
CLD (b)	18/28 (64%)	25/53 (47%)	
CLD (c)	12/28 (43%)	10/53 (19%)	0.03
CLD (c) and deaths	14/30 (47%)	26/69 (38%)	

*Steroids given to mother before delivery for fetal lung maturation.
†Antibiotics given to mother before (within 1 week of) delivery.
‡PROM; premature rupture of membranes.
††Neonatal sepsis as defined by a positive blood culture.

Table 5 Independent predictors of chronic lung disease (c) by multivariate logistic regression analysis

Factor	*Odds Ratio*	*Confidence Interval*	*Significance*
All infants			
Gestational age	0.47*	0.30–0.75	0.001
Multiple gestation	3.1	1.2–8.1	0.02
Antibiotics	2.7	1.0–7.4	0.05
Singletons only			
Gestational age	0.46*	0.26–0.80	0.006
U urealyticum isolated	3.0	1.0–9.1	0.05

*The odds ratio is the factor by which CLD (c) decreases for each week of gestational age over 24 weeks.

PCR was performed on ureaplasma culture broths, from 50 different infants; *U urealyticum* had been isolated from 22. *U urealyticum* was identified by PCR in 19 (86%) culture positive and no culture negative specimens; biovar 1 was found in 16 (84%) and biovar 2 in four cultures (one culture had both). Serovars 3/14 were detected most commonly (7/16; 43.8%) among cultures containing biovar 1.

Discussion

This study primarily aimed to investigate the independent role of *U urealyticum* infection in the development of respiratory disease in high risk infants (those less than 28 weeks of gestation). The study was limited to infants in the gestational age group in which both ureaplasma colonisation and respiratory disease (RDS and CLD) are most common. Our findings confirmed and extended those of others, in showing significant differences in outcomes in colonised, compared with non-colonised infants.

U urealyticum was isolated from the ETA of 27% of infants, a rate comparable with that reported before. A single culture at birth would have underestimated the colonisation rate. A lower rate of recovery of ureaplasmas from cultures taken on the first day of life, compared with later,[20 25] probably reflects the antibacterial effects of amniotic fluid or intrapartum exposure, rather than nosocomial infection. We have shown that *U urealyticum* is inhibited, in vitro, by surfactant (unpublished observation), therefore we collected the first culture before surfactant was given. Maternal antibiotics did not prevent neonatal colonisation; significantly more mothers of the colonised infants had been given antibiotics—often erythromycin—in the week before delivery.

RDS occurred significantly less often among colonised infants compared with non-colonised

infants, despite the fact that similar proportions of mothers in both groups had been given steroids before delivery. This was masked by the effect of multiple gestation, which was associated with a very high incidence of RDS (89%), irrespective of ureaplasma colonisation. After multivariate logistic regression analysis, colonisation with *U urealyticum* remained the only significant independent predictor of RDS in singleton infants. The lower incidence of RDS in colonised infants was reflected in a lower mortality in the first 28 days of life, although the numbers were too small to be significant. However, despite the lower incidence of RDS, CLD occurred more frequently in colonised infants.

Previous studies have attempted to prove an association between ureaplasma colonisation and CLD, with variable results. CLD correlates best with gestational age and iatrogenic complications of respiratory support, including barotrauma and oxygen toxicity. Lung damage and development of CLD may depend on the balance between pro-inflammatory cytokines—interleukin (IL)-1β, IL-8 and tumour necrosis factor α (TNFα)—which are increased in ventilated infants and anti-inflammatory cytokines—IL-10 and IL-6—which may be reduced in premature infants.[16 31] Respiratory infection can aggravate cytokine mediated lung injury[32] and high IL-1β concentrations and ratios of IL-1β to IL-6 and TNF-α to IL-6 have been found in infants colonised with ureaplasma.[16] This supports other evidence that colonised infants often have congenital pneumonia,[10] but the association between these early effects of ureaplasma infection and CLD is inconsistent and probably indirect.[16 30]

In this study multivariate logistic regression analysis showed that both gestational age and colonisation with *U urealyticum* were significant independent predictors of CLD (oxygen dependence at 36 weeks postconceptional age) in singleton infants. These factors are closely correlated,[8 10] and their individual effects difficult to separate. Previous studies of the role of *U urealyticum* in CLD have shown inconsistent results.[26] Most, however, have shown a higher—but not always significantly higher—incidence of CLD in infants colonised with *U urealyticum*.

