

Ureaplasma urealyticum as a cause of pneumonia in preterm infants: analysis of the white cell response

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Abstract

The tracheal isolation of *Ureaplasma urealyticum* from critically ill infants was investigated to determine if the organism was associated with an inflammatory response. Twenty nine neonates consecutively admitted for acute respiratory disease, with birthweights of <1301 g and no evidence of viral, chlamydial, or bacterial infections, were identified. Culture results for ureaplasmas were correlated with white cell counts and clinical and radiographic features. Sixteen infants had tracheal aspirates and/or blood specimens positive for *U urealyticum*. Pneumonia was diagnosed more frequently in the *U urealyticum* positive infants than in the 13 who were negative for the organism. The mean total white cell count, absolute neutrophil, and band form counts were significantly higher in the *U urealyticum* positive group than in the negative group.

These data suggest that *U urealyticum* can induce an inflammatory response in selected individuals who present with clinical, radiographic, and, in some instances, histological features of pneumonia.

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During the past decade there has been increased interest in *Ureaplasma urealyticum* and its possible role in perinatal morbidity and mortality, including sepsis, meningitis, and pneumonia.¹⁻⁴ Therefore, given the pathogenic potential of this organism, the ability to target this population for a marker of severity of inflammation in order to direct treatment against *U urealyticum* would be welcome.

U urealyticum colonisation of the nasopharynx and/or trachea of preterm neonates in the absence of other microbial pathogens is associated with an increased white cell count, indicative of a true inflammatory process.⁵ During the course of an ongoing prospective study on the incidence and outcome of *U urealyticum* infection in neonates, we discovered three neonates with *U urealyticum* infection of the respiratory tract who had a substantial increase in white cell count.⁶ These observations needed to be confirmed and extended, and it was with this aim that we reviewed all neonates with acute respiratory distress recently admitted to the neonatal

intensive care unit who had had specimens cultured for mycoplasmas, and we correlated culture results with white cell counts, clinical and chest radiograph findings, paying particular attention to pneumonic changes.

Methods

All infants with acute respiratory distress admitted consecutively to the neonatal intensive care unit at the University of Rome, Italy, from October 1993 to September 1994 (n=55) were included in the study. Data on maternal and perinatal risk factors (rupture of membranes, maternal fever, perinatal asphyxia and Apgar scores) were obtained by chart abstraction. The gestational age of all infants was determined using the Dubowitz score.

Samples were obtained for cultures of *U urealyticum* within the first day of life, during the third day of life, and subsequently in some cases if the patient's respiratory condition deteriorated. Tracheal aspirates were collected by mechanical suction through a catheter passed into the distal trachea through an endotracheal tube and were immediately placed into 2 ml of urea containing mycoplasma broths.⁷ Blood was collected for culture of mycoplasmas either by peripheral venepuncture or from an umbilical catheter and inoculated into mycoplasma broths. Subcultures for identification of *U urealyticum* were done on differential agar medium A7.⁸ Broths were incubated at 37°C under atmospheric conditions, and agar plates under 5% CO₂. All cultures were held for seven days. *U urealyticum* was identified by its characteristic morphological colonies and urease production.

Tracheal and blood specimens were cultured for aerobic and anaerobic organisms. Nasopharyngeal and tracheal specimens were also cultured for *Chlamydia trachomatis* and viruses.

White cell counts were performed by automated methods and were corrected for the presence of increased nucleated red blood cells. Differential counts were determined manually by duty personnel in accordance with standard methods.

RADIOGRAPHIC ANALYSIS

Chest radiographs were retrieved from the archives and reviewed by a paediatric radiologist (MR) who had no knowledge of the clinical history. There was agreement between the first and second interpretations.