A meta-analysis of 17 studies, of variable quality, showed an overall relative risk (RR) of 1.72 (95% confidence interval 1.50–1.96).[33] The mean RR for studies performed since surfactant replacement therapy has been used, was significantly less (1.24; 95% CI, 1.10–1.49) than in earlier studies (1.92; 95% CI, 1.59-2.32). Several well designed studies reported more recently, using a requirement for supplemental oxygen at 36 weeks gestational age as the definition of CLD,[29] have still shown variable results.[22 34]

Circumstantial evidence suggests that *U urealyticum* colonisation in premature infants usually reflects intrauterine infection and a causative role in preterm delivery. *U urealyticum* can cause chronic intrauterine infection,[35] chorioamnionitis,[8] amniotic fluid infection[36] and congenital pneumonia.[14] Its isolation from placentas, following caesarean section with intact membranes,[10] and from infants, correlates strongly with spontaneous preterm birth. However, although colonised infants often have radiological and or laboratory evidence of pneumonia or systemic infection, most do not have obvious clinical signs of sepsis.

The most likely explanation for the protective effect of ureaplasma colonisation against HMD might be a stimulatory effect of subacute intrauterine infection on lung maturation and surfactant production. This is supported by a recent report that only four of nine (44%) infants colonised with *U urealyticum* required surfactant, compared with 84% of 51 non-colonised infants ($p = 0.04$).[30] However, in most previous studies, RDS has not been mentioned specifically or the incidence of RDS has been higher in infants colonised with ureaplasmas.[11 15] No previous study has analysed outcomes in singleton infants separately. In our study, the very high incidence of RDS in multiple births overshadowed the effect of ureaplasma infection, but this did not alter the overall result.

Treatment with both steroids and antibiotics (including erythromycin in many of our patients), at the onset of premature labour or rupture of membranes, is widespread. We suggest that this could suppress the inflammation caused by ureaplasma infection and so delay delivery for long enough to permit lung maturation. This is supported by our observation that colonisation of infants with *U urealyticum* was associated with an interval between membrane rupture and delivery (1–7 days); the usual outcome of intrauterine bacterial infection is prompt delivery.

It has been suggested that a randomised, controlled trial of appropriate antibiotic treatment is needed to determine whether *U urealyticum* has a causative role in CLD.[26] However, inconsistent results, small relative risks in previous studies, and undefined effects of other interventions, mean that large numbers of colonised premature infants would be needed to show a significantly improved outcome. A small, randomised, controlled trial of erythromycin showed no beneficial effect of treatment on the incidence of CLD,[30] suggesting that an antibiotic regimen at birth may be too late to significantly affect outcome.

There have been significant improvements in outcomes following preterm delivery, but less progress in understanding the causes and reducing the incidence. Intrauterine infection with *U urealyticum* has a causative role in a significant proportion of deliveries before 28 weeks. The determinants of adverse outcomes, including CLD, are complex, but gestational age at birth is the most important. Prevention or early treatment of intrauterine infection, to prevent preterm delivery, is only intervention likely to improve outcomes in pregnancies where *U urealyticum* infection is a factor.

Some serovars of *U urealyticum* have been implicated in disease more than others.[37] The use of PCR based serotyping and recombinant antigens for antibody assays, in future studies,

could help to overcome technical difficulties involved in confirming these associations.

Our research has provided important new insights into the complex interactions between *U urealyticum* infection (presumably in utero) and various treatment modalities that can affect the outcome of preterm birth. The challenge is to identify the small subset of women at risk from uterine infection among the much larger proportion who carry *U urealyticum* in the vagina without ill effect, and treat them before premature delivery occurs.