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Characteristics of infants studied with and without *U urealyticum* infection

	U urealyticum infection		P value
	Present (n=16)	Absent (n=13)	
Birthweight (g)*	963 (150)	978 (201)	NS
Gestational age (week)*	27 (1.4)	29 (1.4)	0.001
Gender M:F	7:9	3:10	NS
Vaginal delivery	10	2	0.047
Birth asphyxia	12	6	NS
Exogenous surfactant	9	6	NS
Evidence of pneumonia†	14	4	0.0017
Total days of assisted ventilation*	16 (10)	7 (9)	0.033
Total days of oxygen therapy*	43 (40)	12 (20)	0.026
Intraventricular haemorrhage	5	1	NS
Patent ductus arteriosus	3	3	NS
Death	4	1	NS

*Mean (SD). †Radiographic and/or postmortem. NS=not significant.

Pneumonia 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.^{9 10}

STATISTICS

The means of the numerical data were tested for significant differences using the two tailed independent *t* test. Frequency incidences were analysed using the χ^2 test incorporating Yates' correction, where indicated. The level of significance was set at 0.05.

Results

Specimens were obtained for the culture of *U urealyticum* from the lower respiratory tract as well as from the blood of 55 infants.

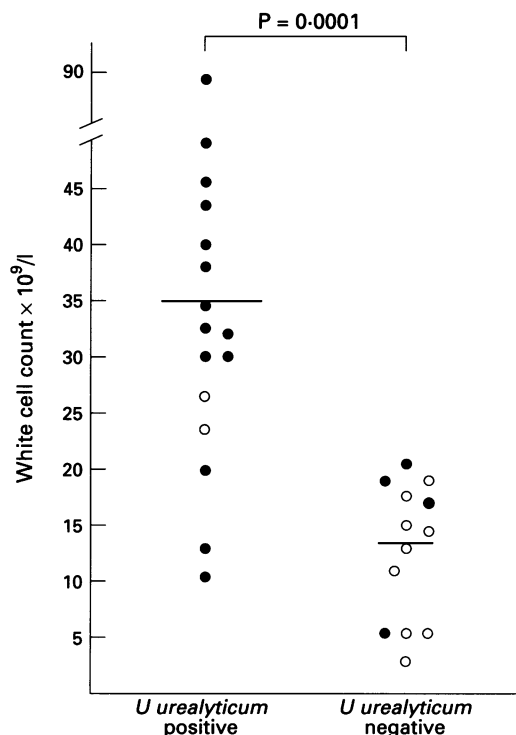


Figure 1 Total white cell count on day 3 of life in 16 neonates positive for *U urealyticum* compared with 13 neonates negative for *U urealyticum*. Solid line represents mean values: ●=with evidence of pneumonia; ○=without evidence of pneumonia.

Neonates with birthweights of >1300 g (n=17), systemic bacterial infections (n=3), a tracheal aspirate positive for other potential respiratory pathogens, such as *Streptococcus agalactiae*, *Escherichia coli*, *Staphylococcus aureus*, and *Chlamydia trachomatis* (n=4), or serious congenital malformations (n=2) were excluded for further analysis.

Among the 29 neonates eligible for this study, 16 were positive and 13 negative for *U urealyticum*. Of the 16 positive newborns, 15 grew *U urealyticum* from tracheal aspirates and one from blood as well as tracheal samples. Ten of these patients showed *U urealyticum* growth in the aspirate specimens taken within the first 24 hours. The specimens of the remaining six patients became positive later; in five patients the second specimen (at the age of 48–72 hours) was the first positive one, and in one, the third (at the age of 5 days). Ureaplasmas were always isolated in quantities exceeding 10^4 colony forming units/ml.

Blood and urine cultures for bacteria, and tracheal aspirate cultures for bacteria, chlamydiae and viruses were negative in all 29 neonates, as were serological studies for toxoplasmosis, rubella, cytomegalovirus infection, and herpes simplex virus infection.

The characteristics of the study patients are shown in the table. Infants with *U urealyticum* were of significantly lower gestation. There were no differences between *U urealyticum* positive and negative newborns with respect to gender, birth weight, incidence of maternal fever, as well as prolonged rupture of membranes and maternal hypertension. The isolation rate was significantly higher in neonates born by vaginal delivery. All the infants in both groups presented with acute respiratory disease requiring assisted ventilation and/or supplemental oxygen. Exogenous surfactant was used in nine of the 16 infants with, and in six of the 13 infants without, *U urealyticum*. The incidence of patent ductus arteriosus and intraventricular haemorrhage was similar in the two groups.

A roentgenographic diagnosis of pneumonia was established in 13 of the 16 *U urealyticum* positive infants and in four of the 13 *U urealyticum* negative infants (81% v 31%; $P=0.006$).

Compared with infants whose ureaplasma cultures were negative, those with *U urealyticum* had a longer duration of respiratory symptoms, ventilatory support, and oxygen supplementation. Death occurred in four of the 16 *Ureaplasma* positive infants. The first two infants died on postnatal days 4 and 7, respectively. In the first infant pneumonia was seen only at necropsy, while in the second one this was when the roentgenographic diagnosis of pneumonia was confirmed. Postmortem examination of the lung tissues showed the presence of patchy bilateral pneumonitis with polymorphonuclear cell infiltrates and areas of alveolar destruction, and yielded *U urealyticum* in pure culture. The third infant who soon after birth had a chest x ray picture indicative of pneumonia died at 24 hours of life because

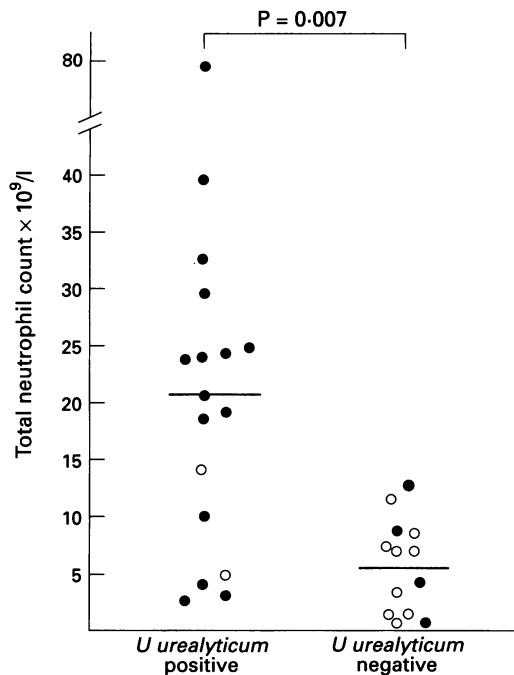


Figure 2 Total absolute neutrophil (polymorphonuclear plus band) count on day 3 of life in 16 neonates with *U urealyticum* and 13 neonates without. Solid line represents mean values: ●=with evidence of pneumonia; ○=without evidence of pneumonia.

of respiratory failure and intraventricular haemorrhage. Necropsy was not performed. The fourth infant, whose chest radiograph taken at 3 days of life showed bilateral pneumonitis, died of respiratory failure on day 28. A postmortem examination of the lungs showed extensive bilateral consolidation, multiple haemorrhages, moderate acute and chronic inflammation, necrosis of the bronchial epithelium, and interstitial fibrosis. Postmortem cultures were not performed. Among the 13 *Ureaplasma* negative infants, one died at 7 days of age because of hypertensive pneumothorax.

On day 3 of life, significant increases in the counts of total white cells and total absolute neutrophils (polymorphonuclear cells plus band forms) were observed in *U urealyticum* positive infants compared with those who were negative (mean (SD) $35.2 (18.6) \times 10^9/l$ and $21.5 (18.6) \times 10^9/l$ v $13.5 (5.5) \times 10^9/l$ and $6.2 (3.9) \times 10^9/l$; $P=0.0001$ and $P=0.007$, respectively) (figs 1 and 2). The band form counts were also significantly higher in the positive than in the negative group (mean (SD) $4.4 (5.2) \times 10^9/l$ v $1.2 (0.9) \times 10^9/l$; $P=0.009$). In the neonates positive for *U urealyticum*, repeated white cell counts remained invariably raised until day 7 of life but gradually decreased.