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1 Burnard ED, John E, Todd DA, Gratten-Smith P. A 15 year survey of chronic lung disease of prematurity. *Acta Paediatrica Scandinavia* 1989; **310 (suppl)**:120–6.
2 Todd DA, John E. Lung injury and repair in rabbits from ventilation with moist air. *Br J Exp Pathol* 1989;**70**:637-45.
3 Todd DA, John E, Osborn RA. Epithelial damage beyond the tip of the endotracheal tube. Early *Hum Dev* 1990;**24**:187-200.
4 Kresch MJ, Lin WH, Thrall RS. Surfactant replacement therapy. *Thorax* 1996;**51**:1137-54.
5 Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. *N Engl J Med* 1988;**319**:972-8.
6 Andrews WW, Shah SR, Goldenberg RL, Cliver SP, Hauth JC, Cassell GH. Association of post-cesarean delivery endometritis with colonization of the chorioamnion by Ureaplasma urealyticum. *Obstet Gynecol* 1995;**85**:509-14.
7 Kundsin RB, Leviton A, Allred EN, Poulin SA. Ureaplasma urealyticum infection of the placenta in pregnancies that ended prematurely. *Obstet Gynecol* 1996;**87**:122-7.
8 Eschenbach DA, Nugent RP, Rao AV, *et al*. A randomized placebo-controlled trial of erythromycin for the treatment of Ureaplasma urealyticum to prevent premature delivery. The Vaginal Infections and Prematurity Study Group. *Am J Obstet Gynecol* 1991;**164**:734-42.
9 Cassell GH, Waites KB, Watson HL, Crouse DT, Harasawa R. Ureaplasma urealyticum intrauterine infection: role in prematurity and disease in newborns. *Clin Microbiol Rev* 1993;**6**:69-87.
10 Cassell GH, Waites KB, Crouse DT, Rudd PT, Canupp KC, Stagno S, Cutter, GR. Association of Ureaplasma urealyticum infection of the lower respiratory tract with chronic lung disease and death in very-low-birth-weight infants. *Lancet* 1988;**ii**:240-5.
11 Waites KB, Crouse DT, Philips JB, Canupp KC, Cassell GH. Ureaplasmal pneumonia and sepsis associated with persistent pulmonary hypertension of the newborn. *Pediatrics* 1989;**83**:79-85.
12 Crouse DT, Odrezin GT, Cutter GR, Reese JM, Hamrick WB, Waites KB, Cassell GH. Radiographic changes associated with tracheal isolation of Ureaplasma urealyticum from neonates. *Clin Infect Dis* 1993; **17 Suppl 1**:S122-S30.
13 Panero A, Pacifico L, Rossi N, Roggini M, Chiesa C. Ureaplasma urealyticum as a cause of pneumonia in preterm infants: analysis of the white cell response. *Arch Dis Child Fetal Neonatal Ed* 1995;**73**:F37-F40.
14 Ollikainen J, Hiekkaniemi H, Korppi M, Sarkkinen H, Heinonen K. Ureaplasma urealyticum infection associated with acute respiratory insufficiency and death in premature infants. *J Pediatr* 1993;**122**:756-60.
15 Patterson AM, Taciak V, Lovchik J, Fox RE, Campbell AB, Viscardi RM. Ureaplasma urealyticum respiratory tract colonization is associated with an increase in interleukin 1-beta and tumour necrosis factor alpha relative to interleukin 6 in tracheal aspirates of preterm infants. *Pediatr Infect Dis J* 1998;**17:**321-8.
16 Arnon S, Grigg J, Silverman M. Pulmonary inflammatory cells in ventilated preterm infants: effects of surfactant treatment. *Arch Dis Child* 1993;**69**:44-8.
17 Kotecha S, Chan B, Azam N, Silverman M, Shaw RJ. Increase in interleukin-8 and solouble intercellular adhesion molecule-1 in bronchoalveolar lavage fluid from prematuire infants who develop chronic lung disease. *Arch Dis Child Fetal Neonatal Ed* 1995;**72**:F90-F96.
18 Wang EE, 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.
19 Sanchez PJ, Regan JA. Ureaplasma urealyticum colonization and chronic lung disease in low birth weight infants. *Pediatr Infect Dis J* 1988;7:542-6.
20 Payne NR, Steinberg SS, Ackerman P, *et al*. New prospective studies of the association of Ureaplasma urealyticum colonization and chronic lung disease. *Clin Infect Dis* 1993; *17 Suppl 1*:S117-S21.
21 Pacifico L, Panero A, Roggini M, Rossi N, Bucci G, Chiesa C. Ureaplasma urealyticum and pulmonary outcome in a neonatal intensive care population. *Pediatr Infect Dis J* 1997;**16**:579-86.
22 Saxen H, Hakkarainen K, Pohjavuori M, Miettinen A. Chronic lung disease of preterm infants in Finland is not associated with Ureaplasma urealyticum colonization. *Acta Paediatr* 1993;**82**:198-201.
23 Jonsson B, Karell AC, Ringertz S, Rylander M, Faxelius G. Neonatal Ureaplasma urealyticum colonization and chronic lung disease. *Acta Paediatr* 1994;**83**:927-30.
24 van Waarde WM, Brus F, Okken A, Kimpen JL. Ureaplasma urealyticum colonization, prematurity and bronchopulmonary dysplasia. *Eur Respir J* 1997;**10**:886-90.
25 Wang EE, Matlow AG, Ohlsson A, Nelson SC. Ureaplasma urealyticum infections in the perinatal period. *Clin Perinatol* 1997;**24**:91-105.
26 Kong F, Zhu X, Wang W, Zhou ZD, Gordon S, Gilbert GL. Comparative analysis andserovar-specific identification of the multiple-banded antigen genes of Ureaplasma urealyticum biovar one. *J Clin Microbiol* 1999;**37**:538-43.
27 Bancalari E, Abdenouiz GE, Feller, R, Gannon J. Bronchopulmonary dysplasia: clinical presentation. *J Pediatr* 1977;**95**:819-23.
28 Shennan AT, Dunn MS, Ohlsson A, Lennan K, Hoskins EM. Abnormal pulmonary outcomes in premature infants: prediction from oxygen requirement in the neonatal period. *Pediatrics* 1988;**82**:527-32.
29 Lyon AJ, McColm J, Middlemist L, Fergusson S, McIntosh N, Ross PW. Randomised trial of erythromycin on the development of chronic lung disease in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1998;**78**:F10-F14.
30 Jones CA, Cayabyab RG, Kwong KY, *et al*. Undetectable interleukin (IL)-10 and persistent IL-8 expression early in hyaline membrane disease: a possible developmental basis for the predisposition to chronic lung inflammation in preterm newborns. *Pediatr Res* 1996;**39**:966-75.
31 Groneck P, Goetze-Speer B, Speer CP. Inflammatory bronchopulmonary response of preterm infants with microbial colonisation of the airways at birth. *Arch Dis Child Fetal Neonatal Ed* 1996;**74**:F51-F5.
32 Wang EE, Ohlsson A, Kellner JD. Association of Ureaplasma urealyticum colonization with chronic lung disease of prematurity: results of a metaanalysis. *J Pediatr* 1995;**127**:640-4.
33 Perzigian RW, Adams JT, Weiner GM, Dipietro MA, Blythe LK, Pierson CL, Fais RG. Ureaplasma urealyticum and chronic lung disease in very low birth weight infants during the exogenous surfactant era. *Pediatr Infect Dis J* 1998;**17**:620-5.
34 Cassell GH, Davis RO, Waites KB, *et al*. Isolation of Mycoplasma hominis and Ureaplasma urealyticum from amniotic fluid at 16-20 weeks of gestation: potential effect on outcome of pregnancy. *Sex Trans Dis* 1983;**10 Suppl**:294-302.
35 Yoon BH, Chang JW, Romero R. Isolation of Ureaplasma urealyticum from the amniotic cavity and adverse outcome in preterm labor. *Obstet Gynecol* 1998;**92**:77-82.
36 Quinn PA, Li HC, Dunn M, Butany J. Serological response to Ureaplasma urealyticum in the neonate. *Clin Infect Dis* 1993; **17 Suppl 1**:S136–S43.