When analysing the leucocyte response in accordance with pneumonic changes, the 14 *U urealyticum* positive infants with roentgenographic ($n=13$) and/or postmortem ($n=2$) findings of pneumonia had a mean white cell count of $36.2 (18.2) \times 10^9/l$ while the four *U urealyticum* negative infants with a roentgenographic diagnosis of pneumonia had a mean white cell count of $13.5 (5.9) \times 10^9/l$ ($P=0.004$).

Discussion

The cause of lower respiratory disease in newborn babies is a diagnostic dilemma because pneumonia in the early neonatal period cannot be distinguished readily either clinically or radiologically from surfactant deficiency distress syndrome. In most nurseries it is therefore common practice to consider the possibility of sepsis or pneumonia in all clinically ill newborns with respiratory distress and to treat them with conventional antibiotics.

Although some investigators have reported apparently contradictory results in terms of the importance of *U urealyticum* as a pathogen in newborns, recent prospective studies confirm the validity of case reports. These findings contend that under certain conditions *U urealyticum* is a true pathogen capable of causing respiratory disease in newborn infants.^{4 11 12} Additional evidence of the pathogenic potential of ureaplasmas in the respiratory tract comes from animal studies. Newborn pathogen-free mice inoculated intranasally with ureaplasma strains isolated from the lungs of newborn human infants developed an acute, self-limiting, interstitial pneumonia. The lesions were most severe three days after inoculation and increased in severity by exposure to oxygen.^{13 14}

That the *U urealyticum* isolated from the tracheal aspirates of our patients represented true infection is suggested by the following reasons: (1) the organism was always isolated in pure culture and in high numbers; (2) *U urealyticum* was the only organism isolated from the lung tissue of two infants with histological evidence of pneumonitis at postmortem examination; (3) compared with *U urealyticum* negative infants, those who were positive had a significantly higher percentage of radiographic changes of pneumonia; (4) an inflammatory response, as shown by an increase in the mean total white cell count with both polymorphonuclear leucocytes and immature cells increasing in number, was observed in the *U urealyticum* positive group.

Previous studies have shown that the leucocyte count is a sensitive screening test for neonatal infection.^{15 16} However, most studies were performed before *U urealyticum* was implicated as a neonatal pathogen. Recently, Ohlsson *et al* observed an association between nasopharyngeal or endotracheal colonisation with *U urealyticum* and raised white cell counts, but they did not interpret the leucocyte counts in conjunction with the clinical picture.⁵ Our results confirm and expand on the findings of the above report. In fact, in our study, the increase in white cell counts was a reliable indicator of the ability of the organism to induce an inflammatory response in selected individuals who presented with clinical, radiographic, and, in some instances, histological findings of pneumonia.

Routine screening for *U urealyticum* is not warranted because many infants with ureaplasmas are asymptomatic and the organism often disappears without treatment.¹⁷ However, clinical and radiographic evidence of pneumonia, particularly in a preterm neonate,

is an adequate justification for obtaining the appropriate specimens for mycoplasmal cultures. It is even more important to consider ureaplasma infection when an ill neonate exhibits a leucocyte response and no other verifiable microbiological aetiologies of pneumonitis have been identified. Such neonates should be considered for antimicrobial treatment. Despite controversy stemming from non-standardised susceptibility testing, erythromycin is the drug of choice for treating neonatal ureaplasma infections not involving the central nervous system. The use of erythromycin is supported by its activity in vitro, limited data from clinical experience, and preliminary pharmacokinetic and safety studies.¹⁸

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